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Research and Development in Pharmaceutical Science

Volume II

Editor:

Prof. R. Uma Maheswari

Dr. Narayan D. Totewad

Dr. K. V. Madhusudhan

Mr. Umesh D. Laddha

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Editors

Prof. R.Uma Maheswari

Department of Pharmacy,

Ultra College of Pharmacy,

Madurai - 625 020,

Tamilnadu, India

Dr. Narayan D. Totewad

Department of Microbiology,

B. K. Birla College of Arts,

Science and Commerce (Autonomous),

Kalyan – 421 301, Dist. Thane, M.S., India

Dr. K. V. Madhusudhan

Department of Botany,

Government College for Men,

Kurnool - 518 002, AP, India

Mr. Umesh D. Laddha

Department of Pharmaceutics,

MET's Institute of Pharmacy,

Nashik – 422 003, M. S., India

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PREFACE

Pharmaceutical field encloses a wide number of themens ranging from drug discovery to manufacturing, techniques and technology, regulation and marketing and involves several disciplines such as chemistry, physics, biology biotechnology, pharmaceutics and engineering. The introduction of complex compounds in to medicine resulted in pharmacy becoming a speciazed branch.

To contribute the recent dimensions of the subject, we decided to publish the present book entitled "Research and Development in Pharmaceutical Science (Volume ll)". This book is the unified approach to various research areas. The prime goal behind the publication of the book is to bring awareness and exposure to research scholars, academicians and professionals in various topics of current research so that they can actively persue or incorporate the novel methods in their area of work. lt is the platform to exchange information and ideas on the recent trends in pharmaceutical sciences and research.

The articles in the book have been contributed by eminent scientists, academicians. Our special thanks and appreciation goes to experts and research workers whose contributions have enriched this book. We thank our publisher Bhumi Publishing, lndia for taking pains in bringing out the book.

Finally, we will always remain a debtor to all our well-wishers for their blessings, without which this book would not have come into existence.

- Editorial Team

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CONTENTS

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PHYTOCHEMISTRY AND PHARMACOLOGY OF *C. FASCICULARIS* LAM.

Y. K. Patil[1], C. Y. Patil[1], P. B. Koli[2] and T. A. Rajput[1*]

[1]Arts, Commerce and Science College,

Onde, Tal Vikramgad Dist. Palghar (India)

[2]Karmaveer Abasaheb and N. M. Sonawane Arts, Commerce and Science College,

Satana, Tal. Baglan Dist. Nashik (India)

*Corresponding author E-mail: tejenrajput@gmail.com

Abstract:

Phytochemical studies of leaves of *C. fascicularis* Lam. which contains physico-chemical factors like total ash values, extractive values and wetness content. Phytochemical investigation of n-hexane, chloroform and ethanol extract discovered the presence of glycosides, terpenoids, steroids, carbohydrate, alkaloids, saponins and protein. The antimicrobial activity was decided in all three extracts by using agar -agar disc diffusion method. Extracts were founding very effective on tested micro-organisms. The anti-microbial activities of various solvent extracts of *C. fascicularis L.* were tested against gram positive, gram negative human pathogenic bacteria and fungus. For isolation of medicaments, the dried powder of *C. fascicularis* Lam. was subjected for cold maceration at room temperature with solvent ethanol and did column chromatography. Finally, two compounds were isolated and purified by methanol. Mass spectrum of EEC1 and EEC2 showed corresponds to molecular formula $C_{15}H_{14}O_7$ and $C_{15}H_{14}O_6$. The structures were determined as Quercetin and Catechin by physical, chemical tests and spectral characterization such as Elemental analysis, Nuclear magnetic Resonance and Infrared spectrometry.

Keywords: Quercetin, Catechin, cold maceration, ethanol extracts, Antibacterial activity, Antifungal activity, *Corchorus fascicularis L.*

Introduction:

The development of medicinal plant as medicine is good way. The most common bacteria causing food generated illness are *S. aureus, E. coli* and others[1,2]. Natural products of medicinal plants may give new foundation of antimicrobial mediators with new mechanisms of action[3, 4].

Corchorus fascicularis L. is an once yearly herb found in all over India and also many steamy countries. The leaves are succulent and bitter. It shows pharmacological activity of

purgative, stimulant, energizer and aphrodisiacal. The seeds were removed tumors, lower the pain stomach troubles, skin diseases and itching. It is also useful in discharging ulcers[5]. Powder of entire plant is used as tonic to anemic patient[6]. *C. fascicularis* L. shows physiological activities[7]. Primary phyto-chemical studies of leaves of *C. fascicularis* L. reveals that presence of flavonoids, terpenoids, steroids, phenol, saponins, glycosides and alkaloids[8]. In Ayurvedic therapy with regards with medicines this plant has a huge demand due to uses in the treatment of many chronic, acute diseases disorders. In prolongation of work of phyto-chemical studies of several plants we are presenting of this paper on *C. fascicularis L*[9, 10].

Material and Methods:

Plant material collection and authentication:

The plant *C. fascicularis* L. were gathered from small town Tande of Tehsil Shirpur Dist. Dhule (Maharashtra state). The specimens of plants were confirmed by Dr. L. K. Kshirsagar, Department of Botany, S.S.V.P. S's Science College, Dhule.

Drying and pulverization:

Leaves of *C. fascicularis* L. were dark dried and powdered and deposited in an air tight container for future usage.

Extraction of powdered leaves:

The extraction of *C. fascicularis L.* leaves was carried out using established model practices [11]. The powdered leaves were uninterruptedly extracted by using cold maceration process using solvents like ethanol, n-Hexane and chloroform. All the extracts were vaporized to dryness and stored for future use.

Preliminary phytochemical screening:

All the extracts were subjected to primary Phyto-chemical testing for the existence of different types of chemical groups of compounds. The air-dried powdered plant material were screened for the presence of saponins, flavonoids, steroids, triterpenoids, proteins, glycosides, as described in various literatures[12,13].

Test microorganisms and growth media:

Bacteria strain *S. aureus* (NCIM 2079), *E. coli* (NCIM 2169) and fungal strain *Candida albicans* (NCIM 3471) were taken which one based upon their medical and pharmacological importance[14]. The above bacterial and fungal strains were acquired from NCIM, Pune were used for estimating antimicrobial activity. The bacterial and fungal store cultures were incubated for 24 Hrs. at around 37^0C in nutrient Agar and MGYP correspondingly, following freezing storage

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at temperature 4^0C. The bacterial strains were grown in Muller - Hinton agar at 37^0C whereas the fungal yeast was grown in MGYP respectively at temperature 28^0C. The hoard cultures were maintained at temperature 4^0C.

Antimicrobial activity[15]:

In vitro antibacterial and antifungal activities were scrutinized for ethanol, n-Hexane, chloroform and water extracts. *In vitro* antibacterial and antifungal activities of these extracts were investigated by the very accurate Agar Disk Diffusion Method[16, 17, 18]. All the four extracts of plant were screened for their antibacterial and antifungal activities against the bacterial strains *S. aureus*, *E. coli* and fungal strain *C. albicans.* The solutions of *C. fascicularis L.* extracts and used standard drug were prepared in distilled water using nutrient agar-agar tubes. The Muller Hinton sterile agar plates were incubated with two bacterial strains (1×10^8 bacteria/ ml) and allowed to remain at temperature $37\ ^0$C for about 3 hrs. Control tests were conducted under same conditions by using standard drug Chloroamphenicol for anti-bacterial activity and standard Nyastatin for anti-fungal activity All the Muller Hinton plates were incubated at temperature 37°C for about 18 to 24 hrs for taken bacteria and at temperature 28°C for about 48 to 96 hrs for fungi stain. The zones of growth inhibition around the disks were measured after 18 to 24 hrs of incubation at temperature 37°C for bacteria and 48 to 96 h for fungi at 28°C, respectively. The sensitivity of the microorganism species to the plant extracts was determined by measuring the sizes of inhibitory zones on the agar-agar surface around the taken disks.

Isolation and purification of compounds:

A small quantity of ethanol extract was dissolved in ethanol and this solution was spotted on TLC Plates. Silica gel 60F-254 size precoated plates (Merck) were used for TLC experiment. The spots were detected by spraying agent 70% Ethanolic-H_2SO_4 reagent followed by heating. In this study; chemicals and reagents used for TLC plates were of analytical grade reagents. Then the TLC plates were run by particular solvent system and were watched independently in iodine chamber and with the 70% Ethanolic–H_2SO_4 spraying reagent. Through several about 10 to 17 pilot experiments, it was discovered that the compounds of chloroform extract portion were divided by solvent system of Chloroform: Methanol: Ethyl acetate in the proportion of 7:2:1. The ethanol fraction, was subjected to column chromatography on silica gel (size 60-120 mesh) with gradient elution using Chloroform: Methanol: Ethyl acetate[19].

Two fractions were found homogeneous on TLC plate by using Chloroform: ethyl acetate (9.2:0.8), Petroleum ether: chloroform (9.5:0.5), Toluene: ethyl acetate:Metahnol (7:2:1) solvent

systems. These fractions were crystallized[20] and named as EEC-1 (Ethanol extract compound-1) and EEC-2 (Ethanol extract compound-2) respectively.

Test for Flavonoids:

Shinoda Test (Magnesium Hydrochloride Reduction Test)**:**

A few crystals of EEC1 and EEC2 were dissolved in Ethanol and a magnesium ribbon and drop wise concentrated Hydrochloric acid drop wise added to the solution, for both EEC1 and EEC2 formed a crimson red color after few minutes it converted to blue color indicating presence of flavonoids[11].

Zinc-Hydrochloride Reduction Test:

A few crystals of EEC1 and EEC2 were dissolved in Ethanol; then mixture of Zinc dust and conc. Hydrochloric acid solution was added, both EEC1 and EEC2 developed red color after few minutes indicating presence of flavonoids[11].

Alkaline Reagent Test:

A few crystals of EEC-1 and EEC-2 were dissolved in Ethanol. In this solution few drops of Sodium hydroxide solution were added, both EEC-1 and EEC-2 formed an intense yellow color which turns to colorless on addition of few drops of dilute acetic acid indicating the presence of flavonoids.

Spectroscopic characterization:

Different spectroscopic methods were used to elucidate the structure of EEC1 and EEC2. Among the spectroscopic technique IR, ^{1}H-NMR, ^{13}C-NMR and LCMS were carried out. The infrared spectrum was verified on FTIR 8400s (Shimadzu), ^{1}HNMR spectra were recorded on a Varian-400 MHZ NMR spectrometer (Shimadzu), ^{13}CNMR spectra were recorded on a Varian-400 MHZ NMR spectrometer (Shimadzu) at Aurangabad, India. The ^{1}HNMR and ^{13}CNMR spectra were recorded using $CDC1_3$, as solvent with Tetramethyl silane (TMS) as an internal standard. Mass spectrums were documented at high determination on a mass spectrometer (Perkin Elmer Auto system XL) at Aurangabad, the data were provided in m/z values. Elemental analysis was recorded on instrument namely Vario Micro Cube using oxygen and helium as combustion and carrier gas respectively at the temperature of around 1150°C at Wockhardt Research and Development Centre, Aurangabad, India.

Result and Discussion:

Physical appearance, color and odor of different extracts were recorded in (Table 1).

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Table 1: Shows characteristics of *Corchorus fascicularis* L. extracts

Sr. No.	Extract	Physical Appearance	Color	Odor
1	Ethanol	Semi-Solid mass	Dark Green	Pungent Aromatic
2	N- Hexane	Syrupy mass	Light Green	Aromatic
3	Chloroform	Semi-Solid mass	Dark Green	Aromatic
4	Water	Semi-Solid mass	Greenish Brown	Pungent Aromatic

The physical constants evaluation of drugs is an important parameter in detecting adulteration or improper handling of drugs. The total ash value is important in evaluation of purity of drugs i.e. presence or absence of foreign inorganic matter. The ash values, extractive values and moisture content of leaves were determined, and results are shown in (Table – 2)

Table 2: Shows physicochemical parameters of *Corchorus fascicularis* leaves.

Sr. No.	Parameters	Values (%) w/w
1	Loss on drying	3.01%
2	Ash values:	
	Total ash	5.40%
	Acid insoluble ash	2.96%
	Water soluble ash	1.85%
	sulphated ash	0.64
3	Extractive values:	
	Water soluble extractives	5.54%
	Alcohol soluble extractives	2.90%
	Petroleum ether soluble extractives	1.73%

Phytochemical tests for the presence of secondary phytoconstituents showed following results (Table -3).

The anti microbial activity of all the extracts of *C. fascicularis L.* were studied with concentration 100 µg/ml against two pathogenic bacterial strains and one fungal strain. Antibacterial and antifungal potential of extracts assessed in terms of zone of inhibition of bacterial growth. The results of antimicrobial activities are presented in Table 1-2. The growth of inhibition zone measured ranged from 15-18 mm for sensitive bacteria and ranged from 08-10 mm for fungal strains. The graphical results are presented in figure 1 and 2.The inhibitory effect

of *C. fascicularis L*. leaves ethanol, n-Hexane, chloroform and aqueous extracts showed at 17.76, 13.61, 15.00, 15.01 mm for *E. coli*, 16.30, 15.41, 14.18, 15.09 mm for *S. aureus* and 9.80, 8.15, 9.73, 8.92 for *C. albicans* respectively, The results showed that *C. fascicularis L*. leaves extracts were found to be effective against all the microbes tested.

Table 3: Show preliminary Phytochemical screening of *Corchorus fascicularis* leaves powder

Sr. No.	Phytoconstituents	Ethanol	N-Hexane	Chloroform	Water
1	Alkaloids	_	+	+	+
2	Carbohydrates	+	_	_	+
3	Glycosides	+	_	_	+
4	Flavonoids	+	_	_	_
5	Phenol& Tannins	+	_	_	+
6	Steroids	_	_	+	_
7	Terpenoids	+	+	_	+
8	Saponins	_	_	_	+
9	Proteins	+	_	_	+
10	Amino Acids	+	_	_	+

From the positive tests for Flavonoids given by the EEC-1 and EEC-2, they were assumed to be flavonoids. The melting point of EEC-1 and EEC-2 were 140 ^{0}C and 273 ^{0}C respectively. The UV λ_{max} value of EEC-1 and EEC-2 was formed at 251 and 276 nm, respectively. Mass spectrum of EEC-1 and EEC-2 showed a parent molecular ion peak at 302 and 290 respectively, which corresponds to the molecular formula $C_{15}H_{10}O_7$ (fig.1) and $C_{15}H_{14}O_6$ (fig.2).

In the IR spectrum of EEC-1 and EEC-2, an intensely broad band at 3368 and 3220 cm^{-1} showed presence of OH stretching and in the ^{1}H-NMR spectrum of EEC-1 it was seen that H-3 proton appeared at δ12.49 as hydroxyl proton with carbonyl system. The signal at 10.74 strong singlet for H-5 due to aromatic hydroxyl group and downfield due to near electronic withdrawing group. In ^{13}C-NMRspectrum, the signal corresponding to the ketonic carbonyl group C-4 appeared at δ 176.51.The signals at δ164.56, δ161.40, δ148.37, δ145.73, δ136.40 for five hydroxyl groups (Table-1).

The ^{1}H-NMR data of EEC-2 showed that the signal at δ 9.14 due to aromatic phenolic groups. The signals at δ 6.70, δ 6.58, δ6.56, δ 5.86, δ 5.66 due to different five aromatic protons.

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In the ^{13}C-NMR spectra, signals appeared at δ 28.52, 66.98, 81.64 due to C-4, C-3, C-2 carbons respectively and other aromatic carbons showed peaks at δ 94.52, δ 95.79, δ 99.73, δ 115.19, δ 115.75, δ 119.10, δ 131.27, δ 144.01, δ 145.51, δ 156.02, δ 156.84, δ 157.12 (Table-2). From above observation EEC-1 and EEC-2 were found to be Quercetin with reported values[21] and Catechin with reported values[22].

Table 4: Antibacterial activity of *C. fascicularis* Lam against bacterial test organism.

Microorganism	Zone Of Inhibition in mm Concentration in 100 µg/ml				
	Ethanol Extract	n-Hexane Extract	Chloroform Extract	Aqueous Extract	Chloroamphenicol Standard
E. coli	17.76	13.61	15.00	15.01	20.52
S. aureus	16.30	15.41	14.18	15.09	30.94

Table 5: Antifungal activity of extracts of *C. fascicularis L.* against bacterial test Organism.

Microorganism	Zone Of Inhibition in mm Concentration in 100 µg/ml				
	Ethanol Extract	n-Hexane Extract	Chloroform Extract	Aqueous Extract	Nyastatin Standard
C. albicans	9.80	8.15	9.73	8.92	9.53

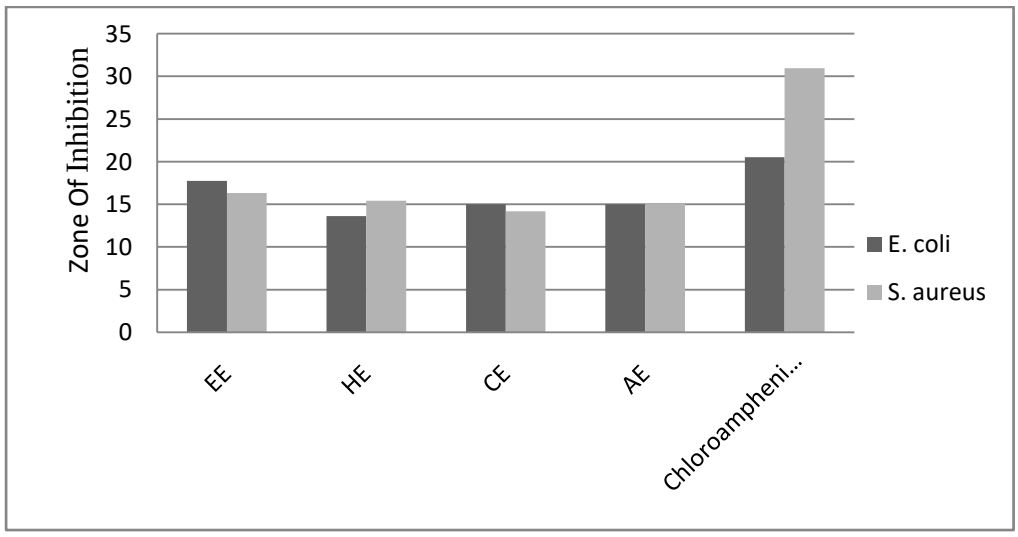

Figure 1: Antibacterial Activity Against *E. coli* and *S. Aureus*

(#EE- Ethanol extract, HE- n-Hexane extract, CE- Chloroform extract, AE- Aqueous extract)

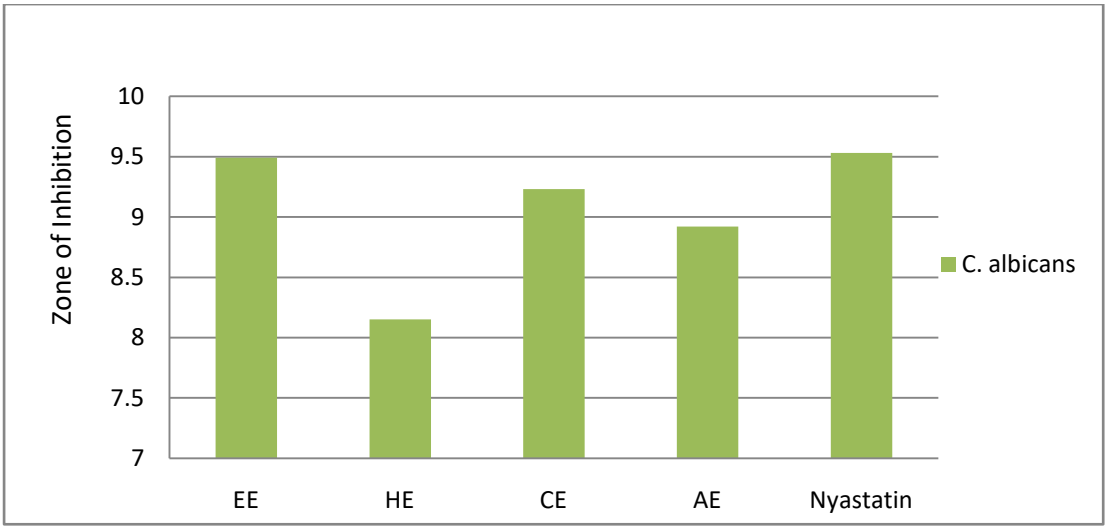

Figure 2: Antifungal Activity Against *C. albicans*

(#EE- Ethanol extract, HE- n-Hexane extract, CE- Chloroform extract, AE- Aqueous extract)

Table 6: Spectroscopic data of EEC-1 (Quercetin)

Spectroscopic Technique	**Data**
CHN Analysis	C= 59.56 %, H= 3.518%
UV λ max	256 nm
IR: (CHCl3):	3368, 3082, 2840, 1760, 1673,1522,1457, 1430, 1365, 1096, 1014, 716, 691 cm^{-1}
LCMS	302
^{1}HNMR ($CDCl_3$)	δ 12.49 (ss, OH C-5), 10.74 (ss, OH C-7), 9.55 (s, OH C3'), 9.33 (s, OHC-4'), 9.27 (s, OH C-3), 7.66 (d, 1H H-2'), 7.53 (dd, 1H, H-6'),6.87 (d, 1H, H-5'), 6.38 (d, 1H, H-8), 6.16 (d, 1H, H-6)
^{13}CMR ($CDCl_3$)	δ 176.5 (C-4), δ 164.56 (C-7), δ 161.40 (C-5), δ 156.81 (C-9), δ 148.37 (C-4'), δ 147.48 (C-2), δ 145.73 (C-3'), δ136.40 (C-3), δ 122.63 (C-1'), δ 120.65 (C-6'), δ 166.28 (C-5'), δ 115.74 (C-2'), δ 103.69 (C-10), δ 98.85 (C-6), δ 94.02 (C-8).

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Table 7: Spectroscopic data of EEC-2 (Catechin)

Spectroscopic Technique	**Data**
CHN Analysis	C=61.13 %, H= 5.080 %,
UV λ max	251 nm
IR: (CHCl3):	3240, 2853, 1629, 1522, 1473,1376, 1237, 1080, 1030, 732, 674.
LCMS	290
^{1}HNMR ($CDCl_3$)	δ 9.14 (m, Phenolic Protons), δ 6.70 (d, 2'H), δ 6.58 (d, 5' H), (Chemical shift in δ ppm)δ 6.56 (dd, 6'H), δ 5.86 (d, 6H), δ5.66 (d, 8H), δ4.46 (d, H2), δ 3.79 (ddd, 3H), δ 2.64 (dd, 4 H), δ 2.33 (dd, 4' H).
^{13}CMR ($CDCl_3$)	δ 157.12 (C-9), δ156.84 (C-7), δ 156.02 (C-5), δ 145.51 (C-3'), δ 144.01 (C-4'), δ 131.27 (C-1'), δ 119.10 (C-6'), δ115.75 (C-5'), δ 115.19 (C-2'), δ 99.73 (C-10), δ 95.79 (C-6), δ 94.52 (C-8), δ 81.64 (C-2), δ 66.98 (C-3), δ 28.52 (C-4).

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References:

1. Van T. T. H., Moutafis G., Tran L. T., Coloe P.J., 2007.Antibiotics resistance in food borne diseases from food animals in Thailand. J.Vet. Med. Sci, 70, 873-879.
2. Gerner-Smidt P., Whichard J.M., Foodborne diseases trends and report: Foodborne Pathogens Dis, 2008, 5: 551-554.
3. Runyoro D., Matee M., Olipa N., Joseph C., Mbwambo H., Screening of Tanzanian medicinal plants for antiCandida activity. BMC Complement Altern Med, 2006, 6 (11).
4. Shahidi B. H., Evaluation of antimicrobial properties of Iranian medicinal plants against Micrococcus luteus, Serratia marcescens, Klebsiella Pneumoniae and Bordetella bronch-oseptica. Asian J Plant Sci, 2004, 3, 82-86.
5. Kirtikar K.R., Basu B.D., Indian Medicinal Plants, International Book Distributor, Dehradun, 1996,401-402..

6. Patil D.A., Flora of Dhule and Nandurbar Districts, Sing Bishen Publishers, Dehradun, 2003, 112-113.
7. Hossen M, Ali M.S., Begum M, Khatton and Halim A., Jour. Innov Dev Strategy 2008, 3, 71-73.
8. Rajput A. P., Rajput T. A., Pharmacognostic & preliminary phytochemical investigation of *corchorus fascicularis* Lam. Leaves. Int J Pharmcy Pharm Sci, 2011, 3(5).
9. Rajput A.P. and Yadav S.S., Jour Phyto Res, 2000, 3,161-66.
10. Rajput A. P., Asian Journal of Chemistry, 2000, 14,795-98.
11. Harborne J.B., Phytochemical Methods, Chapmann and Hall, London, 1984, 168-170.
12. Khandelwal K.R., Practical Pharmacognosy, Nirali Prakashan, Pune, 2005, 117-30.
13. Kokate C.K., Gokhale S.B., Purohit A.P., Pharmacognosy, Nirali Prakashan, Pune, 2004,101-110.
14. Mc Cracken W.A., Cowsan R.A., Clinical and Oral Microbiology, New York, Hemisphere Publishing Corporation, 1983,512.
15. Rajput A. P., Rajput Tejendra A., 2011. Antibacterial and Antifungal activity from leaves extract of Corchorus fascicularis Lam. Int. J. PharmTech Res., 3(4):2195-2198.
16. Kumar A., Ilavarasan R., Jayachandran, Decaraman M., Aravindhan P.,2009 Phytochemical Investigation on a tropical plant in South India, Pakistan Journal of Nutrition, 8(1),83-85.
17. Alzoreky N.S., Nakahara K., Antibacterial activity of extracts from some edible plants commonly consumed in Asia. International Journal of Food Microbiology, 2003, 80, 223–230.
18. Bauer A. W., Kirby W.M.M., Sherris, J.C., Turck,M., Antibiotic susceptibility testing by standardized single disc method. American Journal of Clinical Pathology. 1966, 36, 493–496.
19. Stahl, E., 1969. Thin Layer Chromatography, a Laboratory Handbook. 2nd Edn., Springer, New York, ISBN: 0387047360.
20. Bahl, B. S.and A. Bahl, 1992. A Text book of Organic Chemistry. 13th Edn., Schand and Company Ltd., New Delhi, pp: 11-14.
21. Jung, E.K., S.K. Sang, G.H. Chang and H.L. Nam,. 2012, Antioxidative Chemical Constituents from the Stems of *Cleyera japonica* Thunberg. Int. J. Pharmacol., 8(5): 410-415.
22. Li, Y.L., J. Li, N.L. Wang and X.S. Yao,. 2008. Flavonoids and a new polyacetylene from *Bidens parviflora* Willd, Molecules, 13:1931-1941.

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REVIEW ON MICROBIAL SURFACTANTS AS PROMISING BIOACTIVE MOLECULES

Hemlata Bhosale* and Rahim Pinjari

School of Life Sciences,

Swami Ramanand Teerth Marathwada University, Nanded, M.S., India

*Corresponding author E-mail: bhoslehemlata@gmail.com

Abstract:

Bioactive Molecules have activity related to living organisms. Biosurfactant is one of the bioactive molecules. Biosurfactants are surface active amphiphilic molecules with hydrophobic and hydrophilic moiety which are produced as secondary metabolites by a wide variety of microbes to reduce surface tension and interfacial tension. It is classified as glycolipids, lipopeptides, lipoproteins, phospholipids, polymeric biosurfactants on the basis of structure and origin. Glycolipids and lipopeptides are the most common biosurfactants studied. It is produced by a range of Gram positive, Gram negative and yeast. *Bacillus subtilis* is one common source of biosurfactants. It shows different bioactivities such as antibacterial, antifungal, antibiofilm, anti-adhesives, antioxidant and anti-proliferative. Antibacterials covers range from Gram positive to Gram negative bacteria.Antifungals lipopeptides have potent activity against pathogenic fungi.Some of biosurfactants are protease resistant, thermostable,stable at variable pH. Biosurfactants are also effective at low concentrations. These biosurfactants can degrade the biofilm formation of various pathogens including *Streptococcus mutans. Lactobacillus* species exhibit good antibiofilm activity against MDR and MRSA. Anti-adhesives activity of biosurfactants is effective against pathogenic bacteria such as *S. typhimurium, S. aureus* and *E. coli.* The antioxidant activity exhibit effective cytoprotectivity in the cell lines. The antiproliferative activity was specific to type of cancer cell and dose dependent activity. These biosurfactants led to novel approaches for anticancer drugs.

Keywords: Bioactive, Biosurfactants, Glycolipids, lipopeptides, lipoproteins, Hydrophilic, Hydrophobic, antibiofilm, anti-adhesives, antioxidant, antiproliferative.

Introduction:

The term "bioactivity" presents all the phenomena from which make a form of life, a live organism. In strictly scientific terms, the term "bioactive" is an alternative term for" biologically

active" (Abdelkarim *et al.,* 2014). A bioactive compound is simply a substance that has a biological activity. A compound having biological activity,if it has a direct effect on a living organism. These effects may be positive or negative depending on the substance, the dose or the bioavailability. It is produced by fungi, acinetobacter, Cyanobacteria, microalgae and mycobacteria. These are the most important source for production of bioactive molecules (Marinelli *et al.,* 2015). The major bioactive molecules present in soil ecosystems are flavonoids, exopolysaccharides, antibiotics and quorum sensing signals (Zhuang *et al.,* 2013). Actinomycetes produces many bioactive molecules as secondary metabolites viz. aminoglycosides (Streptomycin and Kanamycin), ansamycins (Rifampicin), anthracyclines (Doxorubicin), Beta-lactams (Cephalosporins), Macrolides (Erythromycin and Tetracycline) and many other. These bioactive molecules have biological activities viz.antimicrobial, antimalarial, antitumor, antioxidant, insecticidal, antidiabeti (Jadon *et al.,* 2014). Biosurfactant are surface active amphiphilic i.e. having both hydrophobic and hydrophilic moiety and produced as secondary metabolites by wide variety of microbes to reduce surface and interfacial tension (Cameotra *et al.,* 201; Rivardo *et al.,* 2009; Hemlata *et al.,* 2015; Gudiña *et al.,* 2010). It occurs in nature as glycolipids, lipopeptides and lipoproteins (Sen *et al.,* 2010).

Classification of biosurfactant compounds:

These are generally made up of hydrophobic and hydrophilic. The hydrophilic moiety comprises of anions, cations, monosaccharides, disaccharides and polysaccharides. The hydrophobic moiety is made up of saturated and unsaturated fatty acids chain (Banat *et al.,* 2010). There are many criteria to classify biosurfactants (Neu *et al.,* 1996). It can be grouped by molecular weight. The low molecular weights are generally glycolipids or lipopeptides (Rosenberg *et al.,* 1999). The high molecular weight includes proteins, lipoproteins, polysaccharides or lipopolysaccharides or mixture of biopolymers and these are called as bioemulsans.*Acinetobacter* was best studied for bioemulsans. The lower molecular weight molecules acts efficiently reduce surface and interfacial tension. The high molecular weight i.e bioemulsans can stabilize the oil-water emulsions instead of lowering surface and interfacial tension (Rosenberg *et al.,* 1999). The best studied Surfactin was the first biosurfactant first reported by Arima *et al,* (1969) and Kakinuma *et al.* (1969). The best studied glycolipids are bioemulsifiers, trehalolipids, rhamnolipids, sophorolipids.

Glycolipids are more intensively studied than other types of Biosurfactants (Heyd *et al.,* 2008). Rhamnolipids are the type of glycolipids. These are produced by *Pseudomonas aeruginosa (*Rahman *et al.,* 2008). It is composed of one or two rhamnoses and upto three

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molecules of hydroxy fatty acids having carbon chain length C8-C14 (Rahman *et al.,* 2008; Zhang *et al.,* 1992). It can be measured by simply colorimetric measurement of rhamnose moiety (Zhang *et al.,* 1992). *Pseudomonas aeruginosa* GS-119 and DS10-129 showed higher yield of Rhamnolipids (Zhang *et al.,* 1992).

Sophorolipids are the high yielding biosurfactants after rhamnolipids (Van Bogaert *et al.,* 2011). The backbone of sophorolipids is the disaccharide sophorose. This backbone is hydrophilic in nature and have bonding of beta-1,2 bonds. The hydrophobic part of this amphiphilic molecule is made up of terminal hydroxylated fatty acids. The linkage present to link fatty acid to sugar is beta glycosidic bond. These are produced by non-pathogenic yeasts *Candida bombicola ATCC 22214.* In producing organisms, it is synthesized in the form of various mixtures. There are two types of sophorolipids depending on the acetylation and lactonization. The acidic form of sophorolipids is better than lactonised. The lactonic sophorolipids forms crystal. Lactonic sophorolipids show instability pH above 7.0-7.5.At pH 6 show optimum stability (Van Bogaert, 2011). Trehalose Lipids are made up of trehalose non-reducing sugar (2 glucose units) linked by alpha,alpha-1,1-glycosidic linkage. It is the basic component of cell wall of *Mycobacterium* and *Corynebacterium* (Franzetti *et al.,* 2017). *Rhodococcus erythropolis* DSM 43215 produced a surface active agent Trehalose Lipids formation induced by n-alkanes (C12-18). Trehalolipids made up of mycolic acids and trehalose sugar (Rapp *et al.,* 1979). The most Trehalolipids are produced Trehalose 6,6' dimycolate (alpha branched Mycolic acid esterified to C6 position of each glucose). It is produced by *Mycolates, Arthrobacter, Nocardia* and *Rhodococcus* (C20-C90) (Franzetti *et al.,* 2010).

Lipopeptides and lipoproteins are another type of biosurfactants[Desai and Banat 1997].A large number of cyclic lipopeptides including decapeptides antibiotics (germicidins) and lipopeptides antibiotics (polymyxins) produced by *Bacillus brevis* and *Bacillus polymyxa* respectively (Desai And Banat, 1997). Cyclic lipopeptide surfactin produced by *Bacillus subtilis* ATCC 21332 is the most powerful biosurfactant. It is made up of 7 amino acids and 8-9 methylene molecules. It has a linear and branched tails (Desai and Banat, 1997). *Bacillus licheniformis* produces several biosurfactants which act synergistically and exhibit excellent stability at various temperatures, pH and salt concentration *(*Desai and Banat, 1997). Lichenysin A has a long chain and beta hydroxylated fatty acids (Desai and Banat, 1997).

Fatty acids, phospholipids, neutral lipids:

Several bacteria and yeasts produce large quantities of fatty acids and phospholipids surfactants during growth on n-alkanes (Desai and Banat, 1997; Yakimov *et al.,* 1995).

Acinetobacter strain HO1-N produces phosphatidylethanolamine rich vesicles. *Aspergillus, Thiobacillus thiooxidans, Arthrobacter, Pseudomonas aeruginosa* accumulate 40-80% on hexadecane and olive oil respectively. *Rhodococcus erythropolis* produces phosphatidylethanolamine on n-alkane (Yakimov *et al.,* 1995).

Polymeric Biosurfactants are the best-studied polymeric biosurfactants emulsan, liposan, mannoprotein and other polysaccharide-protein complexes. *Acinetobacter calcoaceticus* RAG-1 produces a potent polyanionic heteropolysaccharide bioemulsifier called 'Emulsan'. This emulsan has a backbone containing a repeating trisaccharide of N-acetyl-D-galactosamine ironic acid and N-acetyl amino sugar. Fatty acids linked by o-ester linkage to sugar. It is one of the most powerful emulsion stabilizers known today. It resists inversion upto 1:4 water-oil mixture. Biodispersan is an extracellular, nondialyzable agent by *Acinetobacter calcoaceticus* A2. It is anionic heteropolysaccharide glucosamine, 6-methyl-amino hexose,galactosamine and ironic acid. Alasan is an anionic alanine containing heteropolysaccharide produced by *Acinetobacter radioresistens* KA-53. It shows 2.5 to 3 times activity after 100°C under neutral or alkaline conditions. Liposan is an extracellular water soluble emulsifier produced by *Candida lipolytica*. It is made up of 83% carbohydrates and 17% of proteins. It is composed of glucose,galactose, galactosamine and galacturonic acid (Desai and Banat 1997).*Saccharomyces cerevisiae* synthesize mannoprotein which is composed of 44% of mannose sugar and 17% of proteins. It shows emulsifying activity against oils, alkanes and organic solvents (Cameron *et al.,* 1988).

Particulate Biosurfactants are the extracellular, membrane vesicle partition hydrocarbons to form a microemulsion which plays an important role in alkane by microbial calls. *Acinetobacter* species HO1-N produces vesicles of 20-50 nm composed of proteins, phospholipids and lipopolysaccharides. The membrane vesicle contains 5 times phospholipids and 350 times polysaccharides compared to the outer membrane of the same organism (Desai and Banat, 1997).

Bioactive Properties of Biosurfactants:

Antibacterial activity of biosurfactants:

Biosurfactants reported as antimicrobial agents against *Bacillus subtilis, S.epidermidis* and *P. acnes.* Effect of biosurfactants as an antibacterial is different for gram positives like *Bacillus subtilis* and CTC 40400 and *S.aureus* ATCC 9144. Sophorolipids is a broad spectrum antibacterial agent against gram positive and gram negative (Diaz *et al.,* 2015). Surfactants producer *Bacillus subtilis* VSG4 and *Bacillus licheniformis* V16 showed the largest inhibition

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zone against *E. coli* and *S. aureus* effective against gram positive and gram negative (Giri *et al.,* 2019).

Antifungal activity:

B. subtilis derived lipopeptides is a potent antifungal against *F. solani* (Potato tuber causing fungi) on PDA.It shows moderate to higher antifungal activity at 2-3 mg/ml MIC and B-FSO1 at 20 microgram/ml activity against *Fusarium moniliforme.* Iturin A (amino fatty acid C14-C17 cyclic heptapeptide biosurfactant) produced by *B. subtilis LB-5* inhibits *Pestalotiopsis eugenile* spore and *Colletotrichum gloeosporioides conidia* germination respectively. Protease resistant thermostable glycolipid fungicide from *Pseudozyma fusiformata* is active against more than 80% of 280 species of yeast and yeast-like species under acidic conditions at 20 to 30° C (Mnif *et al.,* 2015). Rhamnolipids exhibit excellent antifungal activity against many fungi at 16 to 32 microgram/ml (Cameotra *et al.,* 2018).

Antibiofilm activity:

S. marcescens BS showed antibiofilm activity against *Candida albicans* and *P. aeruginosa*. This antifungal activity was studied on Luria Bertani and YEPD media respectively. 50 microgram/ml of glycolipids have potent action to reduce biofilm formation. These glycolipids can reduce biofilm formation in *Candida albicans* (59.8%), *P. aeruginosa* (70.6%) and *B.pumilus* (69.5%) (Dusane *et al.,* 2011). *L.helveticus MRC1* showed antibiofilm activity against *S. aureus, L. monocytogenes, L. innocua* and *B. cereus* (Giri 2019). Biosurfactants produced by *Lactobacillus reuteri* against *S.mutans* present on tooth surface. *Pseudomonas* species producing rhamnolipids exhibit antibiofilm activity against *S.aureus* at 0.1% in nutrient broth. Rhamnolipids are able to exhibit 86.9% at 25°C.In skim milk its ability to reduce biofilm formation is more than in nutrient broth. *Pseudomonas aeruginosa* produced mixed rhamnolipids against *S.mutans* and *N.mucosa.* This activity was detected by anti-adhesion assay at 0.8 to 1.25 mg/ml. At low MIC value,it exhibits good antibiofilm activity. *L.jensenii* and *L.rhamnosus* at 25-100 mg/ml exhibit good antibiofilm activity against MDT, MRSA and *A.baumannii* (Katarzyna *et al.,* 2019).

Anti-adhesives activity:

Anti-adhesives activity of lipopeptide was evaluated against four bacterial strains *E. coli, S. aureus, P. aeruginosa B. cereus.* It exhibits highest activity against *P. aeruginosa* 67.1% at 40 g/L and lower against *S.aureus* 32.3% at 40 g/L (Hajfaraj *et al.,* 2014). *B. subtilis BS-VSG4* and *BS VS16* exhibit highest antiadhesive activity against *S. Typhimurium* and *S. aureus* respectively. Biosurfactants produced by *L. helveticus* are highly active against bacterial pathogens at 69.2%

to 87% for 25 mg/ml biosurfactants concentration. *B.circulans* produced antiadhesive exhibit activity against *S. Typhimurium* 80% inhibition at 5 mg/ml. *E.coli* and *S.aureus* inhibited by two lipopeptides produced by *B. subtilis* and *B. licheniformis* (Giri *et al.,* 2019).

Antioxidant activity:

Mannosylerythritol lipid (MEL) produced by *Pseudozyma* yeast exhibit concentration dependent antioxidant activity. This activity was assayed by using DDPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity studied for MEL. P.cubensis grown on soybean oil produced MEL exhibited 50.3% at 10 mg/ml. MEL-C exhibited in vitro effects on NB1RGB and H2O2 treated cells. MEL-C exhibits higher cytoprotection than arbutin which is a strong scavenger (Takahashi *et al.,* 2012). *Lactobacillus casei* BS-B1 and BS-Z9 exhibit antioxidant activity and this activity were assayed by the DPPH method. By this method antioxidant values calculated 0.15 to 5.0 mg/ml (Merghini *et al.,* 2017). The antioxidant activity measured by DPPH 2,2-diphenyl-1-picrylhydrazyl and hydroxyl radical scavenging assay(HRSA) for biosurfactants produced by *B. subtilis* VSG4 at 0.5 to 5.0 mg/ml 69.1% to 62.3% DPPH and HRSA respectively. BSVS16 at highest concentration 5mg/ml exhibit 73.5% and 68.9% DPPH and HRSA respectively (Giri *et al.,* 2019).

Antiproliferative activity:

A Biosurfactant Monoolein produced by *Exophiala dermatitidis* was tested against four cell lines. Two of them HeLa and U937 didn't affect growth by Monoolein. This Monoolein exhibits antiproliferative activity specific to types of cancer cells. Most prominent effects were against cervical cancer (HeLa) and leukaemia (U937) and were detected to be dose dependent cell lines. The Monoolein exhibits prominent characterization of cytotoxicity induced by it. It was examined under phase contrast microscope. It causes cell rounding, cell shrinkage, membrane blebbing, loss of adhesion of cells in both cells observed. The fragmentation of DNA confirmed by DNA ladder assay. Monoolein triggers death by activating apoptosis in cell lines. This study opened a novel approach to find new anticancer drugs in the future(Paramaporn *et al.,* 2010). Cell mediated antiproliferative activity studied in human epithelial cells HEp-2. These cells were treated with biosurfactants from BS-B1 and BS-Z9 in the range of concentration of 25,50,100 and 200 mg/ml for 48 hrs incubation. This activity was confirmed by the MTT reduction assay method with IC50 from 109.1 to 129.7 mg/ml. BS-B1 exhibits higher antiproliferative activity than BS-Z9 (Merghini *et al.,* 2017).

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Conclusion:

Biosurfactants are the surface active amphiphilic molecules. These molecules are classified in several groups depending upon its different structures and microbial origin. It includes different biosurfactants such as glycolipids, lipopeptides, lipoproteins,polymeric biosurfactants and phospholipids. Low molecular biosurfactants referred exhibit activity mostly as active to reduce surface tension and interfacial tension. High molecular weight surfactants used as bioemulsans. The widely used biosurfactants are glycolipids including Trehalolipids, rhamnolipids and sophorolipids. Whereas lipopeptides such as germicidins, polymyxins, and surfactins are produced widely. Surfactin is the most powerful surface active cyclic lipopeptides used today. Polymeric biosurfactants are used to disperse or emulsify oil-water emulsion. Particulate biosurfactants are small group but have significance to stabilize microemulsions. Biosurfactants have various bioactive properties including antibacterials including Gram positive to Gram negative bacteria such as *B. subtilis* and *S.epidermidis.* Antifungals are active against pathogenic plant fungi like *F. solani.* Antibiofilm against biofilm forming bacteria and fungi such as *P. aeruginosa, S. mutans* (on tooth surface), *B. pumilus* and *Candida albicans.* Anti-adhesives activity exhibited against mostly *P. aeruginosa.* Mannosylerythritol lipids exhibit a stronger scavenger than arbutin and are cytoprotective in cell lines treated with H2O2. Monoolein produced by *Exophiala dermatitidis* exhibited dose dependent antiproliferative activity against some types of cancer leukaemia and cervical cancer. The limitations of this study includes more emphasis should be given on the cost effective production of biosurfactants using many low cost residues and optimizing the production process parameters. The goal to study biosurfactants is to explore bioactive properties to use in the development of sustainable human life.

References:

1. Banat, I. M., Franzetti, A., Gandolfi, I., Bestetti, G., Martinotti, M. G., Fracchia, L., Smyth, T. J., and Marchant, R. (2010). Microbial biosurfactants production, applications and future potential. Applied Microbiology and Biotechnology, 87(2), 427–444. https://doi.org/10.1007/s00253-010-2589-0
2. Cameotra, S., and Amp; Makkar, R. (2004). Recent applications of biosurfactants as biological and immunological molecules. Current Opinion in Microbiology, 7(3), 262–266. https://doi.org/10.1016/j.mib.2004.04.006

3. Cameotra, S. S., Makkar, R. S., Kaur, J., and Mehta, S. K. (2010). Synthesis of Biosurfactants and Their Advantages to Microorganisms and Mankind. Advances in Experimental Medicine and Biology, 261–280. https://doi.org/10.1007/978-1-4419-5979-9_20
4. Cameron, D. R., Cooper, D. G., and Neufeld, R. J. (1988). The mannoprotein of Saccharomyces cerevisiae is an effective bioemulsifier. Applied and Environmental Microbiology, 54(6), 1420–1425. https://doi.org/10.1128/aem.54.6.1420-1425.1988
5. Desai, J. D., and Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews: MMBR, 61(1), 47–64. https://doi.org/10.1128/.61.1.47-64.1997
6. Díaz De Rienzo, M. A., Stevenson, P., Marchant, R., and amp; Banat, I. M. (2015). Antibacterial properties of biosurfactants against selected Gram-positive and -negative bacteria. FEMS Microbiology Letters, 363(2). https://doi.org/10.1093/femsle/fnv224
7. Dusane, D. H., Pawar, V. S., Nancharaiah, Y. V., Venugopalan, V. P., Kumar, A. R., and amp; Zinjarde, S. S. (2011). Anti-biofilm potential of a glycolipid surfactant produced by a tropical marine strain ofSerratia marcescens. Biofouling, 27(6), 645–654. https://doi.org/10.1080/08927014.2011.594883
8. Franzetti, A., Gandolfi, I., Bestetti, G., Smyth, T. J., and Banat, I. M. (2010). Production and applications of trehalose lipid biosurfactants. European Journal of Lipid Science and Technology, 112(6), 617–627. https://doi.org/10.1002/ejlt.200900162
9. Giri, S. S., Ryu, E. C., Sukumaran, V., and amp; Park, S. C. (2019). Antioxidant, antibacterial, and anti-adhesive activities of biosurfactants isolated from Bacillus strains. Microbial Pathogenesis, 132, 66–72. https://doi.org/10.1016/j.micpath.2019.04.035
10. Guaadaoui Abdelkarim, Soumaya Benaicha, Naima Elmajdoub, Mohammed Bellaoui, and Abdellah Hamal. 2014. "What Is A Bioactive Compound? A Combined Definition For A Preliminary Consensus". International Journal of Nutrition And Food Sciences 3 (3): 174. doi:10.11648/j.ijnfs.20140303.16.
11. Gudiña, E.J., V. Rocha, J.A. Teixeira, and L.R. Rodrigues. 2010. "Antimicrobial And Antiadhesive Properties Of A Biosurfactant Isolated Fromlactobacillus Paracaseissp.Paracaseia20". Letters In Applied Microbiology 50 (4): 419-424. doi:10.1111/j.1472-765x.2010.02818.x
12. Hajfarajollah, H., Mokhtarani, B., and amp; Noghabi, K. A. (2014). Newly Antibacterial and Antiadhesive Lipopeptide Biosurfactant Secreted by a Probiotic Strain, Propionibacterium Freudenreichii. Applied Biochemistry and Biotechnology, 174(8), 2725–2740. https://doi.org/10.1007/s12010-014-1221-7

13. Hemlata, B., Selvin, J., and Tukaram, K. (2015). Optimization of iron chelating biosurfactant production by Stenotrophomonas maltophilia NBS-11. Biocatalysis and Agricultural Biotechnology, 4(2), 135–143. https://doi.org/10.1016/j.bcab.2015.02.002
14. Heyd, M., Kohnert, A., Tan, T.-H., Nusser, M., Kirschhöfer, F., Brenner-Weiss, G., Franzreb, M., and Berensmeier, S. (2008). Development and trends of biosurfactant analysis and purification using rhamnolipids as an example. Analytical and Bioanalytical Chemistry, 391(5), 1579–1590. https://doi.org/10.1007/s00216-007-1828-4
15. Jadon, R., Singh, V., and Chaudhary, H. S. (2014). Update on Bioactive Molecules of Actinomycetes. Biosciences Biotechnology Research Asia, 11(2), 705–714. https://doi.org/10.13005/bbra/1325
16. Kakinuma, A., Ouchida, A., Shima, T., Sugino, H., Isono, M., Tamura, G., and Arima, K. (1969). Confirmation of the Structure of Surfactin by Mass Spectrometry. Agricultural and Biological Chemistry, 33(11), 1669–1671. https://doi.org/10.1271/bbb1961.33.1669
17. Marinelli, Flavia, Olga Genilloud, Victor Fedorenko, and Eliora Z. Ron. 2015. "Specialized Bioactive Microbial Metabolites: From Gene To Product". Biomed Research International 2015: 1-2. doi:10.1155/2015/276964.
18. Merghni, A., Dallel, I., Noumi, E., Kadmi, Y., Hentati, H., Tobji, S., Ben Amor, A., and amp; Mastouri, M. (2017). Antioxidant and antiproliferative potential of biosurfactants isolated from Lactobacillus casei and their anti-biofilm effect in oral Staphylococcus aureus strains. Microbial Pathogenesis, 104, 84–89. https://doi.org/10.1016/j.micpath.2017.01.017
19. Mnif, I., Hammami, I., Triki, M. A., Azabou, M. C., Ellouze-Chaabouni, S., and amp; Ghribi, D. (2015). Antifungal efficiency of a lipopeptide biosurfactant derived from Bacillus subtilis SPB1 versus the phytopathogenic fungus, Fusarium solani. Environmental Science and Pollution Research, 22(22), 18137–18147. https://doi.org/10.1007/s11356-015-5005-6
20. Neu, T. R. (1996). Significance of bacterial surface-active compounds in interaction of bacteria with interfaces. Microbiological Reviews, 60(1), 151–166. https://doi.org/10.1128/mr.60.1.151-166.1996
21. Paraszkiewicz, K., Moryl, M., Płaza, G., Bhagat, D., K. Satpute, S., and amp; Bernat, P. (2019). Surfactants of microbial origin as antibiofilm agents. International Journal of Environmental Health Research, 31(4), 401–420. https://doi.org/10.1080/09603123.2019.1664729

22. Rahman, P. K. S. M., and Gakpe, E. (2008). Production, Characterisation and Applications of Biosurfactants-Review. Biotechnology(Faisalabad), 7(2), 360–370. https://doi.org/10.3923/biotech.2008.360.370
23. RAPP, P., BOCK, H., WRAY, V., and WAGNER, F. (1979). Formation, Isolation and Characterization of Trehalose Dimycolate from Rhodococcus erythropolis Grown on n-Alkanes. Journal of General Microbiology, 115(2), 491–503. https://doi.org/10.1099/00221287-115-2-491
24. Rivardo, F., Turner, R. J., Allegrone, G., Ceri, H., and Martinotti, M. G. (2009). Anti-adhesion activity of two biosurfactants produced by Bacillus spp. prevents biofilm formation of human bacterial pathogens. Applied Microbiology and Biotechnology, 83(3), 541–553. https://doi.org/10.1007/s00253-009-1987-7
25. Rosenberg, E., and Ron, E. Z. (1999). High- and low-molecular-mass microbial surfactants. Applied Microbiology and Biotechnology, 52(2), 154–162. https://doi.org/10.1007/s002530051502
26. Sen, R. (2010). Surfactin: Biosynthesis, Genetics and Potential Applications. Advances in Experimental Medicine and Biology, 316–323. https://doi.org/10.1007/978-1-4419-5979-9_24
27. Takahashi, M., Morita, T., Fukuoka, T., Imura, T., and amp; Kitamoto, D. (2012). Glycolipid Biosurfactants, Mannosylerythritol Lipids, Show Antioxidant and Protective Effects against H2O2-Induced Oxidative Stress in Cultured Human Skin Fibroblasts. Journal of Oleo Science, 61(8), 457–464. https://doi.org/10.5650/jos.61.457
28. Van Bogaert, I. N. A., Zhang, J., and Soetaert, W. (2011). Microbial synthesis of sophorolipids. Process Biochemistry, 46(4), 821–833. https://doi.org/10.1016/j.procbio.2011.01.010
29. Yakimov, M. M., Timmis, K. N., Wray, V., and Fredrickson, H. L. (1995). Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface Bacillus licheniformis BAS50. Applied and Environmental Microbiology, 61(5), 1706–1713. https://doi.org/10.1128/aem.61.5.1706-1713.1995
30. Zhang, Y., and Miller, R. M. (1992). Enhanced octadecane dispersion and biodegradation by a Pseudomonas rhamnolipid surfactant (biosurfactant). Applied and Environmental Microbiology, 58(10), 3276–3282. https://doi.org/10.1128/aem.58.10.3276-3282.1992
31. Zhuang, X., Gao, J., Ma, A., Fu, S., and Zhuang, G. (2013). Bioactive Molecules in Soil Ecosystems: Masters of the Underground. International Journal of Molecular Sciences, 14(5), 8841–8868. https://doi.org/10.3390/ijms14058841

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VESICULAR DRUG DELIVERY SYSTEM

S. Muthukumar[1]*, R. Gayathri[1], R. Vinesha[2] and C. Sankar[1]

[1]Department of Pharmaceutics,

KMCH College of Pharmacy, Coimbatore, Tamilnadu

[2]Department of Pharmaceutics,

Karpagam College of Pharmacy, Coimbatore, Tamilnadu

*Corresponding author E-mail: pharmmuthu@gmail.com

Vesicular drug delivery system:[1]

Vesicular drug delivery systems have gained a lot of interest as a carrier for advance drug delivery. Encapsulation of the drug in carrier structures is one such system, which delays the drug release and reduces the toxicity by selective uptake. The colloidal particulate carriers like liposomes possess numerous merits over any other conventional dosage form as they can act as drug reservoir and can deliver both hydrophilic and hydrophobic drugs. The vesicles dispersed in aqueous systems can hold few problems like degrade by hydrolysis or oxidation, sedimentation, aggregation or fusion of liposomes during storage. Two novel approaches adopted to avoid the possible problems are: to develop proliposomes and to develop niosomes incorporating non-ionic surfactants alternative to phospholipids in preparing vesicles. Though proliposomes are advanced over conventional liposomes some physical instability still persist, so a vacuum or nitrogen atmosphere is optional during preparation and storage to prevent the oxidation of phospholipids. In later approach, niosomes display appreciable chemical stability during storage but the aqueous suspension of niosomes exhibit physical stability problems like leaking of entrapped drug, aggregation, fusion or hydrolysis of encapsulated drugs etc. The recent trend in the vesicular delivery is combining the two previously mentioned techniques by extending the pro-vesicular approach to niosomes through the formation of "proniosomes" which are converted to niosomes.

Types of vesicular drug delivery system: [2,3,4]

1. Liposomes
2. Virosomes
3. Niosomes
4. Proniosomes
5. Transfersomes

6. Proteasomes
7. Sphingosomes
8. Ethosomes

Liposome:

They are artificially prepared vesicles composed of lipid bilayer. They can be formulated by disrupting the biological membrane. They are composed of natural phospholipids and can also contain lipid chains with surfactant properties.

Virosome:

They consist of uni lamellar phospholipid membrane which is either a mono or bi layer vesicle incorporating virus derived proteins to allow virosomes to fuse with target cells.

Niosome:

They are non- ionic surfactant based liposome. Niosomes are mostly formed by cholesterol incorporation. They have similar structure to that of liposomes.

Transfersome:

It is an artificial vesicle designed to be such as a cell vesicle or a cell engage in exocytosis & thus suitable for controlled & potentially targeted drug delivery.

Proteasome:

They are cytoplasmic organelles that are responsible for degradation of endogenous proteins due to the presence of ubiquitin conjugated to the targeted protein's lysine residue.

Sphingosome:

They are vesicles of bilayer in which an aqueous phase is entirely enclosed by lipid bi-layer membrane mainly composed of natural or synthetic sphingolipid.

Archaesome:

Archeasomes are liposomes that are made from Archaea, that are basically polar ether lipids.

Ethosome:

They are modified forms of liposomes which have high ethanol content. They are flexible, malleable vesicles adapted for enhanced delivery of active agents.

Proniosome[8]:

Proniosomes are dry, free flowing, granular product that could be hydrated immediately before use by agitation in aqueous media to form the niosomal suspension.

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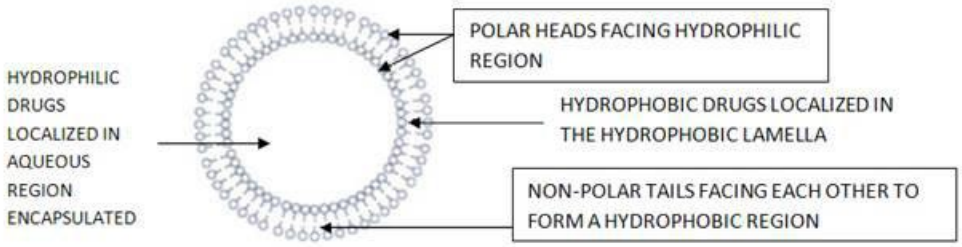

Figure 1: Schematic diagram of proniosome

Advantages of proniosomes[1]:

- The difficulties of physical stability like fusion, aggregation, sedimentation,&leakage on storage can be reduced.
- They help in avoiding hydrolysis of encapsulated drug which affects the shelf life of drug.
- Since it's a dry formulation provide convenience in storage and transportation.
- Provides drug delivery with improved bioavailability &reduced side effects.
- Both hydrophobic, as well as, hydrophilic drugs can be entrapped.
- Exhibits controlled and sustained release of drugs as a result of depot formation.
- Biodegradable, biocompatible & non-immunogenic to the body.

Interaction between proniosome and skin:[5]

There is a direct contact of the proniosomal formulation with the skin after it is applied. Since the proniosomes are made of non-ionic surfactants, it is important to study the possible interactions between the non-ionic surfactants and the skin. The non-ionic surfactants are amphipathic molecules containing hydrophobic (alkylated phenol derivatives, fatty acids, long chain linear alcohol etc) & a hydrophilic part (ethylene oxide chains in variable length). Skin consists of a range of bio active materials like membrane phospholipids, proteins, amino acids, peptides etc. Surfactants are employed to increase the permeability of vesicles and phospholipid membranes, causing low molecular mass compounds to leak. The non-ionic surfactants are included in the pharmaceutical formulations to increase their stability, solubility & permeation.

Factors affecting nature of proniosomes:

- Hydration temperature
- Choice of main surfactant
- Nature of drug
- Nature of membrane additives
- Size reduction techniques
- Addition of kinetic energy

Mechanism of drug permeation from vesicles through skin:[6]

The following types of vesicle-skin interactions are observed during in-vitro studies using human skin.

1. Absorption & fusion of vesicles onto skin surface leads to increase in thermodynamic activity gradient of the drug at the interface, which acts as a driving force for absorption of lipophilic drugs across the stratum corneum.
2. Altering the structure of the stratum corneum is also a type of interaction which involves the ultra structural changes in the intracellular lipid section of the skin & its deeper layers which is evaluated by Freeze Fracture Electron Microscopy (FFEM) & Small angle X- ray scattering (SAXS).
3. Bilayer present in the niosomes behave as a rate limiting barrier for drugs.
4. Proniosomes contain both non-ionic surfactant & phospholipids, which can behave as penetration enhancer & are useful in increasing penetrability of many drugs.
5. The penetration enhancer effect of vesicles to reduce stratum corneum barrier properties.

Mechanism of vesicle formation in proniosomes:[6]

Non-ionic surfactants have the ability to form bilayer vesicles which rely on the HLB of surfactant & also on critical packing parameter. CPP is defined as the relationship between structure of surfactant including size of the hydrophilic head group & length of hydrophobic alkyl chain in the ability to form vesicles is described as

$$\textbf{CPP= u/lca} \quad (1)$$

Where, u= the hydrophilic group volume

lc =critical hydrophobic group length

a= area of hydrophilic head group

When the value of CPP is between 0.5 to 1, then the surfactant is likely to form vesicles. CPP below 0.5(indicates that there is high contribution from hydrophilic head groups) gives spherical micelles & value of CPP above 1 (indicates that there is high contribution from the hydrophobic group) gives inverted micelles which on later stage gives precipitation. Spans are most widely used in proniosomal preparation.

All grades of span have same head group but are differentiated on basis of alkyl chain. The entrapment efficiency increases as the alkyl chain length increases.

Span60 (C18) > Span40 (C16) > Span20 (C12) > Span80 (C18).

Span 60 &80 have same head group but there is difference in the alkyl chain of span80 which is unsaturated. The low entrapment efficiency of span80 may be due to introduction of double bond to its paraffin chain. Cholesterol also provides stability to the bilayer membrane by increasing gel liquid transition temperature of vesicles &also attributes to high HLB value & small CPP.

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Categories of proniosomes:

- Dry Granular type of proniosomes
  - Sorbitol based proniosomes
  - Maltodextrin based proniosomes
- Liquid crystalline proniosomes

Dry granular proniosome:[7]

Dry granular type of proniosomes includes the coating of water-soluble carrier like sorbitol and maltodextrin with surfactant. Coating results in developing a dry formulation in which each water-soluble particle is covered with thin film of surfactant. It is necessary to prepare vesicles above the transition temperature of the non-ionic surfactant that is being used in the formulation. These are further categorized as follows

Sorbitol based proniosome:

Sorbitol based proniosomes is a dry formulation that uses sorbitol as the carrier, which is further coated with non-ionic surfactant and is used as niosomes within minutes by addition of hot water followed by agitation. They are produced by spraying surfactant mixture prepared in organic solvent onto the sorbitol powder and then evaporating the solvent. Since the sorbitol carrier is soluble in organic solvent, the process is required to be repeated till the desired surfactant coating has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to form as the carrier dissolves.

Maltodextrin based proniosome:[8]

Here maltodextrin is the carrier. The major advantage is the amount of carrier required to support the surfactant could be easily adjusted and proniosomes with very high mass ratios of surfactant to carrier could be prepared.

Liquid crystalline proniosome:

When the surfactant molecules are in contact with water, the lipohilic chains of surfactants get transformed into a disordered liquid state called lytorophic liquid crystalline state also called as neat phase. This transformation occurs by increasing the temperature at the kraft point, addition of solvent or use of both the temperature & solvent. Neat phase or lamellar phase in bilayers is placed over one another with intervening aqueous layer. These liquid crystalline proniosomes act as a reservoir for transdermal delivery of drug.

Proniosomes as drug carriers:[4]

The proniosomes are better drug carriers as they possess good chemical stability and overcome many disadvantages associated with liposomes. Additional merit is its non –toxic nature due to the use of non-ionic surfactants in its preparation. It does not require special conditions and precautions for formulation and preparation. They are dry free flowing product

which makes them more stable during sterilization and storage & also ease of transfer, distribution and measuring make them a pronouncing versatile delivery system.

Components of proniosomes: [9]

Surfactants:

Surfactants are surface active agents usually organic compounds that are amphiphilic in nature i.e they have have both hydrophobic & hydrophilic groups. They function as solubilizer, wetting agents, emulsifier & permeability enhancer. Only non-ionic surfactans are used for the preparation of proniosomes.

Example:

- Alkyl ethers & alkyl glyceryl ethers
  - Polyoxyethelene 4 lauryl ether
  - Polyoxyethelene cetyl ethers
  - Polyoxyethelene stearyl ethers
- Sorbitan fatty acid esters -Span20,40,60,80
- Polyoxyethelene fatty acid esters -Tween 20,40,60,80

Carrier material:

The carrier which is non- toxic, free flowing, poor solubility in the solvent are used for the preparation of proniosome & good aqueous solubility for hydration.

E.g: Maltodextrin, sorbitol, mannitol, spray dried lactose, glucose monohydrate, lactosemonohydrate, sucrose stearate.

Membrane stabilizer:

Cholesterol and lecithin are mainly used as membrane stabilizer. They influence the stability & permeability of the vesicles. Steroids are components of cell membrane, they bring about significant changes in the bilayer stability, fluidity & permeability. Cholesterol is a naturally occurring steroid used as membrane additive. It stabilises the system by means of electrostatic effects &also by prevention formation of the aggregates. It also leads to change of gel state to liquid state in niosomal system. Phosphatidylcholine is a major constituent of lecithin. It has low solubility in water & can form liposomes, bilayer sheets, micelles structures depending on hydration & temperature. Depending on the source, there are egg lecithin & soya lecithin. The vesicles composed of soya lecithin are larger in size then the one formed from egg lecithin, due to the difference in intrinsic composition.

Solvent and aqueous phase:

Alcohol used in proniosome has a higher effect on vesicle size & drug permeation rate. The size of the vesicles formed from different alcohol is different and are in the order: ethanol>propanol>butanol>isopropanol. Ethanol forms the largest sized vesicles due to its greater solubility in water. Ispropanol forms vesicles of smallest size due to its branched chain.

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Phosphate buffer pH7.4, 0.1% glycerol, hot water is used as the aqueous phase in the preparation of proniosomes.

Preparation of proniosomes:

There are 3 methods of preparation:

Slurry method:[5]

The carrier was taken in RBF and the entire volume of surfactant dissolved in the organic solvent was added to form slurry. The flask was then attached to the rotary evaporator & vacuum was applied until a dry free flowing powder was obtained. The flask was removed from the evaporator and kept under vacuum overnight. The obtained proniosomal powder was stored in sealed container at 4ºC.

Coaservation phase separation method:[9]

Weighed amounts of surfactant, lipid & drug were taken in a clean dry wide mouthed glass vial &0.5ml alcohol was added to it. All the ingredients were mixed well after warming. The vial was closed with a lid to prevent the loss of solvent. The surfactant mixture was dissolved completely by warming it over a water bath at 60-70ºC. Then the aqueous phase was added & warmed on water bath till a clear solution was formed which was then converted into a proniosomal gel.

Slow spray coating method:[5]

This method involves preparation of proniosomes by spraying surfactant in organic solvent onto the carrier (sorbitol) & then evaporating the solvent. As the sorbitol carrier is soluble in organic solvent, it is required to repeat the process until the desired surfactant loading has been achieved. The surfactant coating on the carrier is very thin and hydration of this coating allows multilamellar vesicles to be formed as the carrier dissolves. The resulting niosomes are very similar to those produced by conventional methods and the size distribution is more uniform.

Drug delivery features:[10, 11]**:**

- The physical and chemical features of the conjugate is responsible for the stability of the system.
- Due to presence of hydrophobic and hydrophilic phases, they easily penetrate through the cell membranes and tissues, achieved by endocytosis or exocytosis.
- The degradation rate depends on the particle size, function groups present in the drug molecule, nature of lipid, etc… Variations in these parameters alter the pharmacokinetic properties.
- Ideal for topical, extra and intra vascular, oral routes.
- Wider stability profile and greater shelf life.

- They tend to enhance permeation rate by improving the membrane fluidity. The transition temperature of vesicles might hold an effect on interaction with biomembrane.

References:

1. Trupti Anil, Vikrant P, Kiran K. Proniosome: A novel approach to vesicular drug delivery system. Int J Pharm Pharm Sci.2013; 3(1):1-6.
2. G V Radha , T. Sudha Rani, B. Sarvani – A review on proniosomal drug delivery drug delivery system for targeted drug action. J Basic & Clinical Pharm.2013;4(2):42-43.
3. Patel S. Ethosomes: A promising tool for transdermal delivery of drug. Pharma Info Net.2007; 5(3):233-240.
4. N. Bharti , S. Loona & M.M.U Khan. Proniosomes- A recent development in nanotechnology as a drug carrier. IJPSRR 2012;12(1) :68-75.
5. Nidhi Pandey. Proniosomes & ethosomes: New prospect in transdermal and dermal drug delivery system. *IJPSR* 2011;2 (8):1988-1996.
6. Nirav N Patel, Vikrani, Komal R, Arvind G,. Proniosomes for improved transdermal drug delivery – A review, *JPR*, 2013; 8(2): 62-67.
7. Hu C, Rhodes DG. Proniosomes: A novel drug carrier preparation, Int J Pharm.1999; 185(1):23-35.
8. Welesh AB, Rhodes DG. Maltodextrin based proniosomes. *Pharm Sci.* .2001; 3(1):1-8.
9. Waghmode M, Shruti A. Proniosomal drug delivery systems: An overview. IJPCS 2012; 1(3):1044-1056.
10. M. O. Vaizoglu and P. P. Speiser, “Pharmacosomes—a novel drug delivery system,” Acta Pharmaceutica Suecica, 1986; 23 (3), 163–172.
11. Archana Pandita, Pooja Sharma, "Pharmacosomes: An Emerging Novel Vesicular Drug Delivery System for Poorly Soluble Synthetic and Herbal Drugs", International Scholarly Research Notices, vol. 2013.
12. B. M. Mithal, Ocular Dosage Forms, Text Book of Pharmaceutical Formulations, Vallabh Prakashan, New Delhi, India, 1997.

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BIOCHEMICAL STUDY ON CERTAIN SEAWEEDS OF SOUTH INDIAN COAST

S. M. Prasad*[1] and Cissie Theeblyn David[2]

[1]Post Graduate Department of Nutrition and Dietetics,

SadakathullahAppa College (Autonomous), Rahmath Nagar,

Tirunelveli, Tamil Nadu, India Pin-627 011

[2]Department of Food Science and Nutrition,

ICAR-Krishi VigyanKendra, Tirupathisaram,

Kannyakumari District, Tamil Nadu, India, Pin-629 901

*Corresponding author E-mail: prasadsm33@gmail.com

Abstract:

Seaweeds which are known as sea vegetables are gift of god to the mankind. They provide important treasures of vitamins and minerals which are needed for a healthier living and promote health. They are rich in vitamins, minerals and antioxidants which play an important role in the prevention of cardiovascular diseases, arthritis, diabetes and cancer. In this piece of work biochemical parameters of certain seaweeds were analyzed and linked to good health and medicinal value. It was concluded that the moisture content was highest in SW_3 *(Gracilaria fergusonii)* with 90 per cent and lowest in SW_5 *(Sargassum wightii)* with 83.18 per cent. Calcium was maximum in SW_3 *(Gracilaria fergusonii)* with 632.270 mg and minimum in SW_1 *(Ulva lactuva)* with 95.047 mg. Magnesium recorded maximum in SW_3 *(Gracilaria fergusonii)* with 133.69 mg and minimum in SW_1 *(Ulva lactuva)* with 17.287 mg. The maximum value for Zinc was observed as 20.14 mg in SW_2 *(Ulva fasciata)* and minimum as 13.05 mg in SW_4 *(Gracilaria cortigata).* Iron was highest in SW_3 *(Gracilaria fergusonii)* 16.11 mg and lowest value in SW_4 *(Gracilaria cortigata)* 10.28 mg. Manganese was maximum in SW_3 *(Gracilaria fergusonii)* with 0.1674 mg and minimum in SW_5 *(Sargassum wightii*) with 0.0066 mg). Selenium recorded highest value of 0.0014 mg in SW_4 *(Gracilaria cortigata)* and in traces in SW_5 *(Sargassum wightii).* The results justify the popular use of these sea weeds to be a primary health care medicine and a functional food for the near future.

Key Words: Seaweeds, Vitamins, Minerals, Trace Elements, Life Style Diseases

Introduction:

India has the highest record of seaweeds species from the Indian Ocean region. The country has a coastline of 75,000 km with diverse habitats supporting rich seaweeds biodiversity **(**Bhavanth Jha *et al.,* 2018**)**. Seaweeds grow abundantly along the Tamil Nadu and Gujarat coasts and around Lakshadweep and Andaman and Nicobar islands. There are also rich sea weed beds around Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam and Pulicat inTamil Nadu and Chilka in Orissa (Gulshad Mohammed, 2015).

In contrast to terrestrial plants the vast majority of seaweeds species are edible, but not all of them are equally suitable for human consumption, because they are either tough or simply not particularly tasty. A few species can be eaten if they are completely fresh and collected from areas of clean water. More however need to be processed in some ways by drying cooking or toasting in order to make palatable. This often results in a noticeably improved flavour **(**Ole G. Mouristen, 2013).

In general terms it can be said that a varied diet that includes a proportion of seaweed product up to 10 per cent as in japan promote wellness. This is due principally to the relatively high concentration of important minerals and vitamins-sea vegetables have been described as nutrient dense in this regard. Seaweeds have relatively high concentration of vitamins and minerals (Loenel Pereira, 2018). The inclusion of large amounts of seaweeds in a balanced diet has been connected to decreased rates of many of the western life style diseases (eg Cancer, Cardiovascular diseases) (Joel Fleurence and Ira Levine, 2016).

As shown earlier seaweeds have been used in a variety of ways in industry and for farming. It's most common usage however comes from human consumption seaweeds was considered a seasonal food product for household consumption or sold locally seasonally (Delaney *et al.,* 2016). The sea weeds are economically valuable resources. They are used as food, fodder, fertilizers and medicine and thus useful to mankind in many ways. Agar and align extracted from the sea weeds have a varied of industrial applications (Rajrupa Gosh *et al.,* 2012).

Seaweeds has been used in various dishes including appetizers, casseroles, muffins, pilafs and soups in Korea, China and Japan due to its high mineral content (Se-Kwon Kim, 2015). Seaweeds are taking majority of attention from scientists because of its phenomenon bioactive compounds and its properties like anti-viral, anti-tumor, anti-inflammatory and anti-lipidemic and may more properties (Burtin, 2003).

Materials:

Raw Materials:

The samples were collected from different localities of Tamil Nadu and Kerala namely Mandapam, Tuticorin, Kanyakumari and Vizhinjam seashore for research purpose. They are

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sw_1= *Ulva lactuva, sw*$_2$= *Ulva fasciata,* sw_3 =*Gracilaria fergusonii,* sw_4 =*Gracilaria cortigata,* sw_5 = *Sargassum wightii.*

Methods:

Identification of plants

Fresh seaweeds were identified with the help of a Botanist and a Taxonomist

Procurement of Samples

The sample were collected in quantities of 100 g, washed thoroughly to remove the epiphytes and other contamination, cleaned, shade dried, powdered and sealed in polythene bags and brought to the laboratory for analysis.

Equipment used

Weighing balance

Balance of 2 kg capacity was used for quantifying the sample

Hot air oven

Hot air oven used for drying the sample and also for sterilizing the glass wares

Muffle furnace

With thermostat controlled degree, 100 and 200 was used for ashing the samples

Desiccator

Desiccator was used to preserve the hygroscopic samples used during analysis.

Atomic Absorption Spectrophotometer

The trace element analysis was performed using Atomic Absorption Spectrophotometer

Analysis of biochemical components

Moisture

Moisture content of the samples were estimated by the Hot Air Oven methodsuggested by Ranganna, 1997.

Ash

To determine the ash content a known weight of the sample was dried in the Hot air oven, incinerated and ashed using muffle furnace at a specific temperature of 610 degree centigrade according to Berwal *et al.,* 2004.

Minerals

For the estimation of minerals a known sample was weighed, dried in the hot air oven at 120^0C for 2 hours ashed, in the muffle furnace. A specific quantity of ash was digested with triple acid solution, made up to a known volume and fed into the aspirator of the Atomic

Absorption Spectrophotometer as suggested by (Sadasivam and Manickam, 2004). The absorption of radiation was measured at a specific wave length of 422.7 nm for calcium, 285.2 nm for magnesium, 213.8 nm for zinc, 248.3 nm for iron, 279.5 nm for manganese,196 nm for selenium respectively.

Statistical analysis

Completely Randomized Block Design (CRBD) described by Das and Giri 1979) was employed for analysing the data at 0.01 levels and 0.05 level of significance with duplicate number of sample.

Results and Discussion:

Moisture content of seaweeds (per cent)

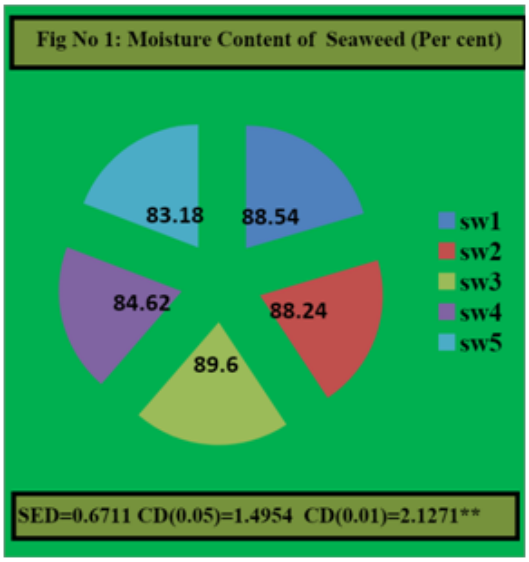

Figure 1: Moisture content of Seaweed

From Fig 1, it was confirmed that the highest per cent of moisture was observed in sw_3 (89.60), followed by sw_1 (88.54), sw_2 (88.24) and sw_4 (84.62) whereas, the lowest moisture per cent was observed in sw_5 (83.18). The statistical analysis of the data revealed that the moisture content among the seaweeds was highly significant at 0.05 and 0.01 % level of significance.

Similar results were obtained from Ruperz (2002**)** where he found the moisture content of *Gracilaria fergusonii* and *Ulva lactuva* within the range of 87.24 to 90.4 per cent. Burkiff (2007) observed the moisture content of *Ulva fasciata* was in between 86.25 to 94.3 per cent

Calcium content of seaweeds (mg/100g)

From Table 1, it was concluded that sw_2 (632.27mg) had the highest calcium content followed by sw_4 (322.30mg), sw_5 (304.70mg) and sw_2 (126.87mg) whereas, lowest calcium

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content was observed in sw_1 (95.04mg).Among the samples analyzed for calcium highly significant difference was observed among the data at both 0.05 and 0.01 level of significance.

Ruperz (2002) has found the calcium content vary between 602.27 mg to 638.48 mg in *Gracilaria fergusonii* and 289.48 mgto 340.00 mg in *Gracilaria cortigata.* Similar results were obtained by Sahoo *et al.* (2001) in Sargassum *wightii* with the calcium range in between 302.00 mg to 320.45 mg

Table 1: Calcium Content of Seaweeds (mg/100g)

Sr. No.	Sample	Calcium
1	SW1	095.04
2	SW2	126.87
3	SW3	632.27
4	SW4	322.30
5	SW5	304.70
SED=0.7515 CD(0.05)=1.6745 CD(0.01)=2.3819**		

Magnesium content of seaweeds (mg/100g)

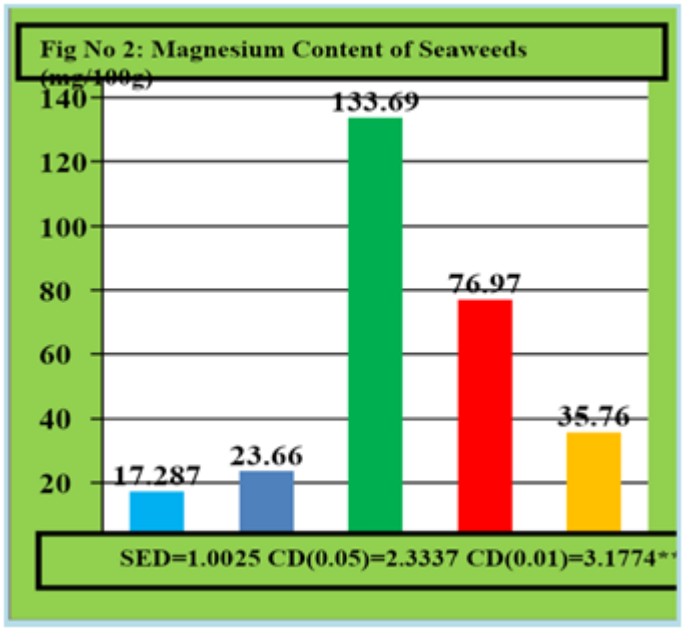

Figure 2: Magnesium content of Seaweed

Among the sample analysed for magnesium (Fig. 2) the highest rating was given to sw_3 (133.69 mg) followed by sw_4 (767.97mg), sw_3 (35.76 mg), sw_2 (923.66 mg), and sw_5 (17.28 mg) had the lowest content. Statistically it was proved that all the seaweeds sample analyzed were highly significant at 0.05 and 0.01 level of significance.

Susman (2006) analyzed the magnesium content in *Ulva* Species and *Sargassum* species to be 25.820 mg and 40.450 mg respectively which was significantly less when compared to the present study

Zinc content of seaweeds (mg/100g)

Table 2: Zinc Content of Seaweeds (mg/100g)		
Sr. No.	**Sample**	**Zinc**
1	SW1	14.280
2	SW2	20.140
3	SW3	14.150
4	SW4	13.055
5	SW5	15.390
SED=0.3320 CD(0.05)=0.7398 CD(0.01)=1.0524**		

The zinc content of the seaweeds analysed showed maximum content of zinc is in sw_2 (20.14mg), followed by sw_5 (15.39mg) and sw1 (14.28mg) howbeit, the minimum wasobserved in sample sw_4 (13.05mg)

Iron content of seaweeds (mg/100g)

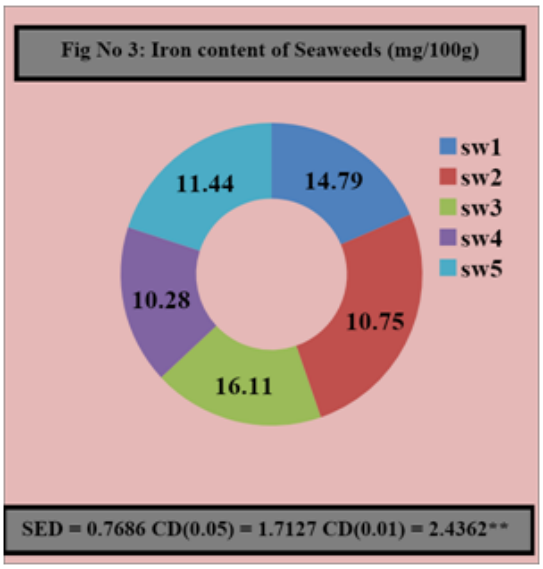

Figure 3: Iron content of Seaweed

Among the samples analysed for iron (Fig No 3) the highest rating was given to sw_3 (16.11mg), followed by sw_1 (14.79 mg), sw_5 (11.44 mg) and sw_2 (10.75 mg) however, sw_4 (10.28 mg) had the lowest content. Statistically it was proved that all the seaweeds were highly significant. Similar results were obtained by Muthu *et al.* (2005) within the range of 9.26 mg to

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20.84 mg in *porphyra* species whereas the present study recorded the highest iron content. The value obtained here was in between 10.28 mg to 14.79 mg.

Manganese content of seaweeds (mg/100g)

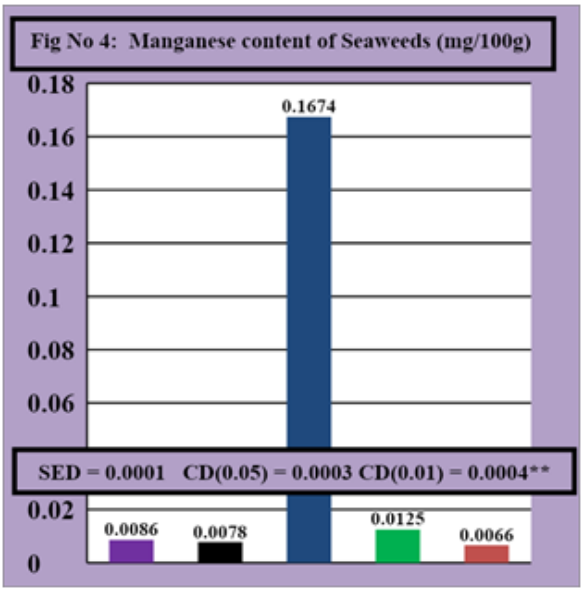

Figure 4: Mangenese content of Seaweed

The manganese content of the seaweeds analysed (Table. 3) showed that the maximum content of manganese was in sw_3 (0.1674mg), followed by sw_4 (10.0125 mg), sw_1 (0.0086 mg) and sw2 (0.0078mg) whereas, the minimum was observed in sample sw_5 (0.0066mg).

From the above data it was statistically proved that highly significant difference existed among the sea vegetables analyzed for manganese at 0.01and 0.05 level of significance. Manganese the most representative mineral was found to be 0.002 mg to 0.003 mg in *Caulpera browni* Ruperz (2002).

Selenium content of seaweeds (mg/100g)

Table 3: Selenium Content of Seaweeds (mg/100g)

Sr. No.	Sample	Selenium
1	SW1	0.0014
2	SW2	0.0012
3	SW3	0.0008
4	SW4	0.0001
5	SW5	In traces

The seaweeds analysed for selenium proved that the lowest values observed in sw_5 (in traces) and the highest in sw_1 (0.0014mg), followed by sw_2 (0.0012mg) sw_3 (0.0008mg) and sw_4 (0.0001mg). Since, *Sargassum* species showed traces of selenium, statistical analysis did not show 0.05 and 0.01 level of significance. Results obtained by Peter (2004) in *Ulva* and *Rhodophyta* species showed values ranged between 0.001 to 0.0010 mg

Summary and Conclusion:

Among the samples analysed, SW3 (*Gracilaria fergusonii*) had maximum moisture content whereas SW5 (*Sargassum*) had the minimum. In the case of calcium, SW3 (*Gracilaria fergusonii*) had highest value and SW1 (Ulva lactuva) had the lowest value. Magnesium recorded the peak value in SW3 (*Gracilaria fergusonii*) and lowest value in SW1 (*Ulva lactuva*). On analysis SW2 (*Ulva fasciata*) had maximum and SW4 (Gracilaria cortigata) had the minimum value for zinc. SW3 (*Gracilaria fergusonii*) had the highest value and SW4 (Gracilaria cortigata) reported to have bare minimum for iron. SW3 (*Gracilaria ferguconii*) had maximum manganese content whereas, SW5 (*Sargassum wightii*) had the minimum. With respect to selenium SW3 (*Gracilaria fergusonii*) had the highest value and SW1 (*Ulva lactuva*) had the lowest value.

Suggestions and Recommendations:

The most commonly used seaweeds should not only be considered as medicinal plant but should rise to the level of being a major crop of cultivation to provide food security for the society by creating a healthy nation. Though Ayurveda has originated several centuries back, there are certain plants, which do not have references and used by the TSM. Such unfamiliar sea vegetables can be taken for clinical research and can be very well used for the welfare of mankind. As said “Let food be thy medicine and medicine thy be food” - Hippocrates.

References:

1. Berwal, J.S., Grewal R. B., Rapoor, C.M., and Garg, M.K.,(2004). Practical Methods in Food Analysis. Agro Tech Publishing Academy, Udaipur. Pp 16-17, 21-2, 28-30, 65-67.
2. Bhavanath Jha, Reddy, C.R.K., Mukund C.Thakur., and Umamaheswara Rao, M. (2009). Seaweeds of India. The diversity and distribution of seaweeds of Gujarat coast. ISBN: 978-90-481-2487-9.P-01
3. Burkif, S., (2007). An Ecological Study of Some Algae.3.Pp 671-680
4. Burtin, P. (2003) Nutritional Value of Seaweeds. Electronic Journal of Environmental, Agricultural and Food Chemistry, 2, 498-503.

5. Das, M.N and Giri N.C (1979).Design and Analysis of Experiments. John Wiley and Sons Publishers Limited, New Delhi. ISBN: 978-0852261583. P-304
6. Delaney, A., Frangoudes, K., and Li, S.A. (2016). Society of Seaweed: Understanding the Past and Present. Chapter 2. Edited by Joel Fleurence and Ira Levine. Seaweed in Health and Diseases Prevention. Academic Press Publications. ISNB: 978—0-12-802772-1.
7. Gulshad Mohamed (2015). ICAR Course Manual. Current Trends and Prospects of Seaweeds Framing in India. Research Centre of CMFTRI, West Hill Calicut. P-78 http://eprints.cmfri.org.in/10671/1/12.%20Gulshad.pdf
8. Joel Fleurence and Ira Levine (2016). Seaweed in Health and Diseases Prevention Academic Press Publications. ISNB: 978—0-12-802772-1.P-2
9. Leonel Pereira (2018).Therapeutic and Nutritional Uses of Algae. Taylor and Francis Publications. ISNB:978-1-4987-5538-2.P-65
10. Muthu B., Chennubhotla, V.S.K., Nair, K.V.K., Sampath, V., and Ravindran, M., (2005). Standing Crop Biomass and Comparative Distribution of Agarophytes, Alginophytes and Other Algae in South Andaman, 2005. Indian Hydrobiology. P 130-138
11. Ole G. Mouritisen (2013). Seaweeds edible available and sustainable. The University of Chicago Press Ltd London. ISNB:978-0226-044453-8.P-68
12. Peters, A F., (2004). Proposal of Ectocarpus siliculosus as a Model Organism for Brown Algal Genetics and Genomics. Journal of Phycology.40.P.1079-1088
13. Rajrupa Gosh, Kakoli Banarjee and Abhijith Mitra., (2012). Eco-Biochemical of Common Seaweeds in the Lower Gangetic Delta. Chapter 3.Edited by Se-Kwon Kim. Hand book of Marine Macro Algae. Biotechnology and Applied Phycology. A John Wiley and Wiley and Sons Limited Publications.P-66
14. Ranganna, S., (1997). Manual for Analysis for Fruits and Vegetables Product .Tata Hill Publishers' Co. West Port.
15. Ruperz, P., (2002). Mineral Content of Edible Seaweed. Food Chemistry.79.P.23-26
16. Sadsasivam, S., and Manickam, A., (2004). Biochemical Methods for Agricultural Science. Wiley Eastern Limited. New Delhi. Pp8-9, 20-21, 178-179.
17. Sahoo, D., Niveditha.G., Debesiah, F., (2001).Seaweeds of the Indian Coast. APH Publishing Corporation New Delhi.P-283
18. Se-Kwon Kim., (2015).Sea Food Science: Advance in Chemistry Technology and Applications. ISBN(13): 978-1-4665-9583-5.P-09
19. Susman, A.V., (2006) Life History of Chlorophyta, in South Western British Columbia and Canada, American Journal of Botany.

STUDY OF *PSEUDOMONAS AERUGINOSA* LIPASE ISOLATION, PURIFICATION AND ENZYME ACTIVITY

Naveen Kumar Kudure Jayanna[1] and Venkatesh[2]*

[1]Department of Studies in Microbiology,

[2]Department of Studies in Food Technology,

Davangere University, Shivagangotri campus,

Davanagere-577007, Karnataka, India

*Corresponding author E-mail: venka.biotech@gmail.com

Abstract:

The study aim to isolate, purify and characterize the lipases from *Pseudomonas aeruginosa*. Lipase secreting bacteria was selected and identified by tributyrin agar plate test. The bacterial culture was inoculated in King's A broth media for the mass production of lipases. Then the enzymes were purified by salt precipitation, ion exchange, chromatography and SDS-PAGE, molecular weight of lipase was determined by SDS-PAGE which shows 37kDa. The enzyme activity of lipase was determined and it was found to be 50 U/ml. The optimization of Lipase activity at different pH, temperature was carried out and it was found that, the optimum pH is 7 and the optimum temperature is 50°C. The optimization of Lipase in the presence of activator i.e., 0.1% calcium chloride and inhibitor i.e., 0.1% Mercuric chloride was carried out and it was found that the volume of 0.1ml, 0.1% calcium chloride and 0.1 and 0.2ml of 0.1% Mercuric Chloride was optimum.

Keywords: Lipases, *Pseudomonas aeruginosa*, SDS-PAGE

Introduction:

Enzymes considered as natures catalyst. Enzymes are essential proteins for the metabolic system of all living organisms and have an important role in the cellular and biocatalytic activities in cell. Several thousand's of enzymes possessing different substrate specificity are known, however only comparatively few enzymes have been isolated in a pure form and crystalloids, and little has been known about their structure and function.

The advancement of protein and engineering techniques makes enzymes has industrial application such as proteases and lipases used in detergents. Amylases and glucose isomerase used in starch processing. Lipases are a class of enzymes which catalyze the hydrolysis of long

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chain triglycerides (Hasan *et al.,* 2006). Lipases constitute the most important group of biocatalysts for bio technological application. Most enzymes are produced through microbial fermentation.

There are many advantages of using microbial enzymes over enzymes from plants or animals, including a large range of catalytic activities, high yields, genetic manipulation, regular availability because of absence of seasonal fluctuations, and rapid growth. Moreover, microbial enzymes are more stable and easier to produce than those found in plants or animals (Wiseman, 1995). There have been a large number of lipases isolated from bacteria, fungi, plants, and animals. Lipases from different sources show wide differences in their specificity in terms of positionality, fatty acids and their size. The lypolytic enzymes are the most important molecular catalysts because of their biotechnological impact (Benjamin and Pandey, 1988). Lipases are commonly used in fat hydrolysis and in organic synthesis, where they are synthesized as catalysts and exhibit regio-specificity and enantio-selectivity (Saxena *et al.,* 2003).

The microbial lipases in powder form will lead the global markets due to its stability, easy handling, packaging, and easy transportation (Arnam and Sutherland, 2001). These are extensively applicable in several another industries such as biocatalytic resolution, esters and amino acid derivatives, fine chemicals production, dairy products, food and drinks, animal feed, cleaning, biofuels, textile cosmetics, perfumery, the flavour industry, biofuels, and pharmaceuticals are only a few examples (Vanleeuw *et al.,* 2019).

Different bacterial lipases can be produced both by Gram-positive and Gram-negative bacteria. Gram-negative bacteria produce the greater part of bacterial lipases, and *Pseudomonas* is by far the most important genus of Gram-negative bacteria (Kojima *et al.,* 2003). The aim of the study to extract, purify and optimize lipase enzyme production from *Pseudomonas aeruginosa*.

Materials and Methods:

A soil sample for this study was collected in shimoga district, Karnataka. Soil sample were serially diluted up to 10^{-9} and aliquots (0.1 ml) was placed on sterilized Nutrient agar plates and incubated for 24 hours at 37°C. After incubation, pick colonies on the plate to streak on the sterilized agar plates and maintained pure culture.

Identification:

Gram's Staining:

Gram stain protocols are followed with slight modification by Smith and Hussey (2005). A Clean glass slide was taken, sterilized with ethanol and dried. The thin Bacterial smear was prepared on a sterile glass slide. Smear was allowed to air dry and was heat fixed. The slide was placed on glass rods and smear was flooded with crystal violet for 1 min. The slide was washed with distilled water to remove excess stain. Smear was flooded with gram's iodine for 1 min. The smear was decolorized with 95% alcohol, washed with distilled water and was counter stained with saffranine for 1min and then washed with distilled water to remove excess stain and dried. Slide was viewed under oil immersion objective and result was recorded.

Motility Test:

Motility test were conducted as per the procedure of Tittsler and Sandholzer (1936). A clean depression slide and cover glass was taken and washed with soap to remove any grease, a small amount of Vaseline was placed near each corner of cover glass. The Vaseline will provide adhesion for the cover glass to the cavity slide. Then a loop full of culture was placed on the center of the cover ship the depression slide was inverted on to the cover slide with the cavity facing down so that depression covers the suspension. The slide was inverted quickly that is hanging drop preparation cover slip facing up so that the culture drop was suspended observed the preparation under oil immersion objective for motility of Bacteria.

Biochemical characterization:

Citrate Utilization Test:

The medium was prepared and dispensed to the test tube and sterilized, after sterilization slants were prepared and inoculated with the culture. The slants were incubated at 37^0C for 24 to 48 hrs. Observed for the growth and coloration of the medium. The slants with blue colour are citrate positive and the slants with green color are citrate negative (Raghavachari, 1926).

Gelatin Hydrolysis Test:

Nutrient Gelatin media was prepared and deeps were made. After solidification of the medium, the bacterial culture was inoculated by means of stab inoculation. The stabbed tubes were incubated at 37^0C for 24 to 48 hrs. After incubation, the tubes were kept in refrigerator at 4^0C for 15mins. After incubation, the tubes were observed for liquefaction of the media (Dela Cruz *et al.*, 2012).

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KOH Solubility Test:

A loop full of bacterial culture was taken in the slide. Few drops of KOH added on to it. The culture was mixed with the help of toothpicks. The mixed bacterial culture and 3% KOH was lifted to observe the threads which are caused due to 3% KOH (Bourgault and Lamothe, 1988).

Catalase Test:

0.3% Hydrogen peroxide was prepared a loop full of bacterial culture was placed on a clean glass slide. It was thoroughly mixed a drop of hydrogen peroxide and observed for the catalase activity (Reiner, 2010).

Lipid Hydrolisis Test:

Nutrient agar media was prepared, sterilized and allowed to cool. After cooling, egg yolk was added and mixed well. Then the egg yolk agar media was poured into presterilized petriplates. After solidification of media, the bacterial culture was inoculated by means of point inoculation. Then the plates were incubated at 37^0C for 24 to 48hrs. After incubation the plates were observed for opaque zone around the colony.

Oxidase Test:

A loop full of culture was placed on a oxidase disc, and time was noted. The disc was observed for change of color that is violet or purple color within 30-60 seconds (Tarrand and Groschel, 1982).

Screening for Lipase Production:

Tributyrin agar media was prepared and autoclaved. Then this autoclaved and cooled (45°C – 50°C) medium was poured into sterile petriplates and allowed these to solidify. The tributyrin agar plates were labeled with name of bacterial organism to be inoculated and one plate kept as control. A streak inoculation was made on to its labeled petriplate across the surface of the medium and the plates were incubated for 24-48 hours at 37°C in an inverted position. Then the plates were observed for clear zone around the colonies or methyl red was overlayered on agar observed for pink colored zone around streaked area.

Mass Production of Lipase Enzyme:

Mass production of the enzyme was done by submerged fermentation. The organism was cultivated in liquid media in the flasks for the enzyme production. King's A Broth medium was

used for production of Lipase enzyme. After inoculation, the flasks were incubated at 37^0C in a shaker at 220 rpm for 24-48 hours.

Purification of the Enzyme:

Purification by salt precipitation technique:

The broth containing enzyme lipase was centrifuged for ten minutes at 6000 rpm and at 4^0c. Supernatant was collected, and then filtered through whatman Filter paper No.1; volume was measured using a graduated cylinder. 90% salt cut was given to the supernatant (93 ml of supernatant was collected from inoculated broth. To this supernatant 52.3 g of ammonium sulphate was added). Ammonium sulphate should be added very slowly with continuous stirring of the solution on a magnetic stirrer in cold conditions. The solution was centrifuged at 10,000 rpm for 10 minutes at 4^0C. The pellet was collected and dissolved in 10 ml of 10mM Tris hydrochloric acid solution and subjected to dialysis. This solution contains the enzymes precipitate by Ammonium sulphate (Dixon and Webb, 1962).

Dialysis:

The dialysis bag was first processed, to activate it. Dialysis bag of about 8 cm was boiled in 100 ml of distilled water for 10 minutes. The bag is then boiled in 100 ml of 2% sodium bicarbonate solution for 10 minutes. The bag was again boiled in 100 ml distilled water for 10 minutes. Now, the mouth of bag was gently rubbed to open it. One end of the bag was lightly tied and the sample was loaded into it. After loading the sample the other end was also sealed. The dialysis bag was then suspended in a beaker containing 10% Tris HCl buffer. This setup was kept in a magnetic stirrer overnight.

Ion Exchange Chromatography:

The chromatography column packed with 2g Diethyl aminoethyl cellulose was washed using distilled water one to two times. The column was then washed with solution `A' (10 ml of 10 mM Tris HCl + 10mM KCl). The dialyzed enzyme sample was poured into the column. The enzymes were then eluted using solution `B' (10 ml of 10 mM Tris HCl + 150mM KCl). The elutants were collected in the same test tubes.

Molecular characterization of lipase by using SDS –PAGE method:

The glass plates were assembled according to the apparatus manufacturer's instructions. The desired concentration of acrylamide and bisacrylamide for the resolving gel and stacking gel were prepared. The samples were mixed with loading dye followed by boiling for 5 min. Then load the samples in the well (already created during preparation of stacking gel) Electrophoreses

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with adding buffer to both upper tank and lower tank. Then connect to power supply. The dye bromophenol blue reached at bottom of electrophoresis. Then the power supply was turned off. The gel was removed and the orientation of the gel was marked by cutting a corner from the bottom of the gel that is closest to the left most well. The gel was immersed at least at 5 volumes of staining solution (Coomassie Brilliant Blue R250) and left for 4 hrs at room temperature. Later the gel was destained by soaking it in to the methanol/acetic acid solution for 4 – 8 hours, by changing the destaining solution three or four times. After destaining, the gel was stored indefinitely in water containing 20% glycerol (Graybosch and Morris, 1990).

Determination of Enzyme activity of Lipase Enzyme by Titrimetric Method:

The quantity of fatty acid released in unit time is measured by the quantity of Sodium hydroxide required to maintain pH constant. The milli equivalent of alkali consumed is taken as a measure of the activity of the enzyme. For substrate preparation 2ml of clear vegetable oil was taken and neutralize to pH 7 and stirred well with 25ml of water in the presence of 100mg of bile salt till an emulsion was formed, then 2g of gum Arabica was added. For titration, 20ml of substrate was taken in a 100ml of beaker, 5ml of phosphate buffer of pH 7 was added. The beaker was set on the top of magnetic stirrer cum hot plate and stirred the contents slowly, the temperature was maintained at 35^0C, then electrodes of pH meter were dipped in reaction mixture, pH was adjusted to 7 and noted. 0.5ml of enzyme extract was added, immediately pH was recorded, timer was set on. At frequent intervals (10 min) or as the pH drops about 0.2 unit 0.1 N NaOH was added to bring pH to the initial value. Continued the titration for 30-60 min period. The volume of alkali consumed was noted. The enzyme activity was calculated according to the following formula.

$$\text{Enzyme activity (U/ml)} = \frac{\text{volume of alkali consumed} \times \text{strength of alkali}}{\text{Weight of sample} \times \text{time in minutes}}$$

Optimization of Lipase Enzyme:

Effect of activator on Lipase activity (Patel *et al.*, 1964):

The addition of activators activates the activity of enzyme. In the present work calcium chloride was used as activator, 0.1% calcium chloride was prepared and was added to substrate in increasing volume that is 0.1ml, 0.2ml, 0.3ml and 0.4ml for different trails to determine the activation level of lipase enzyme. For determining optimum volume of activator for Lipase activation the standard procedure which was followed for determining

the activity of Lipase was used and the optimum activity of Lipase which was activated at a specific volume of calcium chloride was determined and the graph of enzyme activity V/S volume of activation was plotted.

Effect of inhibitor on Lipase activity (Enujiugha *et al.*, 2004):

Addition of inhibitors inhibits the activity of enzyme. In the present work 0.1% Mercuric chloride was used as inhibitor, 0.1% Mercuric chloride was added to substrate in increasing volume that is 0.1ml, 0.2ml, 0.3ml and 0.4ml for different trials to determine inhibition level of Lipase enzyme. For determining optimum volume of inhibitor for Lipase activation. Optimum volume of the standard procedure which was followed for the determining the activity of Lipase was used and the optimum activity of Lipase which was inhibited at a specific volume of Mercuric chloride was determined and the graph of enzyme activity V/S volume of inhibitor were plotted.

Effect of pH on Lipase activity (Fukumoto *et al.*, 1964):

The enzyme activity is influenced by various environment factors including pH. The enzyme activity is peak at a particular pH. For determining the optimum pH of Lipase. The standard procedure which was followed for determining Lipase activity was used. The pH of reaction mixture was adjusted that is pH 4.0, pH 6.0, pH 7.0, pH 9.0 for different trails and titration carried out. The optimum pH required for maximum Lipase activity was found. The graph of enzyme activity v/s pH was plotted.

Effect of temperature on Lipase activity:

The enzyme activity is influenced by temperature. At a particular temperature the enzyme activity is at peak. For determining optimum temperature of Lipase enzyme. The standard procedure which was followed for determining Lipase activity was used. But the temperature of reaction mixture was adjusted that is at (room temperature) 29^0C, at 37^0C, at 50^0C and at 70^0C, for different trials, and the titration was carried out. The optimum temperature required for maximum lipase activity was found and the graph of enzyme activity v/s temperature was plotted.

Immobilization of enzyme:

3.6 grams of sodium Alginate was added was to 0.5% sodium chloride solution with continuous stirring. The mixture was allowed for 6 hours to facilitate escaping air bubbles. Then the enzyme sample was added and mixed thoroughly. 4 % Calcium chloride solution was taken in a beaker and then with help of syringe the sodium alginate and enzyme sample mixture was

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added drop by drop to form gel beads. The bead was allowed in the calcium chloride solution for 10 min to get gelatinized beads and then bead was stored in screw cap bottles.

Result and Discussion:

Soil sample was serially diluted and spreaders on the nutrient agar media colonies of organisms are obtained. The colonies are streaked on the agar media and broth, pure culture was obtained and maintained. Confirmatory tests were carried out by Gram's staining. Morphology of the organism was identified. The organism was gram negative, *Pseudomonas*. Biochemical Tests showed in table 1. The organism was identified and confirmed by Gram's staining and Biochemical tests as *Pseudomonas aeruginosa*.

Table 1: List of results of biochemical tests

Sr. No.	Test	Result
1.	Citrate Utilization	Positive
2.	Gelatin Hydrolysis	Positive
3	KOH Solubility test	Positive
4	Catalase test	Positive
5	Lipid hydrolysis	Positive
6	Oxidase test	Positive

Motility test:

After subjecting the culture for motility test, it was found that the bacteria in motile in nature.

Biochemical characterization:

Citrate Utilization test:

After incubation, the change in colour of media from green to blue was observed (Fig. 1) which confirms that bacteria have the ability to produce citrase enzyme.

Gelatin hydrolysis:

After refrigeration of the incubated tubes, gelatin liquefaction was observed (Fig. 2) which in turn shows the gelatin hydrolyzing capacity of the bacteria.

KOH Solubility test:

After mixing bacterial culture with 3%KOH the threads were observed which confirms that Bacteria was Gram negative.

Catalase test:

After adding hydrogen peroxide to the bacterial culture, bubbles were observed which confirms that bacteria have the ability to produce Catalase enzyme.

Lipid Hydrolysis:

After incubation, clear zone was observed around streaked area (Fig. 3) which confirms that bacteria have the ability to produce lipase enzyme.

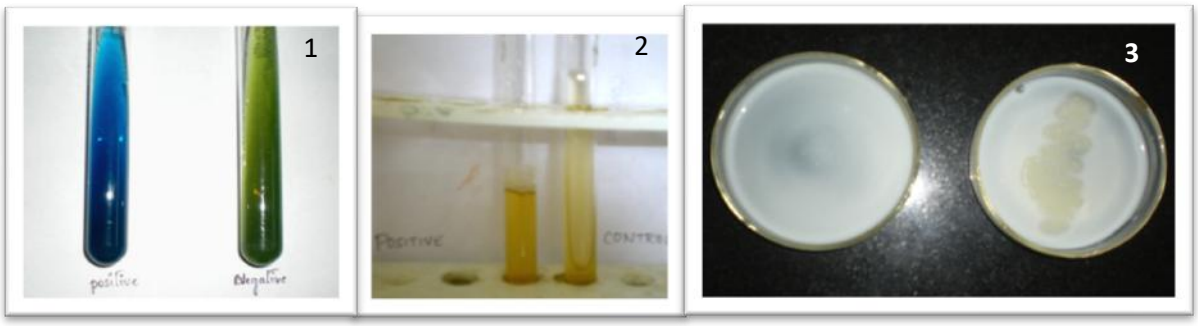

Figure 1: Citrate Utilization test; Fig. 2: Gelatin hydrolysis; Fig. 3: Lipid Hydrolysis

Oxidase test:

After placing a loop full culture on oxidase disc, change of color that is violet color was observed within 30-60 seconds.

Form the above all biochemical tests, it confirms that the given pure culture is *Pseudomonas aeruginosa.*

Lipase assay:

Tributyrin plates were used for lipase assay. The clear zone was observed around the colonies, after adding methyl red indicator pink color zone was observed (Fig. 4). Confirms that the *Pseudomonas aeruginosa* has the capacity to produce lipase enzyme. A similar assay was carried out by Bhumibhamon *et al.* (2002) for lipase using tributyrin agar plates. Adan (2009) isolated lipase producing bacterium from soil using rhodamine B olive oil plate assay.

Figure 4: Lipase assay

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Mass production and purification of lipase:

The lipase enzyme was mass produced by using King's A broth then the enzyme was purified by different purification steps and enzyme activity of lipase enzyme was determined.

Molecular characterization of lipase by using SDS –PAGE method:

From SDS-PAGE the purity of the obtained enzymes can be confirmed. The appearance of single band in the SDS-PAGE confirms the purity of enzymes (Fig. 5); it implies that no other impurities are present with the purified enzyme sample. The results of protein pattern estimated by SDS-PAGE method clearly indicate the molecular weight of the lipase. It is clear by the result that the lipase is of 37 kDa. Adan (2009) studied lipase producing bacteria from soil. The molecular mass of purified lipase was estimated to be approximately 43kDa by SDS-PAGE. Lin *et al.*, (1996) studied an extracellular alkaline lipase. The molecular weight determined by SDS-PAGE was 32kDa.

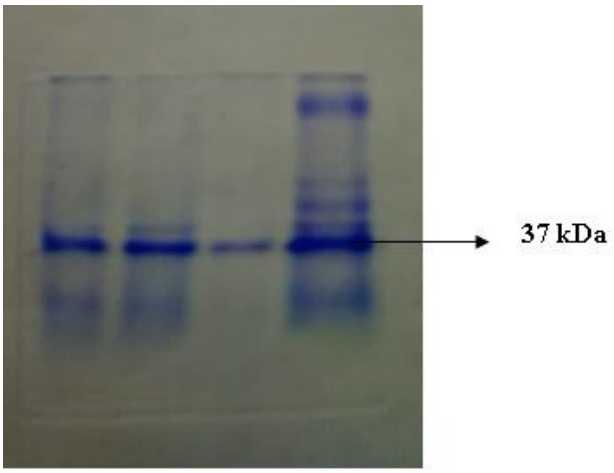

Figure 5: SDS-PAGE

Determination of Enzyme activity of Lipase Enzyme by Titrimetric Method:

The activity of lipase enzyme was found to be 50 U/ml at pH 7. Shukla and Gupta (2007) studied on lipase, their activity was tested titrimetrically and maximum activity was 48 U/ml.

Optimization of lipase:

The effect of effect of activator, inhibitor, pH and temperature on lipase enzyme showed in table 2. The optimum activity of Lipase enzyme in presence of 0.1ml of 0.1% Calcium chloride was found to be 50 U/ml and in presence of 0.1ml and 0.2ml of 0.1% Mercuric Chloride was found to be 35 U/ml. The optimum activity of Lipase enzyme at pH 7 was found to be 20 U/ml and at temperature of 50^0C was found to be 160 U/ml. Gunasekaran *et al.* (2006) worked

on lipase activity that had shown high activity of about 8.8 U/ml and pH of 9.0 and temperature of 40^0 C. Similar work was carried out by Senthikumar and Selvakumar (2008) on lipase its maximum activity at 40^0 C and at pH of 8.0.

Table 2: Effect of activator, inhibitor, pH and temperature on lipase enzyme

Volume of Activator ($CaCl_2$) in ml	**Enzyme Activity (U/ml)**
0.1	50
0.2	0
0.3	20
0.4	40
Volume of Inhibitor($HgCl_2$) in ml	**Enzyme Activity (U/ml)**
0.1	35
0.2	35
0.3	30
0.4	20
pH	**Enzyme Activity (U/ml)**
4	0
6	0
7	20
9	0
Temperature	**Enzyme Activity (U/ml)**
29^0C	5
37^0 C	20
50^0 C	160
70^0 C	100

Immobilization of enzyme

Immobilized lipase enzymes were obtained in the form of sodium alginate beads in calcium chloride solution. The immobilization was done for storage of extracted lipase enzyme for many days and enzyme can be reused for further usage. Similar procedure was carried out by Kanwar *et al.* (2006) for the immobilization of purified lipase efficiently using alginate. Carneiro-da-cunha *et al.* (1999) studied lipase immobilization on to polymeric membranes.

Conclusion:

In the present study, *Pseudomonas aeruginosa* was found to be potent for the production of lipase enzymes. The *Pseudomonas aeruginosa* produces lipase which possesses the novelty to

be applied into many industries. The enzyme production and properties can be improve by taking up further research in fermentation, enzyme engineering based studies to meet the demand of lipase base industries.

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References:

1. Bhumibhamon, O., Koprasertsak, A., and Funthong, S. (2002). Biotreatment of high fat and oil wastewater by lipase producing microorganisms. Agriculture and Natural Resources, 36(3), 261-267.
2. Adan, A. (2009). Isolation and identification of a lipase producing *psychrotrophic bacteria* from soil: cloning and partial characterization of its lipase (Master's thesis, İzmir Institute of Technology).
3. Lin, S. F., Chiou, C. M., Yeh, C. M., and Tsai, Y. C. (1996): Purification and partial characterization of an alkaline lipase from *Pseudomonas pseudoalcaligenes* F-111. Applied and Environmental Microbiology, 62(3), 1093-1095.
4. Shukla, P., and Gupta, K. (2007): Ecological screening for lipolytic molds and process optimization for lipase production from Rhizopus oryzae KG-5. Journal of Applied Sciences in Environmental Sanitation, 2(2), 35-42.
5. Gunasekaran, V., Kotay, S. M., and Das, D. (2006): Alkaline lipase production by Citrobacter freundii IIT-BT L139.
6. Kanwar, S. S., Gupta, M., Gupta, R., Kaushal, R. K., and Chimni, S. S. (2006): Properties of hydrogel-entrapped lipase of thermophilic *Pseudomonas aeruginosa* BTS-2.
7. Carneiro-da-Cunha, M. G., Rocha, J. M. S., Garcia, F. A. P., and Gil, M. H. (1999): Lipase immobilisation on to polymeric membranes. Biotechnology techniques, 13(6), 403-409.
8. Hasan, F., Shah, A. A., and Hameed, A. (2006): Industrial applications of microbial lipases, Enzyme and Microbial technology, 39(2):235-251.
9. Wiseman, A. (1995): Introduction to principles, Handbook of enzyme biotechnology.
10. Benjamin, S., and Pandey, A. (1998): *Candida rugosa* lipases: molecular biology and versatility in biotechnology, Yeast, 14(12):069-1087.
11. Saxena, R. K., Sheoran, A., Giri, B., and Davidson, W. S. (2003): Purification strategies for microbial lipases, Journal of microbiological methods, *52*(1):1-18.

12. Kojima, Y., Kobayashi, M., and Shimizu, S. (2003): A novel lipase from *Pseudomonas fluorescens* HU380: gene cloning, overproduction, renaturation-activation, two-step purification, and characterization, Journal of bioscience and bioengineering, 96(3):242-249.
13. Varnam, A., and Sutherland, J. P. (2001): *Milk and milk products: Technology, chemistry and microbiology* (Vol. 1). Springer Science and Business Media.
14. Vanleeuw, E., Winderickx, S., Thevissen, K., Lagrain, B., Dusselier, M., Cammue, B. P., and Sels, B. F. (2019): Substrate-specificity of Candida rugosa lipase and its industrial application, ACS Sustainable Chemistry and Engineering, 7(19):15828-15844.
15. Raghavachari, T. N. S. (1926): The Applicability of Koser's Citrate Utilization Test to Sanitary Water Analysis in India, Indian Journal of Medical Research, 14:47-51.
16. Dela Cruz, T. E. E., and Torres, J. M. O. (2012): Gelatin hydrolysis test protocol, American Society for Microbiology, 1(10).
17. Bourgault, A. M., and Lamothe, F. R. A. N. Ç. O. I. S. (1988): Evaluation of the KOH test and the antibiotic disk test in routine clinical anaerobic bacteriology, Journal of clinical microbiology, 26(10):2144-2146.
18. Reiner, K. (2010): Catalase test protocol, American society for microbiology, 1-6.
19. Dixon, M., and Webb, E. C. (1962): Enzyme fractionation by salting-out: a theoretical note, In Advances in Protein chemistry, 16: 197-219). Academic Press.
20. Graybosch, R. A., and Morris, R. (1990): An improved SDS-PAGE method for the analysis of wheat endosperm storage proteins, Journal of Cereal Science, 11(3):201-212.
21. Patel, V., Goldberg, H. S., and Blenden, D. (1964): Characterization of Leptospiral lipase, Journal of bacteriology, 88(4):877-884.
22. Enujiugha, V. N., Thani, F. A., Sanni, T. M., and Abigor, R. D. (2004): Lipase activity in dormant seeds of the African oil bean (*Pentaclethra macrophylla* Benth). Food Chemistry, 88(3):405-410.
23. Fukumoto, J., Iwai, M., and Tsujisaka, Y. (1964): Studies on Lipase, The Journal of General and Applied Microbiology, 10(3):257-265.
24. Smith, A. C., and Hussey, M. A. (2005): Gram stain protocols, American Society for Microbiology, 1:14.
25. Tittsler, R. P., and Sandholzer, L. A. (1936): The use of semi-solid agar for the detection of bacterial motility, Journal of bacteriology, 31(6):575-580.
26. Tarrand, J. J., and Gröschel, D. H. (1982): Rapid, modified oxidase test for oxidase-variable bacterial isolates, Journal of Clinical Microbiology, 16(4):772-774.

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PREVALENCE OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE: A GLOBAL REVIEW

Yogesh Joshi[1]*, Saurabh Saklani[2] and Shivam Bisht[2]

[1]Department of Pharmacy Practice, School of Pharmaceutical Sciences,
Shri Guru Ram Rai University, Dehradun-248001, Uttarakhand, India.

[2]Department of Clinical Pharmacy, Shri Mahant Indiresh Hospital,
Dehradun-248001, Uttarakhand, India.

*Corresponding author E-mail: yogeshjoshi1583@rediffmail.com

Abstract:

Chronic obstructive pulmonary disease (COPD) is a major cause of worldwide mortality and disability. COPD is responsible for early mortality, high mortality, and significant costs to health systems. It affects millions of people worldwide and causes significant morbidity and mortality. The projection for 2020 indicates that COPD will be the 3rd leading cause of death worldwide (from 6th in 1990) and lost through early mortality or disability (Disability-Adjusted Life Years) (12th in 1990) the 5th major reason for years. Active smoking remains the main risk factor, but other factors are becoming better known, such as occupational factors, infection, and the role of air pollution. The prevalence of COPD varies by country, age and sex. The disease is also associated with significant co-morbidities. COPD is a disorder involving various phenotypes, the continuation of which remains under debate. The major challenge in the coming years will be the early detection of the disease in the common people as well as stopping the onset of smoking. The goal of this review is to evaluate the overall burden of disease, including prevalence, morbidity, mortality, health care costs, and economic costs.

Key words: COPD, respiratory, prevalence, morbidity, mortality

Introduction:

Chronic obstructive pulmonary disease (COPD) is a progressive, debilitating respiratory condition and is considered the most important respiratory disease globally. Previously, it was thought that COPD would rank 5th in overall burden of disease and 3rd in worldwide mortality by 2020. However, COPD 1st found this mark in the United States in 2008 and become the 3rd largest cause of mortality. Worldwide, 4.5 million people died from COPD in 2008, and in contrast to coronary heart disease and stroke, it is the only leading cause of death that is still increasing. In U.S; COPD results in 15.4 million physician visits, 1.5 million emergency department (ED) visits, and 726,000 hospitalizations every year. The National Heart, Lung, and

Blood Institute estimated that the U.S. had 14.8 million people. With physician diagnosed COPD and another 12 million with undiagnosed COPD. The worldwide unadjusted annual death rate for COPD in 2008 was 45.3 per 100,000 population. Although COPD mortality is difficult to estimate worldwide, COPD is not listed as a cause of death on the death certificate due to naming inconsistencies; for this reason, COPD mortality is underestimated[1-10].

COPD is a major cause of worldwide mortality and disability. COPD is defined by spirometry in average ~5-15% of adults in industrialized countries. In 1990, COPD was considered 12th worldwide as a cause of combined mortality and disability, but is expected to become the 5th cause by the year 2020. COPD has a long-lasting long-term course, with irreversible deterioration of forced respiratory volume in one second (FEV1), increased presence of dyspnea and other progressive deterioration of health status. The 10-year survival rate after diagnosis is ~50%, with more than 1-3rd of patients dying due to respiratory insufficiency[11].

Definition:

The 1995 European Respiratory Society (ERS) consensus statement defined COPD asa disorder characterized by at least maximum respiratory flow and lung emptying; features that do not change markedly over several months. Most airflow limits are slowly progressive and irreversible. Airflow limitation is due to different combinations of airway disease and emphysema; the relative contribution of the two processes is difficult to define. Unfortunately, there is no agreed labeling of the airway disease component which is primarily an inflammatory process. Labeling the airway disease component as obstructive bonchiolitis has some advantages but has not received general acceptance. In 1995, the American thoracic Society (ATS) statement COPD was defined as a "disease state characterized by the presence of airflow obstruction due to bronchitis or emphysema; airflow obstruction is generally progressive, with airway hyper-responses may occur andmay be partially reversible". However, the use of the term Chronic bronchitis in the definition of COPD can easily cause confusion as it is widely used to designate mucus over secretion that mostly arises from large airways.

ERS consensus defines COPD as an atmospheric barrier predicted in males in a 2nd (FEV_1) / forced vital capacity (FVC) L 88% or <89% predicted in females. The following entities are excluded from COPD in this definition: cystic fibrosis, bronchiectases, byssinosis, and bronchiolitis obliterans. However, when both positive and negative criteria are applied, the difference between asthma and COPD is still a major difficulty. Clearly, adopting different definitions or translating different operating criteria to the same definition can lead to different estimates of prevalence rates or risks and may make it more difficult to interpret clearly inconsistent results. One consequence of the ambiguity inherent in the definition of COPD is smoking. Although no prevention of smoking is included in any of the above COPD definitions, many clinical investigations exclude non-smokers[11-13].

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Epidemiology:

Chronic obstructive pulmonary disease (COPD) is a major cause of morbidity and mortality across the globe. According to an estimate by the World health Organization, 65 million people have moderate to severe COPD. COPD killed more than 3 million people in 2005, equivalent to 5% of all deaths globally and is estimated to be the 3rd leading cause of death by 2030.

- An estimated 328 million people have COPD World Wide.
- In 15 years, COPD is expected to become the leading cause of death worldwide.
- 3 billion people worldwide are exposed to toxic amounts of HAP every day and HAP causes 3.5-4 million deaths annually.
- People who have never smoked tobacco can still get COPD-think 'biomass COPD'[14,15].

Global COPD Statistics:

- More than 90% of COPD-related deaths occur in LMICs.
- According to the Global Burden of Disease (GBD), COPD is already the 3rd leading cause of death worldwide, something that the WHO did not predict until 2030.
- The economic impact of COPD among LMICs is expected to increase to £1.7 trillion by 2030.
- In 15 years, COPD is expected to become the leading cause of death worldwide[14,16,17].

Household Air Pollution Statistics:

- Air pollution is the biggest environmental cause of death worldwide.
- 3 billion people worldwide are exposed to toxic amounts of HAP every day.
- HAP accounts for up to 4 million deaths annually[15,18].

Smoking:

Tobacco is a legal drug currently responsible for an estimated 6 million deaths worldwide each year, with many of these deaths occurring prematurely. Tobacco smoking is associated with morbidity and mortality from non-communicable respiratory diseases (NCD's), including approximately 600,000 people who are estimated to die from the effects of second-hand smoke each year. The poor smoke the most. Globally, 84% of smokers live in developing and transitional economy countries. Tobacco smoke reflects harmful effects of exposure to biomass smoke[19,20].

The WHO stated that in 2015, more than 1.1 billion people smoked tobacco, men smoked more tobacco than women, and although it is decreasing worldwide and, in many countries, the prevalence of tobacco smoking in the eastern Mediterranean and growing in Africa[21].

Tobacco and Smoking Statistics:

1. Due to the incomplete combustion of formaldehyde and DEET, one mosquito coil burning for 8 hour releases the same amount of PM2.5 as 100 cigarettes.
2. One hour'hookah' session with 'shisha' tobacco is equivalent to smoking over 100 cigarettes.
3. Those who have never smoked tobacco can still get COPD - think 'biomass COPD[22,23].

Indian Data:

NCD's in India were estimated to account for 53% of all deaths and 44% of disability-adjusted life-years (DALY's) lost in 2005. Of this chronic respiratory disease accounted for 7% deaths and 3% DALYs lost. India had worldwide ignorance in 2005 to face "the most losses in potentially productive years of life". Crude estimates suggest there are 30 million COPD patients in India. India contributes to a significant and increasing percentage of COPD mortality which is estimated to be the highest in the world; That is, an estimated age standardized mortality rate of over 64.7 per 100,000 in both sexes. This would translate to around 556,000 in terms of India (>20%) from the world total of 2,748,000 annually. Such huge blocks of disease have the potential to affect health systems and state economies[24-27].

However, to identify that the prevalence estimate in COPD is not completely accurate. Numerous epidemiological studies have addressed the prevalence of COPD in India, the process of limiting them has been adopted and the definitions employed for diagnosis. Most studies have been done on the basis of questionnaires without validation, which are complementary to the measurement of peak flow. The reported prevalence estimates have ranged from 2 to 22% in men and from 1.2 to 19% in women. A recent Indian study of 'Asthma, Respiratory symptoms and Chronic Bronchitis' (INSEARCH) recorded prevalence of chronic bronchitis in 3.49% (4.29% in men) and 2.7% in 85,105 men and 84,470 women from 12 urban and 11 rural sites female in adults >35years.Thus, the national weight- age was estimated at 14.84 million[28,29].

Prevalence:

An estimated 64-210 million people are living with a diagnosis of COPD worldwide. This substantial population affected with COPD is expected to increase. Most detailed information about prevalence, morbidity, and mortality is from high-income countries, but 90% of COPD related deaths occur in low-and middle-income countries. Different studies in the U.S. have determined COPD prevalence to be between 5%-10%. In 2011, the Behavioral Risk Factor Surveillance System (BRFSS) introduced respondents to the U.S. in a telephone survey of adults, 6.3% said they were diagnosed with COPD, which can be extrapolated for about 15 million years. The same study evaluated risk factors associated with COPD (Table 1). Additionally, a

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systematic review and meta-analysis was conducted looking at articles from 1990 to 2005 and determined that the overall coagulation of COPD was 7.6% with similar risk factors. Once again, the majority of this data is from Europe and North America and there is a lack of data from other regions that may have high mortality rates. COPD was thought to affect men and women equally, but studies have noted COPD may be more prevalent in women and, perhaps, more difficult to treat. U.S data showed that more women died of COPD in 2000 than men. More men died earlier than women. Diagnosis of COPD increased in women in United Kingdom as well as Canada, whereas rates of diagnosis have plateau in males. Recent studies have shown more severe disease than previously noted in both smokers and nonsmokers with a substantial prevalence (3%-11%) in life-long nonsmokers, most likely related to nontobacco environmental exposures or infectious etiologies. As mentioned, COPD is being diagnosed and may relate to patients failing to recognize early symptoms and restricting activities to avoid symptoms. Delay in diagnosis can lead to rapid progression of COPD and inefficient or inappropriate consumption of health care services as diagnosis usually occurs when a patient loses 50% or more of their original lung capacity.COPD was not diagnosed before the study of a high- risk population of smokers older than 39 years of age, with interruption in 24.3% of these patients, and even more interesting, in 14.4% of life-long non-smokers, interruptions were found[1-4,30-39].

Table 1: Common risk factors for developing COPD from two different research data studies[32]

BRFSS U.S. Data	**Systematic Review from 28 Different Countries**
Prevalence of 6.3% Age more than or equal to 65 years old Smokers Gender, female Residence, Ohio and Mississippi river areas Race, whites and blacks Education, no high-school diploma Work status, unable to work Income, lower household income Relationship, not married	Prevalence of 7.6% Age more than or equal to 40 years old Smokers Gender, male Residence, urban areas

Common risk factors for the development of COPD from the BRFSS study conducted in the United States and a systematic review that includes 28 other countries. Common risk factors reported to be older, active smokers, but differ in gender and location[32].

Incidence:

The incidence of COPD varies greatly between countries, but estimates are difficult to compare because they are reported in different units and at different lengths. In most of the studies, the incidence of COPD was greater in men than in women. The incidence of COPD was also greater in older individuals, particularly in those aged 75 years and older. Six articles reported trends in incidence over time for Australia, Canada, Sweden, and the USA. Although the incidence of COPD has increased over the last 20 years, within the last 10-11 years, there has been an overall decrease. Studies in Canada18 and the USA reported that trends in incidence over time were similar between men and women; however, in Australia, COPD incidence decreased in men between 1998 and 2003 but increased in women. Two articles, both conducted in Sweden as part of resistancelung disease in northern Sweden (OLIN), reported incidence rates in smokers. These studies reported a two to three fold higher incidence in smokers than in non-smokers when measured by spirometry and assessed by sleep or BTS criteria. One study also reported that COPD incidence in former smokers was more than double that in nonsmokers[10,40-49].

Screening for COPD:

Initial screening for COPD is based on early detection by a general practitioner. Some authors have developed the application of scores to increase detection of COPD by using spirometers in general practice. The purpose of screening is to detect with accuracy airflow obstruction even in patients with certain symptoms. Thus, the validity of screening in COPD depends directly on the validity of the airflow barrier. The sensitivity, specificity, and values called positive and negative future were calculated for the fixed FEV1 / FVC ratio, considering the LLN definition as the gold standard for the evaluation of airflow obstruction. Sensitivity was 97.9%, specificity 91.2%, positive predictive value 72% and negative predictive value 99.5% for both men and women[50].

The agreement between the two definitions was better for the most severe patients for the subset of 50 years of age for current and former smokers. Screening for COPD was based on performing free spirometric testing on patients at risk of COPD, i.e. those at least 40 years of age and those who consumed cigarette consumption of 010 pack–years. The average age in this population (n=51,10,355) was 53 yrs. Bronchial obstruction was defined with respect to FEV1/FVC,70%. Screening enabled the diagnosis of chronic obstruction in 20.35 of subjects. In terms of severity, 7.6% had mild blockage (FEV1 70% predictive), 6.7% moderate obstruction (FEV1 50-69% predictive), and 5.9% severe blockage (50% predictive). In patients with severe

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obstruction, no diagnosis was previously made by the doctor. The prevalence of bronchial obstruction increased with age and smoking levels. As far as symptoms were concerned, 52.5% of participants presented a moist cough and sputum, whereas the respiratory function was normal. Future challenges in the screening of COPD will be to update the prediction equations so that the most reliable LLN can be obtained.Detection of COPD may provide opportunities for early interventions, including smoking cessation, which may improve quality of life and survival[51].

Morbidity:

Morbidity measures have traditionally included physician visits, emergency department visits, and hospitalizations. Although COPD databases for these outcome parameters are less readily available and generally less reliable than mortality databases, the limited data available suggest that morbidity due to COPD increases with age and is higher in men than women. In these data sets, however, COPD in its early stages (Stage I: Mild COPD and Stage 2: moderate COPD) is not generally recognized, diagnosed, or treated, and is therefore referred to as a diagnosis in a patient's medical record cannot be included[52-54].

Morbidity from COPD may be affected by other co-morbid chronic conditions (e.g., musculoskeletal disease, diabetes mellitus) that are not directly related to COPD but nevertheless may has an effect on the patient's health status, or may negatively interfere with COPD management. In patients with more advanced disease (stage 3: severe COPD and stage 4: very severe COPD), morbidity from COPD may be mistaken for another co-morbid condition[55].

Morbidity data are greatly affected by the availability of resources e.g. hospitalization rates are highly dependent on the availability of hospital beds) and are thus carefully interpreted and given a clear understanding of potential biases inherent in the dataset with. Despite limitations in the data for COPD, the European White Book provides good data on the average number of consultations for major respiratory diseases in 19 countries of the European Economic Community. In most countries, counseling for COPD is very important for asthma, pneumonia, lung and trachea cancers, and tuberculosis. In the United States in 2000, there were 8 million physician office/ hospital outpatient visits for COPD, 1.5 million emergency department visits, and 673,000 hospitalizations[56].

Another way to estimate the disease burden of illness is to calculate the years of living with a disability (YLD). The Global Burden of Disease Study estimates that COPD results in 1.68 YLD per 1,000 population, representing 1.8% of all YLDs, with a greater burden in men than in women (1.93% vs. 1.42%)[57-59].

Mortality:

Although COPD is often a primary cause of death, it is likely to be listed as a contributory cause of death or completely removed from the death certificate, and death is associated with another condition as heart disease has been attributed to. Despite the problems with the accuracy of COPD mortality data, it is clear that COPD is one of the most important causes of death in most countries. This increased death is driven by the smoking epidemic and the changing demographics in most countries, which have a large population. Among these two forces, demography is a strong driver of the trend. Mortality trends over time provide further important information but, again; these data are greatly influenced by terminology, disease awareness, and potential gender bias in its diagnosis. COPD mortality trends generally lag behind smoking trends for several decades. Trends in age-standardized death rates for the six leading causes of death in the United States from 1970 through 2002 indicates that while mortality from several of these chronic conditions declined over that period, COPD mortality increased. The death rate for COPD in Canada, in both men and women, has also been increasing since 1997. In Europe, however, trends differ, with the reduction in mortality from COPD already being seen in many countries. There is no clear reason for the difference between trends in North America and Europe, although these factors probably include factors such as awareness, changing terminology, and clinical bias[57-59].

The mortality rate of COPD is particularly striking for women. In Canada, the death rate from COPD among women accelerated in the 1990's and is expected to soon overtake the rate among men. In the United States, COPD deaths among women have been increasing rapidly since the 1970's. In 2000, the number of deaths from COPD in the United States was higher in women than in men (59,936 vs. 59,118), although the mortality rates among women remain somewhat lower than among men. Worldwide, recent increases in COPD deaths are likely to continue. The Global Burden of Disease Study projected baseline, optimistic, and pessimistic models for COPD mortality from 1990 to 2020 that take into account the expected aging of the world's population, smoking rates are projected to rise, and other causes of death, such as diarrhea and HIV-related illnesses, are projected to decline[60].

Trends in Mortality:

A total of 25 articles reported COPD mortality over various years to allow trends to be observed, of which 14 reported changes in COPD mortality within the overall population. In general, the studies reported an overall increase in COPD mortality rates within the last 30-40 years, with a much greater increase in mortality in women compared with men. Some studies have indicated that more recently (within the last 10 years) mortality rates have increased at a slower rate or have decreased, particularly in men. Some notable differences in COPD mortality exist between countries, particularly regarding differences between men and women. In

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Australia, a study 34 reported a decrease in COPD mortality in men between 1979 and 1997, while an increase was observed in women over the same period. In France, COPD mortality has increased in women over time, while a decrease has been reported in men. The data from many U.S studies show more heterogeneity. Data from two studies showed a clear increase in COPD mortality in women and only a slight increase in men between 1980 and 2000. Data from a later study suggested that COPD mortality decreased between 2000 and 2005 in men, with little change in women[10,61-65].

Social Burden:

Since mortality provides a limited view on the human burden of a disease, it is desirable to find other measures of disease burden that are consistent and measurable across nations. The authors of the Global burden of Disease Study devised a method to estimate mortality and disability quotient due to major illnesses and injuries using an aggregate measure of burden for each health problem, Disability-Adjusted life year (DALY). The DALY's for a specific condition led to premature death because premature mortality and years of life lived with disability are adjusted for severity of disability. In 1990, COPD was the twelfth leading cause of lost DALY's in the world, accounting for 2.1% of the total. According to estimates, COPD will be the 5th leading cause of lost DALY's worldwide by 2020, behind ischemic heart disease, major depression, traffic accidents, and neurological disease. This substantial increase in the global burden of estimated COPD over the next 20 years reflects, in large part, the continued high use of tobacco in many countries and the changing age structure of the population in developing countries[57-59].

Economic Burden:

COPD is an expensive disease with both direct costs (the value of dedicated health care resources for diagnosis and medical management) and indirect costs (monetary consequences of disability, missed work, premature mortality, and caregiver or family costs resulting from the illness). In developed countries, exacerbations of COPD account for the greatest burden on the health care system. In the European Union, the total direct costs of respiratory disease are estimated to be about 6% of the total health care budget, with COPD accounting for 56% (38.6 billion Euros) of this. In the United States in 2002, the direct costs of COPD were $18 billion and the indirect costs totaled $14.1 billion. The cost per patient will vary across countries because these costs depend on how health care is provided and paid. Not surprisingly, there is a striking direct relationship between the severity of COPD and the cost of care, and the distribution of costs changes as the disease progresses. For example, as the severity of COPD increases, the cost of hospitalization and oxygen- rich oxygen increases, as data from Sweden show[66-68].

The presence of COPD greatly increases the total cost of care of patients, especially when the cost of the patient is considered. In a study of COPD related illness costs in the United States based on the 1987 National Medical Expenditure Survey the per capita expenditure for hospitals with COPD patients was 2.7 times the expenditures for patients without COPD ($5,409 vs. $2,001). In a 1992 study of Medicare, the US government health insurance program for individuals over 65, annual per capita expenditures for people with COPD ($8,482) were nearly 2.5 times the expenditures for people without COPD ($3,511)[69,70].

Individuals with COPD often receive professional medical care in their homes. In some countries, national health insurance plans provide coverage for oxygen therapy, nursing services, rehabilitation, and even in house mechanical ventilation, although coverage for specific services varies by country. Any estimate of direct medical expenditures for home care represents the true cost of home care for society, as it ignores the economic value of care provided by family members with COPD. In developing countries, direct medical costs may be less significant than the impact of COPD on workplace and household productivity. Because the health care sector might not provide long-term supportive care services for severely disabled individuals, COPD may force two individuals to leave the workplace the affected individual and a family member who must now stay home to care for the disabled relative. Since human capital is often the most important national asset for developing countries, the indirect costs of COPD may represent a serious threat to their economies[71].

Health Care Costs:

Higher health care costs are associated with COPD maintenance treatment and even more acute treatment with exacerbations. In Canada, patients with COPD were enrolled in a study for three months to assess the cost of healthcare. It found the mean annual total COPD-related cost per patient to be $4147 ($6255). Both the costs for maintenance therapy and acute treatment increased as severity level increased. An interesting retrospective case-control study 36 months before the diagnosis of COPD looked at the costs of healthcare. COPD patients used 1.5-1.6 times more ED services and in-patient services and had more out-patient visits. There was a significant increased use of these services one month prior to diagnosis, suggesting that patients are not diagnosed until they have experienced acute exacerbations or sudden changes in respiratory status. Even when adjusting for the high incidence of co-morbidity in these patients, having a COPD diagnosis was associated with higher resource utilization and treatment costs. The economic burden from COPD is expected to increase secondary to aging populations, tobacco use, and air pollution. A recent review focused on studies that evaluated economic costs, particularly in elderly patients, and found a trend towards direct cost increases in the population. In 2010, the estimated total cost of COPD in the United States was $36 billion with $32.1 billion directly related to health care costs. Research funding directed at COPD is expensive, along with

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the burden of disease and treatment costs. In 2013, the National Institutes of Health funded $102 million for COPD research. However, this is significantly less than the $1.9 billion for cardiovascular disease and $282 million for stroke, even though the rate of death from these diseases is decreasing unlike COPD[72-76].

Medication Costs:

Medication costs contribute to overall health care costs associated with COPD.It is important to note that medication costs related to smoking cessation are cost effective, especially when paired with counseling. Electronic cigarettes have become a very popular smoking cessation aid in the general population and now a billion-dollar industry. Medication costs also increase with severity. When evaluated according to GOLD severity, the percentage of patients taking inhaled corticosteroid/ long-acting β-agonist (LABA) combination, LABA and long-acting anti-cholinergic medications was greater in patients with more severe COPD. In the Canadian study, 70% of the $2475 per patient was from medication for maintenance treatment in moderate to severe COPD patients. In the U.S., prescription costs for COPD in 2008 were $20.4 billion. Costs for maintenance medication can be elevated due to incorrect medication use. This same study found that under 1-3 of patients with GOLD stage, 1 or 2 had more than 2 acute exacerbations in the previous year. However, more than two-thirds of these patients were placed on combination of corticosteroid with LABA inhalers, and intervention was recommended for more severe disease.Overall, mild to moderate COPD is more common than severe COPD, but if providers are using the drug incorrectly in this group, the costs for treatment of this population will increase[77,78].

Exacerbations:

Exacerbations represent another amplification of the inflammatory response in the airways of COPD patients, and can be triggered by bacterial or virus infections or environmental pollutants. There is a relative lack of information about the inflammatory mechanisms involved in exacerbations of COPD. In mild and moderate exacerbations there is an increase in neutrophils and in some studies also eosinophils in sputum and the airway wall. This is associated with increased concentrations of certain mediators, including TNF-α,LTB4 and IL-8, and an increase in biomarkers of oxidative stress. There is even less information about severe exacerbations, although one study showed a marked increase in neutrophils in the airway wall and increased expression of chemokines. Hyper-inflammation and air trapping increase during an exacerbation, with reduced exfoliation flow, thus accounting for increased dyspepsia. The abnormalities of VA/ Q also worsen, resulting in severe hypoxemia[79,80].

Exacerbations negatively affect the quality of life and patients and increase their risk of death. After an exacerbation, symptoms and lung function takes several weeks to recover, and

exacerbations accelerate the rate of irreversible worsening pulmonary function. These not only cause deterioration in lung function, but are costly for hospital treatment. In Canada, COPD is the 3rd leading cause of hospitalization for men. A study published in 2010 described and characterized the costs of COPD in a hospital.It looked at 602 hospitals and calculated the costs for COPD related visits for 2008 in the US based on rates. This determined the average cost of ED travel, simple admission and complex admission to be $6467, $7242 and $20,757, respectively. Intensive care unit admission within tubation was the most expensive at $44,909. A similar study out of Canada looked at moderate to severe COPD patients and determined that the average cost per excision was $3036 Canadian dollars with 80% of the total hospitalization[81,82].

Hospitalization:

However, COPD 1st found this mark in the United States (U.S.) in 2008 and became the 3rd leading cause of mortality. Worldwide, 4.5 million people died from COPD in 2008 and in contrast to coronary heart disease and stroke, it is the only leading cause of death that is still increasing. In the U.S., COPD results in 15.4 million physician visits, 1.5 million emergency department (ED) visits, and 726,000 hospitalizations each year. The National Heart, Lung, and Blood Institute estimated that there were 14.8 million physicians in the US who diagnosed COPD, and another 12 million who had undiagnosed COPD. The worldwide unauthorized annual mortality rate for COPD in 2008 was 45.3 per 100,000 population. Although COPD mortality is difficult to estimate worldwide, COPD is listed as a death certificate due to naming discrepancies. Is not being done; For this reason, COPD mortality is underestimated[3,10,83].

Conclusion:

Respiratory function develops in several main stages, a developmental phase that occurs in the uterus, a pulmonary developmental stage that continues until young adulthood (20 years), a maturation phase between 20 and 30 years of age, and physical decline, A stage, which is thought to begin after the age of 40 years. The available evidence points to the considerable burden of COPD, from the perspectives of society, patients, physicians and healthcare payers. The disability imposed by the condition has a major economic consequence that is likely to increase in the foreseeable future. Despite this, the burden of COPD is not widely recognized, in large part due to poor recognition, diagnosis and knowledge of COPD, and also to nihilistic attitudes towards the condition. Education of both the public and healthcare professionals could help to increase awareness of the burden of COPD, while further research, particularly long-term epidemiological studies demonstrating the impact of different treatment approaches on mortality, would give healthcare payers the information needed to justify expenditure on the condition.

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References:

1. World Health Organization. The global burden of disease: 2004 update. 2008. Available at www.who.int/healthinfo/global_burden_disease/GBD_report_2004update_full.pdf
2. Chronic Obstructive Pulmonary Disease (COPD). Available at http://www.cdc.gov/copd/data.html
3. Michalski JM. Prostaglandin E2 signaling in human lung fibroblasts: mechanisms of signal attenuation and implications in chronic lung diseases. ProQuest, UMI Dissertation Publishing, 2011.
4. Lange P, Marott JL, Vestbo J, Olsen KR, Dahl M. Prediction of the clinical course of chronic obstructive pulmonary disease, using the new GOLD classification: a study of the general population. Am J Respir Crit Care Med 2012; 186:975-981.
5. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, Barnes PJ. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2013; 187:347-365.
6. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007; 176:532-555.
7. Calverley PM,Walker P. Chronic obstructive pulmonary disease. Lancet 2003; 362:1053-1061.
8. World Health Organization. Global status report on non-communicable diseases 2010. Geneva, 2011.
9. National Heart, Lung and Blood Institute. Morbidity and Mortality: 2012 Chartbook on Cardiovascular, lung, and blood diseases. Available at http://www.nhlbi.nih.gov/resources/docs/2012_ChartBook_508.pdf
10. Mannino DM, Homa DM, Akinbami LJ, Ford ES, Redd SCl. Chronic obstructive pulmonary disease surveillance-United States, 1971-2000. MMWR Surveill Summ 2002; 51:1-16.
11. Siafakas NM, Vermeire P, Pride NB, Paoletti P, Gibson J, Howard P, Yernault JC, Decramer M.Optimal assessment and management of chronic obstructive pulmonary disease (COPD). Eur Respir J 1995;8:1398-1420.
12. ATS Statement. Standards for the Diagnosis and Care of Patients with Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 1995;152:S78–S119.
13. Burrows B, Bloom JW, Traver GA, Cline MG. The course and prognosis of different forms of chronic airways obstruction in a sample from the general population. N Engl J Med 1987;317:1309-1314.
14. Alwan A. Global Status Report on Non-Communicable Diseases. WHO, 2010.

15. Gordon SB, Brucr NG, Grigg J, Hibberd PL, Kurmi OP, Mortimer K, Buist S. Respiratory risks from household air pollution in low and middle income countries. The Lancet Respiratory Medicine 2014; 2: 823-860.
16. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. The Lancet 2012; 380: 2095-2128.
17. Bloom DE, Mitgang E, Osher B. Program on the Global Demography of Aging. Oxford University Press: Oxford Textbook of Geriatric Medicine, 2012.
18. Health Effects Institute. 2017. State of Global Air 2017. Special Report. Boston, MA: Health Effects Institute.
19. Britton J. Death, disease and tobacco. The Lancet 2017; 389: 1861-1862.
20. Guindon GE, Boisclair D. Past, Current and Future Trends in Tobacco Use. HNP Discussion paper, Economics of Tobacco Control Paper No.6. February 2003.
21. WHO global report on trends in prevalence of tobacco smoking, 2015.
22. Liu W. Zhang J, Hashim JH, Jalaludin J,Hashim Z, Goldstein BD. Mosquito coil emissionsand health implications. Environ Health Perspect 2003; 111(12): 1454-1460.
23. American Lung Association. Hookah Smoking: A Growing Threat to Public Health Issue Brief, 2011.
24. ICMR-MRC Workshop. Building Indo-UK Collaboration in chronic diseases 2009:16.
25. Reddy KS, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. Lancet 2005;366:1746-1751.
26. Salvi S, Agarwal A. India needs a national COPD prevention and Control program. J Assoc Physicians India 2012;60(Suppl):5-7.
27. Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS. Chronic obstructive airway disease: Current burden and future projections. Eur Resp J 2006;27:397-412.
28. Jindal SK, Aggarwal AN, Gupta D. A review of the population studies from India to estimate national burden of chronic obstructive pulmonary disease and its association with smoking. Indian J Chest Dis Allied Sci 2001;43:139-147.
29. Jindal SK, Aggarwal AN, Gupta D, Agarwal R, Kumar R, Kaur T. Indian study on epidemiology of asthma, respiratory symptoms and chronic bronchitis in adults (INSEARCH) Int J Tuberc Lung Dis 2012;16:1270-1277.
30. Eisner MD, Anthonisen N, Coultas D, Kuenzli N, Postma D, Balmes JR. An official American Thoracic Society public policy statement: novel risk factors and the global burden of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 182:693-718.

31. Centers for Disease Control and Prevention. Chronic obstructive pulmonary disease among adults-United States, 2011.Morbidity and Mortality Weekly Report (MMWR); 2012;61(46): 938-943.
32. Halbert RJ, Natoli JL, Gano A, Badamgarav E. Buist S, Mannino DM. Global burden of COPD: systematic review and meta-analysis. Eur Respir J 2006; 28:523-532.
33. Soriano JB, Maier WC, Egger P, Visick G, Thakrar B, Sykes J. Recent trends in physician diagnosed COPD in women and men in the UK. Thorax, 2000; 55:789-794.
34. Sorheim IC, Johannessen A, Gulsvik A, Bakke PS.Gender differences in COPD: are women more susceptible to smoking effects than men? Thorax, 2010; 65:480-485.
35. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Mannino DM. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. Lancet 2007; 370:741-750.
36. Rennard SI, Farmer SG. COPD in 2001: a major challenge for medicine, the pharmaceutical industry, and society. Chest 2002; 121:113S-115S.
37. Rennard S, Decramer M, Calverley PM, Pride NB, Soriano JB. Impact of COPD in North America and Europe in 2000: subjects' perspective of Confronting COPD International Survey. Eur Respir J 2002; 20:799-805.
38. Ferguson GT, Petty TL. Screening and early intervention for COPD. Hosp Pract 1998; 33:67-72.
39. Zielinski J, Bednarek M. Early detection of COPD in a high-risk population using spirometric screening. Chest 2001; 119:731-736.
40. de Marco R, Accordini S, Cerveri I, Corsico A, Janson C, Sunyer J. Incidence of chronic obstructive pulmonary disease in a cohort of young adults according to the presence of chronic cough and phlegm. Am J Respir Crit Care Med 2007;175(1):32-39.
41. Gershon AS, Wang C, Wilton AS, Raut R, To T. Trends in chronic obstructive pulmonary disease prevalence, incidence, and mortality in Ontario, Canada, 1996 to 2007: a population-based study. Arch Intern Med 2010;170(6):560-565.
42. Kojima S, Sakakibara H, Motani S, Hirose K, Mizuno F, Ochiai M. Incidence of chronic obstructive pulmonary disease, and the relationship between age and smoking in a Japanese population. J Epidemiol 2007;17(2): 54-60.
43. Lindberg A, Jonsson AC, Ronmark E, Lundgren R, Larsson LG, Lundback B. Ten-year cumulative incidence of COPD and risk factors for incident disease in a symptomatic cohort. Chest 2005;127(5): 1544-1552.

44. Garcia Rodriguez LA, Wallander MA, Tolosa LB, Johansson S. Chronic obstructive pulmonary disease in UK primary care: incidence and risk factors. COPD2009;6(5):369-379.
45. Tan WC, Seale P, Ip M, Abisheganadan J, Mahayiddin A. Trends in COPD mortality and hospitalizations in countries and regions of Asia-Pacific. Respirology 2009;14(1):90-97.
46. Lindgren B. Trends in obstructive lung disease in hospital registers in Sweden 1987-1996. Eur Respir Rev 2000;10(75):423.
47. Lipton R, Banerjee A. The geography of chronic obstructive pulmonary disease across time: California in 1993 and 1999. Int J Med Sci 2007; 4(4):179-189.
48. Kabir Z, Connolly GN, Koh HK, Clancy L. Chronic obstructive pulmonary disease hospitalization rates in Massachusetts: a trend analysis. QJM 2010;103(3):163-168.
49. Lindberg A, Eriksson B, Larsson LG, Ronmark E, Sandstrom T, Lundback B. Seven-year cumulative incidence of COPD in an agestratified general population sample. Chest2006;129(4):879-885.
50. Price DB, Tinkelman DG, Nordyke RJ, Isonaka S, Halbert RJ. Scoring system and clinical application of COPD diagnostic questionnaires. Chest 2006; 129: 1531-1539.
51. Zielinski J, Bednarek M, Gorecka D, Viegi G, Hurd SS. Increasing COPD awareness. Eur Respir J 2006; 27: 833-852.
52. National Heart, Lung, and Blood Institute. Morbidity & Mortality: Chartbook on Cardiovascular, Lung, and Blood Diseases. Bethesda, MD: US Department. of Health and Human Services, Public Health Service, National Institutes of Health; 1998.
53. Soriano JR, Maier WC, Egger P, Visick G, Thakrar B, Sykes J. Recent trends in physician diagnosed COPD in women and men in the UK. Thorax 2000;55:789-794.
54. Chapman KR. Chronic obstructive pulmonary disease: are women more susceptible than men? Clin Chest Med 2004;25(2):331-341.
55. Schellevis FG, Van de Lisdonk EH, Van der Velden J, Hoogbergen SH, Van Eijk JT, Van Weel C. Consultation rates and incidence of intercurrent morbidity among patients with chronic disease in general practice. Br J Gen Pract 1994; 44(383):259-262.
56. Centers for Disease Control and Prevention. Surveillance Summaries. MMWR 2002:51(SS-6).
57. Murray CJL, Lopez AD, editors. In: The global burden of disease: a comprehensive assessment of mortality and disability from diseases, injuries and risk factors in 1990 and projected to 2020. Cambridge, MA: Harvard University Press; 1996.
58. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997;349(9064):1498-1504.

59. Lopez AD, Shibuya K, Rao C, Mathers CD, Hansell AL, Held LS. Chronic obstructive pulmonary disease: current burden and future projections. Eur Respir J 2006;27(2):397-412.
60. Jemal A, Ward E, Hao Y, Thun M. Trends in the leading causes of death in the United States, 1970-2002. JAMA 2005; 294(10):1255-1259.
61. Berend N. Epidemiological survey of chronic obstructive pulmonary disease and alpha-1-antitrypsin deficiency in Australia. Respirology 2001; (Suppl 6):S21-S25.
62. Fuhrman C, Jougla E, Nicolau J, Eilstein D, Delmas MC. Deaths from chronic obstructive pulmonary disease in France, 1979-2002: a multiple cause analysis. Thorax. 2006;61(11):930-934.
63. Miller N, Simoes EJ, Chang JC, Robling AG. Trends in chronic obstructive pulmonary disease mortality. Mo Med 2000;97(3):87-90.
64. Centers for Disease Control and Prevention (CDC). Deaths from chronic obstructive pulmonary disease: United States, 2000-2005. MMWR Morb Mortal Wkly Rep 2008;57 (45):1229-1232.
65. Singh GK, Hiatt RA. Trends and disparities in socioeconomic and behavioural characteristics, life expectancy, and cause-specific mortality of native-born and foreign-born populations in the United States, 1979-2003. Int J Epidemiol 2006; 35(4):903-919.
66. European Respiratory Society. European Lung White Book: Huddersfield, European Respiratory Society Journals, Ltd; 2003.
67. National Heart, Lung, and Blood Institute. Morbidity and mortality chartbook on cardiovascular, lung and blood diseases. Bethesda, Maryland: US Department of Health and Human Services, Public Health Service, National Institutes of Health, 2004.
68. Jansson SA, Andersson F, Borg S, Ericsson A, Jonsson E, Lundback B. Costs of COPD in Sweden according to disease severity. Chest 2002; 122(6):1994-2002.
69. Sullivan SD, Strassels S, Smith DH. Characterization of the incidence and cost of COPD in the US. Eur Respir J 1996;9(Supplement 23):S421.
70. Grasso ME, Weller WE, Shaffer TJ, Diette GB, Anderson GF. Capitation, managed care, and chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998;158:133-138.
71. Fauroux B, Howard P, Muir JF. Home treatment for chronic respiratory insufficiency: the situation in Europe in 1992. The European Working Group on Home Treatment for Chronic Respiratory Insufficiency. Eur Respir J 1994; 7: 1721-1726.
72. Maleki-Yazdi MR, Kelly SM, Lam SY, Marin M, Barbeau M, The burden of illness in patients with moderate to severe chronic obstructive pulmonary disease in Canada. Can Respir J 2012; 19:319-324.

73. Akazawa M, Halpern R, Riedel AA, Stanford RH, Dalal A. Economic burden prior to COPD diagnosis: a matched case-control study in the United States. Respir Med 2008; 102:1744-1752.
74. Bustacchini S, Chiatti C, Furneri G, Lattanzio F. The economic burden of chronic obstructive pulmonary disease in the elderly: results from a systematic review of the literature. Curr. Opin. Pulm. Med., 2011; 17 (Suppl.1):S35-S41.
75. Ford ES, Murphy LB, Khavjou O, Giles WH, Holt JB, Croft JB. Total and state-specific medical and absenteeism costs of chronic obstructive pulmonary disease among adults aged ≥18 years in the United States for 2010 and projections through 2020. Chest 2015; 147(1): 31-45.
76. National Institute of Health. Estimates of Funding for Various Research, Condition, and Disease Categories (RCDC). 2014.
77. Feenstra TL, Hamberg-van Reenen HH, Hoogenveen RT,Rutten-vanMolken MPMH. Cost-effectiveness offace-to-face smoking cessation interventions: a dynamic modeling study. Value Health 2005; 8(3):178-190.
78. Nickels AS, Joshi AY,Dinakar C. Electronic cigarettes: navigating the vapor. Ann Allergy Asthma Immunol2014; 112(6):481-483.
79. Wedzicha JA. Exacerbations: etiology and pathophysiologic mechanisms. Chest 2002;121(5 Suppl):136S-141S.
80. Drost EM, Skwarski KM, Sauleda J, Soler N, Roca J, Agusti AG. Oxidative stress and airway inflammation in severe exacerbations of COPD. Thorax 2005;60(4):293-300.
81. Dalal AA, Shah M, D'Souza AO, Rane P. Costs of COPD exacerbations in the emergency department and inpatient setting. Respir Med 2011; 105:454-460.
82. Maleki-Yazdi MR, Kelly SM, Lam SY, Marin M, Barbeau, Walker V. The burden of illness in patients with moderate to severe chronic obstructive pulmonary disease in Canada. Can Respir J 2012; 19:319-324.
83. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Culverley P, Fukuchi Y. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. Am J Respir Crit Care Med 2007; 176:532-555.

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BIOACTIVE METABOLITES FROM MICROORGANISMS AND ITS APPLICATIONS: A REVIEW

A. Parameswari*, M. Sanjivkumar, K. Nagajothi,
S. Sayen Merlin Sophia and D. Kannan
Department of Microbiology,
K. R. College of Arts & Science,
K. R. Nagar, Kovilpatti - 628503, Tamilnadu, India
*Corresponding author E-mail: parameswari_micro@krcollege.net

Abstract:

Bioactive natural products are the most important substances synthesized by living organisms. They are found in nature and are obtained from various resources like plants, animals and microorganisms (bacteria, fungi and algae), etc. Among these, microbial derived natural byproducts are quite specific and they are traditionally used as novel therapeutics. Thus, the searches for bioactive metabolite from microorganisms continue to play an important role in fashioning new medicinal agents. They have various bioproperties such as antimicrobial, antioxidant, antitumor, anti-inflammatory, immunomodulatory, antidepressant, antihyper-glycaemic, antihypertensive, anti-HIV, antiparasidal activities, etc. Apart from these, the natural products are widely used in several product preparations mainly as flavoring agents, fragrances and functional additives by the food, cosmetic and pharmaceutical industries. Some groups of microorganisms could able to synthesize different types of nanoparticles in order to reduce or oxidize the metal atoms, effectively used in food, cosmetics and pharmaceutical industries. This review encompasses the roles of microorganisms and their derivatives on the advances in the healthcare industry, especially in pharmaceutical industry and allows discovering various biomedically active components that would prevent an escalating number of communicable diseases.

Keywords: Microorganisms, Bioactive metabolites, antimicrobial activity, anticancer activity

Introduction:

Bioactive compound is simply act as a substance or chemical compound that has various biological activities. These are experiencing a growing interest in wide range of applications: geo-medicine, plant science, modern pharmacology, agrochemicals, cosmetics, food industry, nano-bio-science etc. (Manivasagan *et al.*, 2014). This is a very promising area in full development, which has resulted in research works more and more numerous, designed to diversify the resources of bioactive compounds and improve their salvage pathways or synthesis (Mostafa, 2012). Bioactive compounds contain chemicals that are found in small quantities in

plants and certain foods (such as fruits, vegetables, nuts, oils and whole grains); they have actions in the body that can promote good health (Mo *et al.,* 2010). Bioactive compounds in the plants can be defined, then, as secondary plant metabolites eliciting pharmacological or toxicological effects in humans and animals. Bioactive compounds are physiologically active substances with functional properties in the human body. There is, therefore, great enthusiasm for the development and manufacture of various bioactive compounds that can potentially be used as functional ingredients such as carotenoids, phycocyanins (PC), polyphenols, fatty acids, and polyunsaturated compounds (Plaza *et al.*, 2010).

An interest in the production of bioactive compounds from natural sources has recently emerged, driven by a growing number of scientific studies that demonstrate the beneficial effects of these compounds on health benefits (Herrero *et al.,* 2013). These natural products are important in the search for new pharmacologically active compounds and play an important role in new drug discovery for the treatment of human diseases (Newman and Cragg, 2010). Many clinically viable and commercially available drugs with antitumor and anti-infective activity originated as natural products. Bioactive compounds from microbial resources can be attained directly as a primary metabolism, such as proteins, fatty acids, vitamins, and pigments, or as secondary metabolism. Some examples are cyanovirin, oleic acid, linolenic acid, palmitoleic acid, vitamin E, B12, β-carotene, PC, lutein, and zeaxanthin etc., have antimicrobial, antioxidant, and anti-inflammatory properties, with the potential for the reduction and prevention of diseases (Volk, 2008).

Bioactive compounds from different microorganisms:

Actinobacteria:

Microbial mediated natural byproducts are an eminent resource of both existing and new drugs with a wide range of activity (Berdy, 2005). This is particularly noteworthy for the phylum Actinobacteria with the well know representative genera namely *Streptomyces* sp. These are filamentous Gram-positive bacteria belonging to actinobateria that represents one of the largest taxonomic units among the bacteria domain (Usha *et al.,* 2012). They are the most economically and biotechnologically valuable prokaryotes and are responsible for the production of about half of the discovered bioactive secondary metabolites, antibiotics, anticancer agents and enzymes. Around 23,000 bioactive secondary metabolites produced by microorganisms have been reported and over 10,000 of the compounds are produced by actinobacteria, representing 45% of all bioactive microbial metabolites discovered. Among actinobacteria, around 7,600 compounds are produced by *Streptomyces* species (Olano *et al.,* 2009). Kato *et al.* (1993) reported phenazoviridin produced from *Streptomyces* sp. has higher defending activity against KCN-induced acute hypoxia in mice comparatively than indeloxazine induced mice. In another study, Fukami *et al.* (2000) reported the two motile actinobacterial strains, K95-5561T and K95-5562,

from a soil sample, produced a novel antimicrobial agent, 2-hydroxyethyl-3-methyl-1,4-naphthoquinone showed anti-HIV activity. This was also extensively studied by Chiba *et al.* (2001).

Maskey *et al.* (2003) portrayed the isolation and extractionof pigment (phenazostatin D) from a marine associated actinobacterium *Pseudonocardia* sp. B6273 active against the various gram positive organisms. Li *et al.* (2005) enumerated an efficient marine associated actinobacterium *Streptomyces* sp. B6921 for the extraction of a bioactive secondary metabolite namely himalomycin A and B displayed strong antibacterial activity against both Gram positive and Gram negative bacteria. Herold *et al.* (2005) attained the novel polyketide glycoside compounds like Cervimycins A, B, C and D from *Streptomyces tendae* strain HKI 0179 under laboratory condition expressed maximum activity against Gram positive bacteria (*B. subtilis* and *S. aureus*) and multi-drug-resistant bacteria like *S. aureus* (MRSA). He *et al.* (2007) documented the derivatives of aromatic polyketide compound like Griseusin A and B from *Nocardiopsis* sp. YIM80133 showed anticancer property against various cell lines such as breast, melanoma and renal. Chen *et al.* (2011) reported the active metabolites such as benzamide, 3-hydroxyl-2-*N*-iso-butyryl-anthranilamide, benzamide-3-hydroxyl-anthranilamide, anthranilamide and 8-hydroxyl-2,4-dioxoquinazolin from *Sterptomyces* sp.(No.061316) exhibited anticancer activity and reduce Caspase-3 activity under *in vitro* condition.

A red pigment namely undecylprodigiosin extracted from *Streptomyces* sp. JS520 enumerated from sediment soil samples of cave in the mountain Miroc, Serbia, showed antibacterial activity against various clinical pathogenic microorganisms (Stankovic *et al.* 2012). in another report, Yang *et al.* (2013) documented the extraction of a secondary metabolite namely 1”-O-methyl-8-hydroxymethyl-daidzein (isoflavone) from *Streptomyces* sp. YIM 65408 through laboratory condition exhibited ROS scavenging activity. Likewise, Zhou *et al.* (2014) also reported the antioxidant nature of a metabolite namely 2,6-dimethoxy terephthalic acid (IC50 4.61mg/mL) from an endophyticactino bacterium *Streptomyces* sp. YIM66017 through *in-vitro* condition. Recently Chandra *et al.* (2020) reported the different functional groups such as terpenoids, isoflavonoids and 4,7,8-Trihydroxyisoflavone from *Streptomyces* sp. expressed antioxidant property.

Other bacteria:

Other bacterial resources, such as a marine isolate *P. nigrifaciens* (reclassified into *Alteromonas nigrifaciens*) extensively used for the extraction of blue pigment (indigoidine) for bactericidal activity (Norton and Jones, 1969). In accordance with these, Kobayashi *et al.* (2007) studied a pigment with an alkylated structure as like indigoidine from *Shewanella violacea*, enumerated from the sediment samples of Ryukyu Trench (5110m depth). Misawa, *et al.* (1995)

documented an important carotenoid compound namely astaxanthin from a marine associated bacterium *Agrobacterium aurantiacum* under laboratory condition and which has more economical value as a food supplement for animal and human's diet. In an another study, Rettori and Durán (1998) documented an indole derivative component (violacein) from *Chromobacterium* sp. expressed a vast variety of biomedical properties such as antiviral, antimicrobial, anti-ulcerogenic, antileishmanial and anticancer activity through *in-vitro* conditions. Prioult *et al.* (2004) examined beta-lactoglobulin from *Lactobacillus paracasei* stimulates Interleukin 10 (IL-10) production and down regulation of IL-4 and gamma interferon secretion etc.

Algae:

Algae are one of the most important bio-resource of bioactive metabolites has a key role in drug discovery and development area inside pharmaceutical industry. Various researches have been conducted on algae mediated bioactive metabolites from Arthrospira (*Spirulina*), *Botryococcus braunii, Chlorella vulgaris, Dunaliella salina* and *Nostoc* showed high antimicrobial, anticoagulant, antiviral, antifungal, anti-enzymatic, anti-inflammatory, antioxidant, and antitumor activity (Plaza *et al.,* 2010). Algae always had the potential to be beneficial to animal and mankind; especially the use of cyanobacteria (blue-green algae), for antibiotics and pharmacologically active compounds like polyphenols, phycobiliproteins and vitamins etc. Abio-active compound, Scytovirin was isolated and extracted from *Scytonema varium* showed anti-HIV property under *in-vitro* condition (Arment and Carmichael, 1996).Stevenson *et al.* (2002) documented a protein (serine/threonine) kinase inhibitor namely scytonemin, from *Stigonema* sp., regulates cell cycle, inhibits proliferation of human fibroblasts and endothelial cells. This also expressed anti-inflammatory properties.In an another report, Tiwari *et al.* (2009) displayed a polypeptide compound Cyanovirin-N of *Nostoc ellipsosporum* showed antiviral property against HIV andherpes simplex virus etc.

Hasui, *et al.* (1995) assessed the sulfated exopolysaccharides A1 and A2 from a marine algae *Cochlodinium polykrikoides* showed antiviral effects against influenza (types A and B) and parainfluenza viruses. Various scientist started to identify a peptide component Dolastatins from a marine cyanobacterium *Symploca* sp. as an antiproliferative factor, was actively inhibited the cancer cells. An another efficient bioactive metabolite phloroglucinol from *E. bicyclis* used in a high potential elective therapy for diabetic complicated patients through an inhibition of advanced glycation were documented by Okada *et al.* (2004). Asthana, *et al.* (2006) isolated a bioactive compound Hapalindole T from *Fischerella* sp. expressed antituberculosis activity. In an another study, Spolaore *et al.* (2006) attained the most efficient bioactive metabolite β-1,3-glucan from *Chlorella* sp. act as immune stimulator that reduces free radicals and blood cholesterol. In addition, Kamei *et al.* (2009) documented a novel terpenoid compound from the

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methanolic extract of *Sargassum macrocarpum* exhibited antibacterial activity against various clinical pathogens under laboratory conditions using Agar-well diffusion assay.

Apratoxin A, B and C from *Lyngbya majuscule* showed cytotoxicity against different human tumor (KB and LoVo) cell lines were observed by Luesch *et al.* (2002). In an another bioactive compound, bisebromoamide extracted from a cyanobacterium *Lyngbya* sp., expressed cytotoxicity effect against HeLa cells, together with an efficient protein kinase inhibition were denoted by Teruya *et al.* (2009). Similarly, Huskens, *et al.* (2010) enumerated the cyanobacterial lectin compound namely microvirin from *Microcystis aeruginosa* inhibits the HIV-1 strains. Tripathi *et al.* (2011) extracted a bioactive compound Lagunamides A-C from *Lyngbya majuscule* showed antimicrobial activity as well as anticancer activity against different cell lines like P388, A549, PC3, HCT8, and SK-OV3 etc., through in-vitro conditions. Beerling (2012) displayed a lipopeptide compound somocystinamide A extracted from a marine associated cyanobacteria *Lyngbya majuscule*and *Schizothrix* sp. possessed various biological activities such as antimicrobial and anticancer activity. Chen *et al.* (2019) extracted sulfated polysaccharides from filamentous microalgae *Tribonema sp.* (TSP) showed anticancer activity against liver (HepG2) cell lines.The extracted sulphated polysaccharide from *Cladosiphon okamuranus* and *Ulva clathrate* showed antiviral property against Newcastle virus disease were studied by Aguilar Briseno *et al.* (2015). Recently, Sun *et al.* (2020) examined the fucoidans extracted from brown algae *Sargassum henslowianum* exhibited maximum activity herpes simplex (HSV-1 and HSV-2) viruses.

Fungi:

Fungi are a rich source of biologically active natural metabolites, including a wide variety of clinically important drugs. There are numerous bioactive functional components such as alkaloids, benzopyranones, chinones, peptides, phenols, quinones, flavonoids, steroids, terpenoids, tetralones, xanthones etc., exhibited various biological properties, including antibacterial, antifungal, immune-suppressants, antiviral, anti-parasitic, antioxidant, anti-inflammatory, and anticancer activity (Toghueo, 2020). A pentacyclic quinoline alkaloid (Camptothecin) compound was firstly isolated and extracted from *Camptotheca acuminate* by Wall *et al.* (1966). In another report, Wani *et al.* (1971) examined alkaloid bioactive compound camptothecin from Camptotheca acuminate showed anticancer properties. In addition, bianthraquinone derivatives from an endophytic fungi *Alternaria porri* expressed antioxidant property were observed by Suemitsu *et al.* (1989). Suto *et al.* (1994) documented the polysaccharide-K extracted from by *Trametes versicolor* expressed anti-cancer activity through suppression of tumor detachment, cell matrix degrading enzymes, inhibition of angiogenesis as well as oxidation/reduction potential of free radicals under laboratory condition.Lee*et al.* (2006)

studied ROS scavenging property of Hispidin derivatives like methylinoscavin A, inoscavin B, and methylinoscavin-B from *Phellinus* sp. and *Inonotus*sp, through *in-vitro* conditions.

Zhang *et al.* (2011) examined Isoindolones and aspernidines derivatives from an endophytic fungi *Emericella* sp. (HK-ZJ) showed anti-viral property against influenza A viral (H1N1). A bioactive metabolite inonotusin A and Bas a natural antioxidants extracted from the methanolic extract of fruiting bodies of *Inonotus hispidus* was reported by Zan *et al.* (2011). Likewise, Cohen *et al.* (2011) described a novel terpenoid compound from *Psammocinia* sp. as a sponge-associated fungi exhibited both antibacterial and antifungal activities. In accordance with these, another bioactive compound pneumocandins from *Glarea lozoyensis* expressed maximum activity against various clinical fungal pathogens and also involved in the treatment of oropharyngeal and oesophageal candidiasis and aspergillosis were documented by Masurekar *et al.* (2012). A natural anthraquinone compound likely 4-deoxybostrycin extracted from a mangrove endophytic fungi *Alternaria* sp. (SK11) effectively used as anti-tuberculosis was performed by Li *et al.* (2014). Li *et al.* (2015) extracted a natural bioactive metabolite namely hispidin from *Phellinus igniarius* was effectively used in ROS scavenging activity, inhibit erythrocyte hemolysis and lipid peroxidation. The hydroanthraquinone derivatives such as 6-O-demethyl-4-dehydroxy-altersolanol A, azaphilones, 8,11-didehydrochermesinone B, and (7S)-7-hydroxy-3,7-dimethyl-isochromene-6,8-dione were isolated and extracted from *Nigrospora* sp. YE3033 displayed strong antiviral activity against the influenza (H1N1) virus (Zhang *et al.*, 2016).

Yang *et al.* (2018) attained a novel bioactive metabolite from a marine-derived fungi *Aspergillus alabamensis* EN-547 showed antibacterial activity against various clinical pathogens through laboratory condition using agar-well diffusion assay. Likewise, Li *et al.* (2018) obtained six novel peniciphenalenins from fungus *Penicillium* sp. ZZ901 showed antibacterial activity against various clinical human pathogens. Mao and colleagues (2020) described a novel bioactive metabolite hyalodendrins A/B extracted from an endophyticfungi *Hyalodendriella* sp. exhibited anti-plasmodialactivity. In an another report, a bioactive metabolite namely oxylipin (9Z,11E)-13-oxooctadeca-9,11-dienoic acid from *Penicillium herquei* BRS2A-AR showed anti-parasitic activity was reported by Hayibor *et al.* (2019). Manganyi (2019) documented the isolation and extraction of the metabolites such as linoleic acid (9,12-octadecadienoic acid (Z,Z)) and cyclodecasiloxane from *Alternaria* sp. through laboratory condition were effectively inhibited the growth of food borne pathogens. A novel bioactive alkaloid compound namely cytoglobosins C17 from an endophytic fungi *Chaetomium globosum* showed antitumor activity against A549 cell line (Rana *et al*, 2020). Likewise, podophyllotoxin as a bioactive metabolite attained from *Podophyllum peltatum* possessed various biomedical activity such as antiviral, antibacterial, anti-helminthic, and antitumor agents (Torres-Mendoza *et al.*, 2020).

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Table 1: Bioactive components from different bacterial bioresources and its applications

Bacteria	Bioactive Compounds	Applications	References
N. dassonvillei OPC-553	Kalafungin	Antifungal and anticancer activity	Tsujibo *et al.* (1990)
Streptomyces sp. K53	Komodoquinone A	Neuritogenic activity	Itoh *et al.* (2003)
Streptomyces sp. BD21-2	Bonactin	Antibacterial, antifungal activity	Schumacher *et al.* (2003)
V. parahaemolyticus	2,2-Di-(3-indolyl)-3-indolone	Antibacterial and antifungal activity	Veluri *et al.* (2003)
Thermoactinomyces sp. YM3-251	Mechercharmycins	Strong antitumor activity	Kanoh *et al.* (2005)
S. tendae HKI 0179	Cervimycins A, B, C, and D	Antibacterial activity	Herold *et al.* (2005)
Marinispora sp	Marinomycins	Antitumor, antibacteri, antifungal activity	Kwon *et al.* (2006)
Streptomyces sp. KORDI-3238	Streptokordin	Anticancer activity	Jeong *et al.* (2006)
Salinispora arenicola	Saliniketal A, saliniketal B	Anticancer activity	Jensen *et al.* (2007)
Actinobacteriam(N2010-37)	Anthroneandlactones	Antitumor activity	Zhou *et al.*, 2011
S. olivaceus (MSU3)	Ethyl acetate extract	Antibacterial, antioxidant and anti-inflammatory activity	Sanjivkumar *et al.* (2016)
B. pseudomycoides DSM1 2442	Pseudomycoicidin	Antibacterial activity	(Basi-Chipalu *et al.*,2015)
B. subtilis 1779	Amicoumacin	Antibacterial activity	Li *et al.* (2015)
*S. avermitilis*TCC 31267	Avermectins	Antihelminthic activity	Deng *et al.* (2017)
Halobacterium salinarum	Bacterioruberin	Antioxidant activity	Giani *et al.* (2019)
P, fluorescens Pf0-1	Gacamide A	Antibacterial activity	Jahanshah *et al.* (2019)

Table 2: Bioactive compounds from different algal bioresources and its applications

Algae	Bioactive Compounds	Applications	References
Ankistrodesmus sp.	α-linolenic acid	Antidepressant,antitumor activity	Ben-Amotz *et al.* (1985)
Botryococcus braunii	Linear alkadienes (C25, C27, C29, and C31), triene (C29)	Hydrogenation activity	Palavra *et al.* (2011)
Botryococcus sp.	α-linolenic acid	Antidepressant,antitumor activity	Chiang et al, (2004)
Chlorella vulgaris	Canthaxanthin, astaxanthin, peptide, oleic acid	Antioxidant activity	Priyadarshani and Rath, (2012)
C. minutissima	Phytol, Phenol	Antioxidant activity	Custodio *et al.* (2012)
*C. prototheco*ides	Lutein	Free radical activity	Markou and Nerantzis (2013)
Fischerella sp.	Ambiguine I isonitrile	Antibacterial and Antimyco-bacterial activity	Mo *et al.* (2010)
Nostoc sp.	Cryptophycin	cytotoxic activity	Singh and Dhar (2011)
	Nostocyclopeptides A1 and A2	Anticancer activity	Burja et al (2001)
Spirulina platensis	Allophycocyanin	Antioxidant activity	Markou and Nerantzis (2013)
N. muscorum	Phenolic, phycobilins	anti-inflammatory, antiviral, antimicrobial and anticancer activity	Mostafa (2012)

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Table 3: Bioactive compounds from different fungal bioresources and its applications

Fungus	Bioactive Compounds	Applications	References
A. alternata KT380662	Flavone chrysin (5,7-dihydroxy flavone)	Anticancer activity	Ran *et al.* (2017)
D. phaseolorum 92C	18-Des-hydroxy Cytochalasin	Antiparasitic activity	Seetharaman (2017)
Fusarium solani	Fusarubin, 3-O-methylfusarubin,	Antitubercular activity	Shah *et al.* (2017)
F. oxysporum	Vinblastine	Anticancer, anti-angiogenesis	Zhang *et al.* (2000)
L. pseudotheobromae PAK-7	dl-Mevalonic acid lactone, Methyl-6-O-[1-methylpropyl]-á-d-galactopyranoside	Antibacterial activity	Chaithra, *et al.* (2020)
Penicillium commune	Dihydrocompactin	Antibacterial activity	Frisvad and Filtenborg 1989
Phomopsis sp. CGMCC No. 5416	Chromanones	Antiviral activity	Yang *et al.* (2020)
Phomopsis sp.	1,7-Dihydroxy-2-methoxy-3- (3-methylbut-2-enyl)xanthone	Cytotoxic activity	Yang *et al.* (2013)
	(+)-Phomopsichin A	Antimicrobial, Antioxidant activity	Meixiang *et al.* (2016)
T. asperellum T1	6-pentyl-2H-pyran-2-one (6-PP)	Antifungal and plant growth promoting properties	Wonglom and Sunpapao(2020)

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References:

1. Arment, A.R. and Carmichael, W.W. (1996): J. Phycology. 32, 591-597.
2. Asthana, R.K., Srivastava, A., Singh, A.P., Deepali, Singh, S.P., Nath, G., Srivastava, R. and Srivastava, B.S. (2006): J. Appl. Phycology. 18, 33–39.
3. Basi-Chipalu, S., Dischinger, J., Josten, M., Szekat, C., Zweynert, A., Sahl, H.G., and Bierbaum, G. (2015):Appl Environ Microbiol. 81, 3419–3429.
4. Beerling, D.J. (2012): Phil. Trans. R. Soc. 367, 477–482.
5. Ben-Amotz, A., Tornabene, T.G. and Thomas, W.H. (1985):J. Phycology. 21, 72-81.
6. Berdy, J. (2005): Bioactive microbial metabolites. J.Antibiot. 58:1.
7. Burja, A. M., Banaigs, E. B., Abou-Mansour, J.G. and Burgess, P.C. (2001): Tetrahedran. 57, 9347-9377.
8. Chaithra, M., Vanitha, S., Ramanathan, A., Jegadeeshwari, V., Rajesh, V., Hegde, V. andApshara, E. (2020):Curr. J. Appl. Science and Tech. 39, 47–56.
9. Chandra, P., Sharma, R.K. and Arora, D.S. (2020):Food Research Int. 129, 108849.
10. Chen, G., Gao, H., Tang, J., Huang, Y., Chen, Y., Wang, Y., Zhao, H., Lin, H., Xie, Q., Hong, K., Li, J. and Yao, X. (2011): Molecules. 24(2), 322.
11. Chiang, I.Z., Huang, W.Y. and Wu, J.T. (2004):J.Phycology. 40, 474-480.
12. Chiba, H., Inokoshi, J., Okamoto, M., Asanuma, S., Matsuzaki, L., Iwama, M., Mizumoto, K., Tanaka, H., Oheda, M., Fujita, K., Nakashima, H., Shinoses, M., Takahashi, Y. and Omura, S. (2001):282, 595–601.
13. Cohen, E., Koch, L., Thu, K.M., Rahamim, Y., Aluma, Y., Ilan, M., Yarden, O. and Carmeli, S. (2011): Bioorganic Medical Chem. 19, 6587–6593.
14. Custodio, L., Justo, T. and Silvestre, L. (2012):Food Chemist. 131(1), 134–140.
15. Deng, Q., Zhou, L., Luo, M.Z., Deng, Z. X and Zhao, C.M. (2017): Synthetic System Biotech. 2, 59–64.
16. Frisvad, J. C. and Filtenborg, O. (1989): Mycologia. 81, 837–861.
17. Fukami, A., Nakamura, T., Kawaguchi, K., Rho, M. C., Matsumoto, A., Takahashi, Y., Shiomi, K., Hayashi, M., Komiyama, K. andOmura, S. (2000): J.Antibiot (Tokyo). 53, 1212–1214.
18. Giani, M., Garbayo, I., Vílchez, C. andMartínez-Espinosa, R.M. (2019): Marine Drugs. 17, 524.
19. Hasui, M., Matsuda, M., Okutani, K. and Shigeta, S. (1995):Int. J.Biol Macromolecule. 17, 293– 297.

20. Hayibor, K., Kwain, S., Osei, E., Nartey, A.P., Tetevi, G.M., Owusu, K.B.A., Camas, M., Camas, A.S. and Kyeremeh, K. (2019):Int. J. Biology Chem. Science. 13, 1918–1937.
21. He, J., Roemer, E., Lange, C., Huang, X., Maier, A., Kelter, G., Jiang and Y., Xu, L.H. (2007): J. Med. Chem. 50, 51685175.
22. Herold, K., Gollmick, F. A., Groth, I., Roth, M., Menzel, K. D. and Mollmann, U. (2005):J. 11, 5523–5530.
23. Herrero, M., Castro-Puyana, M., Mendiola, J.A. and Ibañez, E.(2013): Trends in Analyt. Chem. 43, 67-83.
24. Huskens, D., Férir, G., Vermeire, K., Kehr, J., Balzarini, J., Dittmann, E. and Schols, D. (2010): J.Biol Chemist. 285, 24845–24854.
25. Itoh, T., Kinoshita, M., Aoki, S. and Kobayashi, M. (2003):J. Nat Product. 66, 1373–1377.
26. Jahanshah, G., Yan, Q., Gerhardt, H., Pataj, Z., Lammer- € hofer, M. and Pianet, I. (2019):J. Nat Product. 82, 301–308.
27. Jensen, P.R., Williams, P.G., Oh, D.C., Zeigler, L. and Fenical, W. (2007):Appl.Env. Microbiol. 73, 1146–1152.
28. Jeong, S.Y., Shin, H.J., Kim, T.S., Lee, H.S., Park, S.K. and Kim, H.M. (2006):J. Antibiot (Tokyo). 59, 234–240.
29. Kamei, Y., Sueyoshi, M., Hayashi, K., Terada, R. and Nozaki, H. (2009):J. Antibiot. 62, 259– 263.
30. Kanoh, K., Matsuo, Y., Adachi, K., Imagawa, H., Nishizawa, M. and Shizuri, Y. (2005):J. Antibiot(Tokyo). 58, 289–292.
31. Kato,S., Shindo, K., Yamagishi,Y., Matsuoka,M., Kawai, H. and Mochizuki, J. (1993):J. Antibiot. 46(10),1485–1493.
32. Kobayashi, H., Nogi, Y. and Horikoshi, K. (2007): Extremophiles. 11(2), 245–250.
33. Kwon, H.C., Kauffman, C.A., Jensen, P.R. and Fenical, W. (2006): J.Ame. Chem. Soci. 128, 1622–32.
34. Lee, I.K., Seok, S.J., Kim, W.K. and Yun, B.S. (2006):J. Nat. Product. 69, 299–301.
35. Li, F., Maskey, R. P. and Qin, S. (2005): J. Nat. Product. 68(3), 349–353.
36. Li, H.X., Jiang, J.Y., Liu, Z.M., Lin, S.E., Xia, G.P., Xia, X.K., Ding, B., He, L. and Lu, Y.J. (2014):J. Nat. Product. 77, 800–806.
37. Li, N., Zhao, L., Ng, T.B., Wong, J.H., Yan, Y., Shi, Z. and Liu, F. (2015):Appl. Microbiol. Biotechnol. 99, 7569–7577.

38. Li, N., Zhao, L., Ng, T.B., Wong, J.H., Yan, Y., Shi, Z. and Liu, F. (2018):Appl. Microbiol. Biotechnol. 99, 7569–7577.
39. Li, Y.X., Li, Z.R., Yamanaka, K., Xu, Y., Zhang, W.P. and Vlamakis, H.(2015): Sci. Report. 5:9383.
40. Luesch, H., Yoshida, W. Y., Moore, R. E. and Paul, V. J. (2002c): *Bioorganic* and *Medicinal Chemist*. 10, 1973–1978.
41. Manganyi, M.C., Tchatchouang, C.D.K., Regnier, T., Bezuidenhout, C.C. and Ateba, C.N. (2019): Mycobiology. 47, 335–339.
42. Manivasagan, P., Kang, K.-H., Sivakumar, K., Li-Chan, E. C., Oh, H.-M., and Kim, S.-K. (2014): Environ. Toxicol.Pharmacol. 38, 172–188.
43. Mao, Z., Wang, W., Su, R., Gu, G., Liu, Z.L., Lai, D. and Zhou, L. (2020): Molecules. 25, 114.
44. Markou, G. and Nerantzis, E. (2013):Biotech. Adv. 31 (8), 1532–1542.
45. Maskey, R.P.,Kock, I. Helmke, E. and Laatsch, H. (2003):ZeitschriftfürNaturforschung. 58(7): 692–694.
46. Masurekar, P.S., Fountoulakis, J.M. and Hallada, T.C. (2012): Planta Med. 78–IL24.
47. Meixiang, H., Jing, L., Lan, L., Sheng, Y., Jun, W. and Yongcheng, L.J.M.D. (2016): Marine Drugs. 14, 215.
48. Misawa, N. Satomi, Y. and Kondo, K. (1995): J. Bacteriol. 177 (22), 6575 – 6584.
49. Mo, S., Krunic, A., Santarsiero, B.D., Franzblau, S.G. and Orjala, J. (2010): Phytochemistry. 71, 2116-2123.
50. Mostafa, S. S. M. (2012): Plant Science. 12, 275–314.
51. Newman, D.J. and Cragg, G.M. (2010): J. Nat Product. 75(3), 311-35.
52. Norton, C. F. and Jones, G. E. (1969):ArchivfürMikrobiologie. 64 (4), 369–376.
53. Okada, Y., Ishimaru, A., Suzuki, R. and Okuyama T. (2004):J. Nat Product. 67, 103-5.
54. Olano, C., Méndez, C. and José, A. (2009): MarineDrug. 7(2), 210-248.
55. Palavra, A. M. F., Coelho, J. P. and Barroso, J. G. (2011): 60, 21–27.
56. Plaza, M., Herrero,M. Alejandro Cifuentes,A. and Iba´nez, E. (2009): J.Agricul. Food Chemistry. 57(16), 7159–7170.
57. Plaza, M., Santoyo, S., Jaime, L., García-Blairsy Reina, G., Herrero, M. andSeñoráns, F.J. (2010): J. Pharm Biomed Analytical. 51(2), 450-5.
58. Prioult, G., Pecquet, S. and Fliss, I. (2004):Int. Dairy J. 15, 439–448.
59. Priyadarshani, I. and Rath, B. (2012): J. Algal Biomass Utilization. 3(4), 89–100.
60. Ran, X., Zhang, G., Li, S. and Wang, J. (2017): African Health Science. 566–574.

61. Rana, K.L., Kour, D., Kaur, T., Devi, R., Negi, C., Yadav, A.N., Yadav, N., Singh, K. and Saxena, A.K. (2020): In Microbial Endophytes; Woodhead Publishing: Cambridge, UK. 273–305.
62. Rettori, D. and Durán, N. (1998): World J. Microbiol Biotechnol.14 (5):685–688.
63. Sanjivkumar, M., Rameshbabu, D., Suganya, A.M., Silambarasan, T., Balagurunathan, R. and Immanuel, G. (2016): Biocatal. Agric. Biotechnol.6, 82–90.
64. Schumacher, R.W., Talmage, S.C., Miller, S.A., Sarris, K.E., Davidson, B.S. and Goldberg, A. (2003): J.Nat Product. 66, 1291–1293.
65. Seetharaman, P., Gnanasekar, S., Chandrasekaran, R., Chandrakasan, G., Kadarkarai, M. and Sivaperumal, S. (2017): Annals of Microbiology 67, 321–331.
66. Shah, A., Rather, M.A., Hassan, Q.P., Aga, M.A., Mushtaq, S., Shah, A.M., Hussain, A., Baba, S.A. and Ahmad, Z. (2017): J. Appl.Microbiol. 122, 1168–1176.
67. Singh, N. K. and Dhar, D. W. (2011):Agronomy Sust. Developm.31(4), 605–629.
68. Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A. (2006): J. Biosci. Bioeng. 101, 87- 96.
69. Stankovic, N., Radulovic, V., Petkovic, M., Vuckovic, I., Jadranin, M. andVasiljevic, B. (2012): Appl. Microbiol.Biotechnol. 96, 1217–1231.
70. Stevenson C.S., Capper E.A., Roshak, A.K., Marquez, B., Eichman, C. and Jackson J.R. (2002): J. Pharmacology Exp. Ther. 303(2), 858.
71. Suemitsu, R., Sakurai, Y., Nakachi, K., Miyoshi, I., Kubota, M. and Ohnishi, K. (1989): Agricult. Biol. Chem. 53, 1302–1304.
72. Sun, Q. L., Li, Y., Ni, L. Q., Li, Y. X., Cui, Y. S., and Jiang, S. L. (2020):Carbohydrate Poly. 229, 115487.
73. Suto, T. (1994): Cancer Chemotherapy and Pharmacology. 33, S145-8.
74. Teruya, T., Sasaki, H., Fukazawa, H. and Suenaga, K. (2009): Organic Lett. 11, 5062–5065.
75. Tiwari, V., Shuklaa, S.Y. and Shuklaa, D. (2009): Antiviral Research. 84, 67–75.
a. Toghueo, R.M.K. (2020): Mycology. 11, 1–21.
76. Torres-Mendoza, D., Ortega, H.E. and Cubilla-Rios, L. (2020):J. Fungi. 6, 58.
77. Tripathi, A., Puddick, J., Prinsep, M. R., Rottmann, M., Chan, K. P. and Chen, D. Y. (2011): Phytochemistry. 72, 2369–2375.
78. Usha, R. Anathasevi, P. Venil, C.K. and Palaniswamy, M. (2012): EuropeanJ. Biol. Sci. 4(4),77 - 83.

79. Veluri, R., Oka, I., Wagner-Döbler, I. and Laatsch, H. (2003): J. Nat Prod. 66, 1520–1523.
80. Volk, R.B. (2008):Microbiol Research. 163(2), 161 - 7.
81. Wall, M.E., Wani, M.C., Cook, C.E., Palmer, K.H., McPhail, A.T. and Sim, G.A. (1966): J.Ame. Chem.Soci. 88, 3888-3890.
82. Wani, M.C., Taylor, H.L., Wall, M.E., Coggon, P. and McPhail, A. (1971):J. Ame. Chem.Soci. 93, 2325–2327.
83. Wonglom, P., Ito, S.I. and Sunpapao, A. (2020):Fungal Ecol. 43, 100867.
84. Yang, H.Y., Gao, Y.H., Niu, D.Y., Yang, L.Y., Gao, X.M., Du, G. and Hu, Q.F. (2013): Fitoterapia. 91, 189–193.
85. Yang, X., Yang, Y., Peng, T., Yang, F., Zhou, H., Zhao, L., Xu, L. and Ding, Z. (2013):Nat. Prod. Commu. 8, 1753–1754.
86. Yang, X., Yang, Y., Peng, T., Yang, F., Zhou, H., Zhao, L., Xu, L. and Ding, Z. (2018):Nat. Prod. Commu. 8, 1753–1754.
87. Yang, Z., Wu, K., Xu, Y., Xia, X., Wang, X., Ge, M. and Shao, L. (2020): J. Antibiotics. 73, 194 –199.
88. Zan, L.F., Qin, J., Zhang, Y., Yao, Y.H., Bao, H.Y. and Li, X. (2011):Chemi. Pharma. Bull. 59, 770–772.
89. Zhang, G., Sun, S., Zhu, T., Lin, Z., Gu, J., Li, D. and Gu, Q. (2011): Phytochem. 72, 1436–1442.
90. Zhang, L.B., Gou, L.H. and Zeng, S.V. (2000): Chinese Trad. Herbal Drugs. 11, 805-807.
91. Zhang, S.P., Huang, R., Li, F.F., Wei, H.X., Fang, X.W., Xie, X.S., Lin, D.G., Wu, S.H. and He, J. (2016):Fitoterapia. 112, 85–89.
92. Zhao, K., Penttinen, P., Guan, T., Xiao, J., Chen, Q. and Xu, J. (2011): Curr.Microbiol. 62, 182–190.
93. Zhong-liu,Z.,Bei, J.,Wen-qing,Y., Fuchun-yan, F. andHua-fen, F. (2011): Chinese Herb. Med. 3(3), 165-167.
94. Zhou, H., Yang, Y., Peng, T., Li, W., Zhao, L., Xu, L. and Ding, Z. (2014):Nat. Prod. Res. 28, 265–267.

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MICROSPONGE DELIVERY SYSTEM

S. Muthukumar[1]*, M. Sridevi[2], C. Sankar[1] and K. S. G. Arulkumaran[3]

[1]Department of Pharmaceutics,

KMCH College of Pharmacy, Coimbatore, Tamil Nadu, India

[2]Department of Pharmaceutics,

SNS College of Pharmacy and Health Sciences, Coimbatore, Tamil Nadu, India

[3]K. T. N. College of Pharmacy, Palakkad, Kerala, India

***Corresponding author E-mail:** pharmmuthu@gmail.com

Abstract:

Microsponge technology is recent novel technique generally used for transfer of active drug into the skin. It is a control release and target specific drug delivery system. It entraps various types of drugs and formulations such as cream, powder, gels and lotions. When applied to the skin; in response to stimulants like rubbing, temperature, pH, etc microsponge releases active ingredient on a time mode. Microsponge technology is used to reduce side effects, improved stability, increased elegance, and enhanced formulation flexibility. Also it is non-irritating, non-mutagenic, non-allergenic, and non-toxic. This review article focuses on microsponge drug delivery along with its method of preparation, characterization, advantages and MDS mechanism of release. Microsponges are characterized by particle size, % drug content and % yield, true density, resiliency, dissolution studies and compatibility studies. It is also preferred to develop a drug with enhanced safety and efficacy wide range of applications.

Keywords: Microsponge, Topical drug delivery, Oral drug delivery, Liquid-liquid Suspension Polymerization and Quasi-emulsion Solvent Diffusion.

Introduction:

A microsponge delivery system (MDS) is, porous, polymeric and highly cross-linked, consisting of porous microsphere which helps to ensnare wide range of API and release them in response to trigger, over a time [1]. This system was suggested earlier for the enhancement of performance of drugs. It is a distinctive technique to release the drug in a controlled manner, which consists of microporous beads loaded with active agents [2]. Moreover, it enhances the stability, reduces side effect and modify drug release favorably. The size of these microsponges

can be mostly varied, usually ranges from 5 to 300 μm in diameter which depends on degree of smoothness [3].

MDS achieves a very high embedding capacity (50–60%), and as their pore size is very small (˜0.25 μm), so the bacteria cannot penetrate inside and they do not need any preservatives to obtain stability. It is usually administered in gel form, and the aforementioned physical and physicochemical tests have to be accomplished with rheological studies (viscoelasticity). The disadvantage is that only organic solvent technologies proved to be effective in case of production [4].

In response to rubbing, Ph, temperature (stimuli), API is released by MDS When applied to the epidermis of skin, in a time mode and used in prescription products, over-the-counter (OTC) skin care, cosmetics and sunscreens.[5].

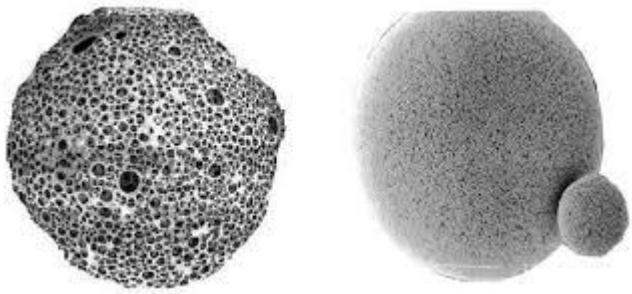

Figure 1: Microsponge

Initial discoveries [6]:

Microsponges were used as oral drug delivery system at the beginning. In 1989, Kawashima developed the ibuprofen microsponges by using quasi-emulsion solvent diffusion method, and from that this type of method was commonly used for preparation of microsponge. And later, the benefits of MDS were found and disclosed by researchers gradually. In 1991, the benzyl peroxide microsponges was designed for Transdermal drug delivery system. And later, for acne and actinic keratoses, Carnac microsponges (0.5% 5-flurouracil) and the Retin-A microsponges (0.1% or 0.04% tretinoin) were approved by Food and Drug Administration (FDA). Afterwards, microsponges for skin topical-target drug delivery are widely developed and employed in topical drug delivery system, such as hydroxyzine hydrochloride microsponge, paeonol microsponge, mupirocin microsponge , and so on.

Advantages [7-10]:

- The surface area is high.
- With its advanced oil control, fascinate 6 times its weight water deprived of drying.
- It may improve grace and aesthetics, efficacy of product.
- It permits loading of immiscible drugs.

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- The novel products can be formulated due to its flexibility.
- It provides ER dosage and countenances novel creation form which can decrease irritation, improved tolerance, hence extensive patient's acceptance
- The production methodology is simple.
    - ✓ The release is programmable.
    - ✓ Pressure triggered system.
    - ✓ Temperature triggered system.
    - ✓ pH triggered system.
    - ✓ Solubility triggered system
- It also increases thermal, physical and chemical stability.

Characteristics of Microsponges [11]:

- Microsponge formulation's average pore size is 0.25 μm through which bacteria cannot penetrate and so it is self sterilizing.
- It is stable over range of pH 1 to 11.
- It is firm at temperature up to 130°C.
- It is compatible with most of the ingredients and vehicles.
- Eventhough it has higher payload (50 to 60%), it is still free flowing.
- It can be cost effective in the cosmetic mass market use where the costs of the materials are very important.

The Microsponge as Programmable Topical Delivery:

Topical drugs of conventional formulations are intended to progress skin's outer layers. Upon application such products release API, producing a highly concentrated layer of API which is rapidly absorbed. The common result is over-medication, followed by a period of under medication until subsequent application.

The release of drug is controlled from the formulation into the epidermis such that the drug remains primarily localized with only a restricted amount entering the systemic circulation, is a means of controlling side-effects [12]. To allow a prolonged rate of release of the active ingredients, microsponge technology is designed, thereby it offers potential reduction in the side effects while maintaining the therapeutic efficacy. It is comprised of polymeric bead having network of pores with an API held within was developed to provide controlled release of the active ingredient whose final target is skin itself.

The system employed the performance improvement of topically applied drugs [13]. The system prevents enormous accumulation of ingredients within the dermis and epidermis. The microsponge systems are polymer-based, microscopic, which will hold together, suspend or capture a wide range of substances and then be incorporated into a formulated product, such as powder, gel, cream or liquid. A single is microsponge is as tiny as a talcum powder particle, measuring less than one-thousandth of an inch in diameter.

The Microsponge for Oral Delivery [12]:

Mostly microsponges are used for topical and recently used for oral administration. In oral applications, rate of solubilization of poorly water-soluble drugs is increased by entrapping such drugs in the microsponge system's pores. As the pores are very small, reduced to microscopic particles and the increased surface area, greatly increases the rate of solubilization.

An extra benefit is that the time takes for the microsponge system to traverse the small and large intestine is significantly increased which maximizes the amount of drug absorption.

Advantages over Ointments [14]:

Ointments are often aesthetically unappealing due to disadvantages such as stickiness, greasiness etc, which often results in lack of patient compliance. For effective therapy vehicles require high concentrations of API because of their low efficiency of delivery system, results into irritation and allergic reactions in significant users. Other drawbacks include unpleasant odor, uncontrolled evaporation of API and potential incompatibility of drugs with the vehicles. The microsponge system increases the amount of time that an API is present either within the epidermis or on skin surface, while minimizing its transdermal penetration into the body.

Table 1: Comparisons between Conventional DDS and MDS [10, 15]

Sr. No.	Parmeters	Conventional Drug Delivery System	Microsponge Delivery System
1	Site	Outer layer of skin	Avoid unnecessary accumulation in epidermis and dermis
2	Absorption	Produce highly concentrated layer and absorb quickly	Drug release is modified
3	Irritation	Irritation is caused	Irritation is reduced

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Table 2: Comparisons between Microcapsule and Microsponge [16]:

Sr. No.	Parameters	Microcapsule	Microsponge
1	Concepts	It is a capsule	It is a sponge
2	Shell	Shell is complete	Shell is Porous
3	Release mechanisms	Cell is ruptured or bursted	Pressure, Partition Coefficient, Temperature
4	Amount released	100% is released	It is Programmable

Table 3: Comparisons between Liposomes and Microsponge [13, 17]:

Sr. No.	Parameters	Liposomes	Microsponges
1	Concepts	Lipid bilayer	Sponge
2	Stability	Potential problems	Stable even change in pH and Temperature.
3	Cost	Expensive	Cost effective
4	Quality control	Require ultra pure raw material	Simply obtainable ingredients of satisfactory pureness
5	Microbiological Stability	Preservation Needed	Preservation not needed
6	Programmable Properties	Chemical Composition and P.S.	Chemical Composition, Small P.S., resiliency in pore size and width
7	Entrapment efficiency	30%	50-60%

Preparation of Microsponges [18]:

Liquid-liquid Suspension Polymerization:

Conveniently, microsponges are prepared using liquid-liquid suspension polymerization method.First solution is produced which consists of monomers or API. As API is not miscible with water, the solution is suspended in aqueous phase with agitation. Usually it contains additives, like dispersants and surfactants. By activating the monomers either by increased temperature, catalysis or irradiation polymerization is initiated when the suspension is established. A spherical structure is produced when the polymerization process continues. The

solid particles are recovered from the suspension when the polymerization is completed. The particles are washed and processed, to use.

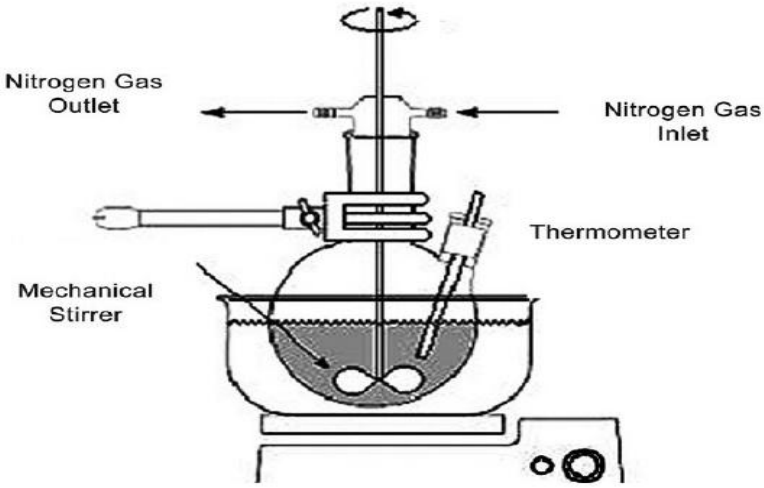

Figure 2: Preparation of Microsponges by Liquid-liquid Suspension Polymerization

Quasi-emulsion Solvent Diffusion [19]:

Quasi-emulsion solvent diffusion method is also used to prepared microsponges by a (two-step process) containing an internal phase of polymers, such as Eudragit, which is dissolved in ethyl alcohol. Next, the drug is added slowly into the polymer solution and dissolved using ultra sonication at 350C. Tri ethyl citrate (TEC) which is a plasticizer was added in order to aid the plasticity. Then internal phase is added into an external phase containing PVP (polyvinyl alcohol) and distilled water and stirred continuously for 2 hours. Inorder to separate the microsponges,the solution is filtered. The microsponges (product) was washed well and dried for 12 hrs in an air- heated oven at 40°C.

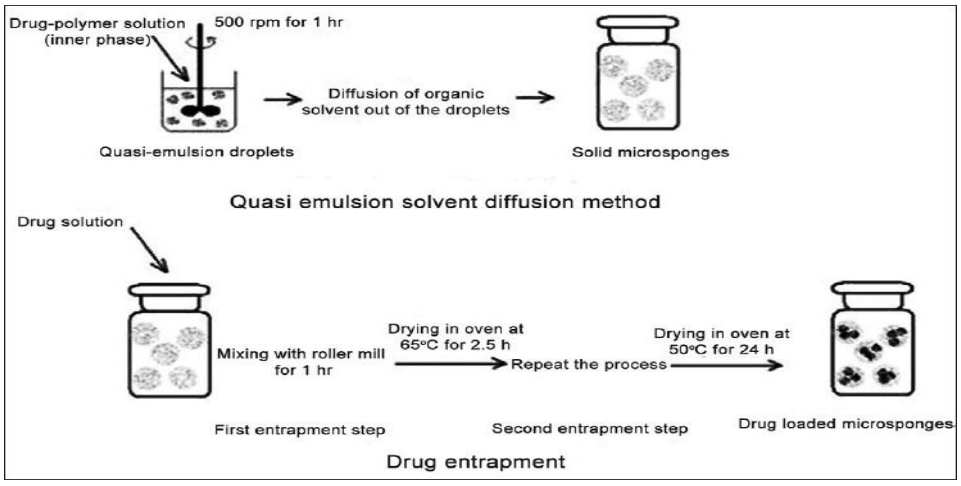

Figure 3: Preparation of Microsponges by Quasi-emulsion Solvent Diffusion

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Characterization of microsponges:

Particle size analysis of microsponges [20]:

By using particle size analyzer, particle size of prepared microsponges was studied. In double distilled water microsponges were dispersed. Light scattering signal should be within the sensitivity range before running the sample in the instrument. The angle of detection is 90°. It is carried out at room temperature.

Morphology and Surface topography of microsponges [21]:

Using scanning electron microscopy (SEM) the surface morphology of the microsponges were studied. Under an argon atmosphere at room temperature the microsponges are coated with gold–palladium and to illustrate its ultrastructure SEM of a fractured microsponge particle is taken.

Determination of production yield and loading efficiency [22]:

The microsponges production yield are determined by calculating accurately the initial weight of the raw materials and the last weight of the obtained microsponge.

$$\text{Production yield (PY)} = \frac{\text{Practical yield of microsponges}}{\text{Theoretical yield of microsponges}} \text{X } 100$$

The microsponges loading efficiency (%) are calculated using the following equation:

$$\text{Loading Efficiency} = \frac{\text{Actual drug contant in microsponge}}{\text{Theoretical drug content}} \text{ X } 100$$

Determination of true density [23]:

It is measured under helium gas using an ultra-pycnometer and it is calculated from a mean of repeated determinations.

Characterization of Pore Structure [22, 24]:

In controlling the duration of effectiveness and API intensity, pore volume and diameter are vital.The microsponges pore diameter also affects the migration of API into the vehicle in which the material is dispersed. To study the effect of pore diameter and volume with rate of drug release from microsponges, mercury intrusion porosimetry can be employed. By using mercury intrusion porosimetry, porosity parameters like intrusion–extrusion isotherms, total pore surface area, pore size distribution, interstitial void volume, average pore diameters,percent porosity filled, percent porosity , shape and morphology of the pores, bulk density and apparent density are determined.

Resiliency [22]:

It (viscoelastic properties) is modified to produce beadlets which is softer or firmer according to the needs of the final formulation. When crosslinking is increased the release rate is slow down hence microsponges resiliency is studied and optimized.

Dissolution Studies [25]:

Microsponges dissolution profile studied using dissolution apparatus (USP XXIII) 5µm stainless steel mesh with a modified basket consisted. Rotation speed is 150 rpm. To ensure sink conditions while considering solubility of actives the dissolution medium is selected. At various intervals samples withdrawn from the dissolution medium is analyzed by using suitable analytical method.

Compatibility studies [26]:

By Fourier transform infra-red spectroscopy (FT-IR) and thin layer chromatography (TLC) compatibility of drug with reaction adjuncts are studied. Using Differential Scanning Colorimetry (DSC) and powder X-ray diffraction (XRD) the effect of polymerization on crystallinity of the drug are studied.

Applications of microsponges as drug delivery system:

Microsponges are applicable mostly for topical application and for oral administration recently as well as biopharmaceutical delivery. The following are some uses, that are either proven or under research.

Burn wound therapy [27]:

Using water in oil in water Quasi-emulsion solvent diffusion method silver sulfadiazine-loaded microsponges were developed. The prepared loaded microsponges were included in the gel base, enhanced the efficacy of the drug without altering the antimicrobial properties by reducing the cytotoxicity towards the keratinocytes and fibroblasts. Microsponges have an ability to minimize cytotoxicity towards host cells and improve the delivery of silver sulfadiazine to burn wounds.

Anti-fungal [28]:

Using the Quasi-emulsion solvent diffusion method eberconazole nitrate-loaded microsponges were developed. The obtained microsponges were dispersed in a gel, and demonstrated an in-vivo skin deposition study and showed that the loaded microsponges had four fold higher retention in the stratum corneum layer compared with the commercial cream.

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Anti-acne [29]:

Microsponges loaded retinoic acid were formulated and tested for anti-acne efficacy and drug release. Notable, higher reductions in non-inflammatory and inflammatory lesions were acquired with tretinoin captured in the microsponge.

Colon-specific drug targeting for treating rheumatoid arthritis [30]:

Using a commercial Microsponge® 5640 system the controlled delivery of flurbiprofen was conducted.The in-vitro studies in the proximal colon of compression-coated colon-specific tablet formulations showed that drug is released for 8h. This is due to the additional enzyme, which follows a modified release pattern.

Anti-glaucoma [31]:

Using Quasi-emulsion solvent diffusion technique stable acetazolamide microsponges were successfully prepared. Ex-vivo studies exhibited that acetazolamide microsponges in-situ gel formulation could be successfully used in the glaucoma treatment, topical ocular administration, and avoiding systemic side effects compared to the oral acetazolamide orally.

Anti-cancer [32]:

To treat skin cancer with increased skin deposition and decreased skin irritation topical gel based 5-Fluorouracil (5-FU) microsponges were formulated. Brunauer-Emmett-Teller analysis signified higher surface area and pore volume of formulated microsponges formulation. Compared to the commercial cream formulation,the optimized formulation revealed greater thixotropy and texture and is used as the control for comparison purposes. Further, Compared to the marketed formulation the optimized formulation demonstrated a 5.5-fold increase in skin deposition reported via in-vivo local bioavailability study, with a significant reduction in skin irritation. Hence, compared to the marketed formulation the developed microsponges based formulation seems to be a viable substitute with increased topical delivery of 5-FU.

References:

1. Chowdary KPR and Rao YS (2004): Mucoadhesive Microspheres for Controlled Drug Delivery, Biol. Pharm. Bull, 27. (11): 1717 - 1724.
2. Riyaz Ali M. Osmani *et al.* (2015): Microsponge based drug delivery system for augmented gastroparesis therapy: Formulation development and evaluation, Asian journal of pharmaceutical sciences, 10. 442 - 451.
3. Nokhodchi *et al.* (2007): Factors affecting the morphology of benzoyl peroxide microsponges, Micron, 38. 834 - 840.

4. Istvan Antal *et al.* (2019): Microparticles, Microspheres, and Microcapsules for Advanced Drug Delivery,Sci. Pharm, 87(3):20. 1-31.
5. Junhui Hong *et al.* (2015): Toward application of microsponges in transdermal drug delivery system, MOJ Bioequiv Availab,1(1):7-12.
6. Manisha K. Tile and Pawar A.Y. (2015): Microsponges: A Novel Strategy for Drug Delivery, International journal of pure & applied bioscience, 3 (1): 224 - 235.
7. Patravale V.B. and Mandawgade S.D. (2008): Novel Cosmetic Delivery Systems: an Application Update, Int J Cosmetic Sci, 30: 19 - 33.
8. Parikh B. N. *et al.* (2010): Microsponge as Novel Topical Drug Delivery System, J Global Pharm Tech, 2 (1): 17 - 29.
9. Parthiban K. *et al.* (2011): Microsponge role in novel drug delivery system, Int. J. of Pharmaceutical Res. and Development, 3(4): 117-125.
10. Panwar A. *et al.* (2011): Microsponge a novel carrier for cosmetics, J. Global Pharma. Tech, 3(7): 15 - 24.
11. Namrata Jadhav *et al.* (2013): Microsponge Delivery System: An updated review, current status and future prospects, Journal of Scientific and Innovative Research, 2 (6): 1097 - 1110.
12. D'souza J. I. and More H. N. (2018): The Microsponge Drug Delivery System: for Delivering an Active Ingredient by Controlled Time Release, Pharmainfo net, 6 (3): 62.
13. Embil K. and Nacht S. (1996): The microsponge delivery system (MDS): A Topical Delivery System with Reduced Irritancy Incorporating Multiple Triggering Mechanisms for the Release of Actives, J Microencapsule,13. 575 - 588.
14. Pradhan S. K. (2011): Microsponges as the Versatile Tool for Drug Delivery System, Int J Res Pharm Chem, 1(2): 243 - 258.
15. Vyas S. and Khar R. (2002): Targeted and controlled drug delivery- Novel carrier system. CBS Publication,1,453.
16. Delattre L. and Delneuville I. (1995), Biopharmaceutical aspects of the formulation of dermatological vehicles, J. Eur. Acad. Dermatol. Venereol, 5:70 - 71.
17. Viral S. (2010), Microsponge drug delivery system: A review, Int. J. Res. Pharm. Sci., 1, 212 - 218.
18. Samyuktha P S. *et al.* (2020): Microsponges- A Novel Drug Delivery System- A Review, European Journal of Molecular & Clinical Medicine,7 (1): 2429 – 2437.
19. Naga Jyothi. *et al.* (2019): Microsponges: A Promising Novel Drug Delivery System Journal of Drug Delivery and Therapeutics, Journal of Drug Delivery & Therapeutics, 9 (5-s): 188 – 194.

20. Afrasim Moin. *et al.* (2016): Fabrication, characterization, and evaluation of microsponge delivery system for facilitated fungal therapy, Journal of Basic and Clinical Pharmacy, 7 (2): 39 - 48.
21. D'Emanuele A and Dinarvand R (1995): Preparation, characterisation and drug release from thermo responsive microspheres, International Journal of Pharmaceutics, 118 (2): 237 - 242.
22. Kilicarslan M and Baykara T (2003): The effect of the drug/polymer ratio on the properties of Verapamil HCl loaded microspheres, International journal of pharmaceutics, 252: 99 - 109.
23. Lendave A. S. (2020): Microsponge Technology for Innovative Drug Delivery System, International Journal of Scientific Research in Science and Technology, 7 (5): 63 - 75.
24. Barkai A. *et al.* (1990): Polyacrylate (Eudragit retard) microspheres for oral controlled release of nifedipine. I. Formulation design and process optimization, Drug Dev. Ind. Pharm, 16: 2057- 2075.
25. Wester R. *et al.* (1991): Controlled release of benzoyl peroxide from a porous microsphere polymeric system can reduce topical irritancy, J. Am. Acad. Derm, 24: 720 - 726.
26. Jones D.S. and Pearce K.J. (1995): Investigation of the effects of some process variables on, microencapsulation of propranolol HCl by solvent evaporation method. Int. J. Pharm, 118: 99 - 205.
27. Kumar P. M. and Ghosh A. (2017): Development and evaluation of silver sulfadiazine loaded microsponge based gel for partial thickness (second degree) burn wounds. Eur J Pharm Sci, 96: 243 - 254.
28. Bothiraja C. *et al.* (2014): Investigation of ethyl cellulose microsponge gel for topical delivery of eberconazole nitrate for fungal therapy, Ther Deliv, 5: 781 - 794.
29. Shah C. N. and Shah D. P. (2014): Microsponges: A revolutionary path breaking modified drug delivery of topical drugs. Int J Pharm Res, 6, 1-13.
30. Orlu M. *et al.* (2006): Design and evaluation of colon specific drug delivery system containing flurbiprofen microsponges, Int J Pharm, 318: 103 - 117.
31. Obiedallah M. M. *et al.* (2018): Ocular administration of acetazolamide microsponges in situ gel formulations, Saudi Pharm J, 26: 909 - 920.
32. Jain S. K. *et al.* (2019): Microsponges enriched gel for enhanced topical delivery of 5-fluorouracil, J Microencapsule, 1-34.

HERBAL IMMUNOBOOSTERS

Hemlata Bhosale* and Apurva Hattekar

School of Life Sciences,

Swami Ramanand Teerth Marathwada University,

Nanded, M.S., India

*Corrsponding author E-mail: bhoslehemlata@gmail.com

Abstract:

Herbal immunoboosters involves the medicinal plants that can help our immune system to fight against any infection or disease. Medicinal plants are those plants of which seed, roots, leaves, bark, stem, rhizome & other parts are used to treat several diseases. India has the great biodiversity and varieties of medicinal plant are abundant in the Indian forests. In recent times researchers have been focused on use of these medicinal plants to treat the variety of diseases like cancer, diabetes, arthritis, neurodegenerative diseases etc. By using herbal medicines we can minimize side effects caused due to the use of antibiotics. The medicinal plants like turmeric (*Curcuma longa),* tulsi (*Ocimum sanctum),* ashwagandha (*Withania somnifera),* neem (*Azadirachta indica),* giloy(*Tinospora cordifolia)* etc. are used since ancient times to treat several diseases and enhance general health and wellbeing. These medicinal plants have anti-oxidant, anti-inflammatory, anti-microbial,anti-cancer, anti-diabetic activity, etc. due to this medicinal plants having great importance in Ayurveda.

Keywords: Medicinal plants, Biodiversity, Side effects, Anti-oxidant, Anti-inflammatory, Anti-microbial, Anti-cancer, Anti-diabetic, Ayurveda.

Introduction:

The immune system evolved to protect multicellular organisms from pathogens. Highly adaptable, it defends the body against invaders as diverse as virus. The immune system generates an enormous variety of cells and molecules capable of specifically recognized and eliminate foreign invaders, all of which act together in dynamic network.

Protection by the immune system can be divided into two related activities – recognition and response. Immune recognition is remarkable for its capacity to distinguish foreign invaders from self. Recognition of a pathogen by the immune system triggers an effector response that eliminates or neutralizes the invader. The multiple components of the immune system are able to converts the initial recognition event into a variety of effector responses, each uniquely suited for

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eliminating a particular type of pathogen. Certain exposures induce a memory response characterized by a more rapid and heightened immune reaction upon lateral attack.

Immunity is typically divided into two categories – innate and adaptive immunity.

Innate (natural/native) immunity is present since birth and consists of many factors that are relatively nonspecific. It operates against any foreign molecules and pathogens. It provides the first line of defence against pathogens. It is not specific to any one pathogen but rather acts against all foreign molecules and pathogens. It also does not rely on previous exposure to a pathogen and response is functional since birth and has no memory.

Adaptive immunity is also known as specific or acquired immunity is capable of recognizing and selectively eliminating specific foreign antigen. It does not come in play until there is an antigenic challenge to the organism. Adaptive immunity displays four characteristics: antigenic specificity, immunological memory, diversity, self and nonself recognition.

In recent times the utilization of medicinal plants for their immune boosting potential has been preferred over pharmaceutical drugs due to their low toxicity and costs. The medicinal plants is used to treat several diseases and as a immunobooster since ancient times. Current research findings have also shown that their toxicity level and side effects are low as compared to their synthetical pharmaceutical counterparts. In the present study the pharmacological applications of commonly used medicinal plants such as turmeric, neem, tulsi and ashwagandgha in ayurvedic formulations are discussed

***Curcuma longa* and curcumin:**

Turmeric is commonly used as spice in curries, food additives and dietary pigment. It has been used to treat various illnesses from ancient times. Turmeric has been used as nontoxic drug in Ayurveda from centuries to treat a wide variety of disorders including body ache, skin diseases, rheumatism, diarrhea, intestinal worms, intermittent fever, urinary discharges, hepatic disorders, inflammations, constipation [1]. It is used to relieve pain and inflammation. It is household remedy to cure injuries and swellings. Turmeric is household remedy for cough, sore throat and common cold, where it is taken orally with hot milk or tea.

Taxonomic position of turmeric:

Kingdom : Plantae

Division : Magnoliophyta

Class : Liliopsida

Subclass : Zingiberidae

Order : Zingiberales

Family : Zingiberaceae (ginger family)

Genus : *Curcuma*

Species : *longa*

Chemical composition:

The major chemical principles of turmeric are curcuminoids, which imparts characteristic yellow color to it. The curcuminoids can be separated from turmeric by ethanol extraction and it usually contains **0.3 – 5.4%** curcumin depending of the season of its harvest [2].

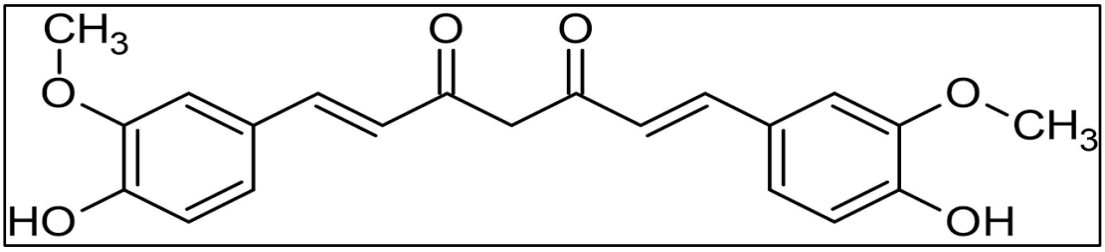

Figure 1: Chemical structure of curcumin (diferuloylmethane)

Curcumin is an orange – yellow crystalline powder that does not dissolve in water. Human consumption of curcumin ranges upto 100mg/day. Clinical trials indicate that human can tolerate a dose of curcumin as high as 12g/day without any toxic side effects. Curcumin possess several pharmacological properties including antimicrobial, antiviral, antifungal, antioxidant, anti-inflammatory, radiosensitizing, antimutagenic, and wound healing activities [3]. Curcumin can suppress tumor initiation, promotion and metastasis in experimental models. It can also act as an antiproliferative agent by interrupting the cell cycle, disrupting mitotic spindle structures and induce apoptosis and micronucleation.

Mechanism of action:

Curcumin is highly pleiotropic molecule with numerous targets and mechanism of action including altering the activity of enzymes, cofactor, growth factor receptors and other molecules. The wide range of action of curcumin can be demonstrated by its activity in inhibiting lipoxygenase by binding lipoxygenase itself or binding to phosphatidylcholine micelles. Curcumin also inhibits tumor invasion and angiogenesis by irreversible binding CD_{13}/aminopeptidase. It affects tumor growth by disrupting the activity of several enzymes that allow for growth and proliferation [3]. Curcumin also have role in regulation of various immune cells including B lymphocytes, T lymphocytes, natural killer cells, dendritic cells, macrophages and other immune cells. Curcumin also has certain effect on cytokines, interleukin.

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Clinical applications of Curcumin:

More recently there has been a significant increase in the number of clinical trials testing the efficacy of curcumin in treating a variety of diseases.

Rheumatoid arthritis:

One of the most important properties of curcumin is its ability as an anti-inflammatory agent. Rheumatoid arthritis is associated with an ongoing inflammatory process. Treating this disease with curcumin has been recently studied. The safety and effectiveness of curcumin make it attractive option to treat rheumatic diseases [5].

Organ transplantation:

Curcumin can be used to modulate the immune response after organ transplantation, as one trial demonstrated curcumin's ability to improve early graft function in post-renal transplant. Studies have demonstrated that curcumin has ability to upregulate the antioxidant hemoxygenase-1 which improves outcomes in kidney graft function.

Diabetes:

Curcumin can suppress blood glucose level, increase the anti oxidant status of pancreatic beta cells and enhance activation of peroxisome proliferator activated receptor (PPAR).

Asthma:

The curcumin can relieve symptoms of asthma has been reported. These effects are linked with reduction of the lymphocytic production of IL-2,IL-5, GM-CSF and IL-4 that is associated with bronchial asthma.

Alzheimer's disease:

Alzheimer is neurodegenerative disorder. Several report suggested that curcumin has potential against this disorder [2].The effect of curcumin in Alzheimer's diseases is mediated through the down modulation of cytokine (i.e. IL-1 beta, TNF-alpha) and chemokine (i.e. MIP-1b, MCP-1 and IL-8) activity [7].

Acquired immunodeficiency syndrome (AIDS):

There are several reports that the curcumin may have potential against AIDS. By inhibiting HIV long terminal repeat and HIV protease curcumin suppress replication of HIV.

Curcumin and cancer treatment:

Now a days there are clinical trials using curcumin to treat pancreatic, hepatocelluclar, gastric, breast, prostrate, skin, lung and colon cancers. Recent clinical trials also demonstrated that a curcumin dose of **8gm/day** when taken with gemcitabine is safe and well tolerated as a supplement. Docetaxel and curcumin used to treat ovarian cancer and breast cancer.

Oxaliplatin and curcumin is used to treat colon cancer. Curcumin can be well tolerated in addition to the chemotherapy [6]. Curcumin has also been shown to decrease risk factors for lung cancer. Curcumin was also well tolerated in doses upto **12gm/day** in patients who were being treated for multiple myeloma.

Side effects (adverse effects) of curcumin at high doses:

1. Gastrointestinal disturbances.
2. Iron chelation.
3. Transient rises in liver enzymes.
4. Inhibition of sperm mobility in-vitro.
5. Suppression of platelet aggregation.
6. Contact dermatitis [3].

***Ocimum sanctum* Queen of herbs in India:**

Cultivation of tulsi plant has both spiritual and practical significance. Tulsi is predominant herb in Ayurveda hence it is also called as "mother of medicine in nature"

Morphology and distribution of tulsi:

The height of tulsi plant is approximately 1 meter but not more than 4-5 feet. Tulsi is a traditional plant having several branches. The leaves of tulsi are green or purple in color. The leaves of tulsi have characteristic smell. Flowers are white-purple in color. The seeds of tulsi can germinate easily in moist place globally. There are 5 species of tulsi isolated by Linneaus.(*Ocimum tenuiflorum*- subtype rama, *Ocimum tenuiflorum*- subtype Krishna, *Ocimum geratissimum*, *Ocimum sacharicum***,** *Ocimum kilmand*) [9]. In Ayurveda tulsi is preeminent and through scientific research it has been proved that tulsi having beneficial effects.

Tulsi protect our organs and tissues from chemical stress from industrial pollutants and heavy metals, prolonged physical exertion. Tulsi also have antidepressant properties, also have ability to normalize the blood glucose level, blood pressure, lipid levels and it also suppress the psychological, against human and animal pathogens. Hence it is used in several preparations like hand sanitizers, mouth wash, toothpasts, soaps and in water purifiers. Tulsi have antimicrobial, anti-inflammatory, cardiaoprotective, adaptogenic, radiaoprotective, anti-allergic, antioxidant, anti-asthmatic properties [9].

It is used to treat several diseases in humans such as fever, mental stress, over body weight (obesity), asthma, bronchitis, headache and some viral diseases (hepatitis). Amongst the physiological benefits of tulsi one most important benefit is it helps in detoxification i.e. having ability to assist with internal housekeeping of body and protect from toxin induced damage.

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According to the experimental studies it is proved that tulsi have ability to protect damaging effect of various toxic compounds. Tulsi have ability to protect liver, brain, kidney by protecting genetic and cellular damage caused by pesticides, insecticides, industrial chemicals. In addition tulsi has been shown to protect against heavy metals and their toxic effects. Heavy metal includes cadmium, chromium, arsenic, lead and mercury [10].

Taxonomic position of tulsi:

Kingdom: Plantae

Division: Mangnolipophyta

Class: Magnolipopsida

Order: Lameales

Genus: *Ocimum*

Species: *sanctum*

Medicinal properties:

Tulsi has antioxidant properties and reduce blood glucose level. Thus it is useful for diabetics. It also reduces blood pressure and blood cholesterol level, so beneficial effect in cardiac diseases. It has been used for treating gastric disorders, cough, headache, malaria, common cold. Leaves of tulsi acts as anti stress agents and studies proved that leaves offer significant protection against stress. Leaves of tulsi acts as nerve tonic and also sharpen memory [8]. Tulsi is major constituent of ayurvedic syrup. It helps to mobilize mucus in bronchitis and asthma. Chewing of tulsi leaves relives cold and flu. Tulsi leaves (juice) and honey is best remedy to treat kidney stone. The leaves are effective to treat ulcer and infections of mouth hence tulsi is widely used in mouth sprays, toothpastes etc. Tulsi has ability to heal skin diseases like pimples and tulsi contain ursolic acid that prevent wrinkles and helps to retain elasticity of skin and prevents aging. The herb is prophylactic properties hence use to treat insect bites [11].

The tulsi is one of the major medicinal plants or widely used medicinal plant used in two ways i.e. tulsi extract and drugs. For adults advice to intake 3-5 leaves of tulsi per day(500-600 mg) and childrens above 3 years 2-3 leaves per day[12]. Tulsi contains flavonoids and phenolic compounds, the important one are eugenol, linalool, apigenin and ursolic acid. The presence of linoleic acid makes it a powerful antioxidant and neutralizes free radicals causing cell damage.

Side effects:

Tulsi has sometime harmful effects due to excess dose. Tulsi has some side effects during pregnancy period, because tulsi has estrogen which contract uterus that reduces the development of embryo. According to WHO the regular intake of tulsi can damage hepatic cells due to

presence of eugenol. There is no more research work carried out on regular longterm intake of tulsi. The presence of eugenol substance in tulsi may cause headache, nausea, diarrhea due to excess use of tulsi [12].

Ashwagandha – A rejuvenator of Ayurveda:

Withania somnifera (Ashwagandha) is used since ancient times as a tonic. *W.sominifera* also called as ashwagandha or winter cheery is one of the most valuable plants in traditional Indian system of medicine it is also called as "Indian ginseng"[13].

Aswagandha is reported with anti-inflammatory, antiarthritic, cardioprotective, antistress, hypoglycemic & thyro-protective activity. It is also useful in certain neurodegerative diseases such as Parkinson's disease, Alzeimer's diseases and huntington's disease. It has the variety of health benefits hence it is also called as Rasayan. It has antioxidant, anxiolytic, adaptogenic, memory enhancing, antitumor, anti-parkinsonian properties.

Ashwagandha commonly available in the form of fine sieved powder (churna) which is mixed with the ghee, water, milk or honey and its consumption in the morning gives various health benefits. It also has roll in enhancing function of brain and improves memory.

Ashwagandha improves cell mediated immunity which acts as body defense against diseases. It also has antioxidant properties that help from free radicles which causes cellular damage [14].

Taxonomic position of Ashwagandha:

Kingdom: Plantae,
Division : Angiosperms
Class : Dicotyledoneae
Order : Tubiflorae
Family : Solanaceae
Genus : *Withania*
Species : *somnifera*

Geographical distribution:

It is a small evergreen shrub that grows to roughly 4-5 feet tall. In India, it is cultivated on a commercial scale in the states of Uttar Pradesh, Punjab, Rajasthan, Gujarat and Madhya Pradesh [13].

Chemical composition:

Laboratory analysis has revealed the roots of *Withania somnifera* contains more than 35 chemicals the biologically active chemical constitutes are alkaloids (isopellertirine, anferine),

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steroidal lactones (withanolides, withaferins) and with anoidoloids. *Withania somnifera* is also rich in iron [12].

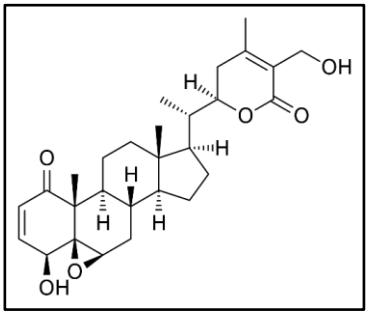

Figure 2: Structure of Withaferin A

Clinical effects of ashwagandha:

Effect on central nervous system:

Ashwagandha is very useful to promote intellectual capacity and memory. It is amazing remedy to treat memory related issues like memory deficits with childrens, in case of head injury or a prolonged illness and in old age.

Effect on neurodegenerative disease:

Ashwagandha has been described as nervine tonic in Ayurveda. It is used to treat neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt - Jakob disease. From studies it is proved that ashwagandha slows, stops, reverse or removes neurotic atrophy and synaptic loss [13].

Anti-inflammatory effect:

Withaferin A and 3-b-hydroxy 2, 3 dihydrowithanolide F isolated form *withania somnifera* shows anti-tumor, antibacterial, immunomodulatory & anti-inflammatory properties.

It is an anti-arthritic agent & found to be useful in clinical cases of osteoarthritis and rheumatoid arthritis. Aswagandha was used to increase energy in ancient period. It helps to treat chronic fatigue, weakness, bone weakness, loose teeth, premature aging and dehydration.

Antiaging:

From clinical trials it has been proved that, dosage of three grams daily for 1 year gives significant improvement in RBC count, haemoglobin and hair melanin and also decrease blood cholesterol level [16].

Cardioprotective activity:

According to clinical studies ashwagandha has diuretic, hypoglycemic, hypocholesterolmic effects. Decrease in serum cholesterol, triglycerides and low density lipoproteins were also seen after consuming powder extract of herb after 30 days [17].

Other therapeutic benefits:

Further studies have also shown ashwangandha to be effective to treat inflammation, stroke, osteoarthritis. It has been shown to be potent antimicrobial agent with antifungal activity and antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [17].

Cancer:

Ashwagandha also used for treatment of breast cancer. The dose of 2 gram for 3 times along with chemotherapy gives best results [18].

Azadirachta indica– the divine tree:

Neem (*Azadirachta indica)* is member of meliaceae family. It is rich source of antioxidants hence it has great importance in Ayurveda and used in prevention and treatment of several diseases. The plant products has important role in disease prevention and treatment by enhancing antioxidant activity, inhibiting bacterial growth and act as modulator in genetic pathway.[19]

Geographical distribution of neem:

Neem tree is found abundantly in tropical and semitropical regions like India, Bangladesh, Nepal & Pakistan. It is fast growing evergreen tree having height 20-30 trunk is straight and diameter ranges from 4-5 feet. The leaves are compound and having 5-15 leaflets. The fruit is green drupes after ripening it appears golden yellow in color.

Taxonomic position of Neem:

Kingdom: Plantae

Division: Magenolipophyta

Class: Magnolipsida

Order: Rutales

Genus: *Azadirachta*

Species: *indica*

Azadirachta indica shows therapeutics role in health management due it is rich source of various ingredients. The one of the most important active constituent is azadiractin and other are nimbin, nimbidin, nimbidol, gedanin, salanin & quercetin. Quercetin and beta- sitosterol, polyphenolic flavonoids were purified from neem leaves having antibacterial and antifungal properties. [19]

Clinical applications of Neem:

Neem has a lot of clinical uses like it has anticancer activity, antidiabetic activity, neuroprotective activity, antiviral, anti-inflammatory, antibacterial, antifungal, anti-malarial,

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antioxidant, hepatoprotective, anti- nephrotoxicity, wound healing effect and immune modulatory effect [20].

Antioxidant activity:

Free radicals and reactive oxygen species cause variety of diseases. Neutralization of these free radicals is important step in disease prevention. Antioxidant stabilize or deactivates these free radicals before itdamage cell and activates the antioxidative enzymes that play role in control in damage caused by reactive oxygen species[20]. Plant fruits, seeds, leaves, bark, root and oil shows an important role in disease prevention due to the rich source of antioxidant.

Anticancer effect:

Cancer is major health problem worldwide. The cancer is progress due to alteration in molecular pathways. The treatment of cancer by using allopathic drug is effective but has certain adverse effects on normal cells. Earlier studies shows that plant and their constituents shows inhibitory effect on growth of malignant cells via modulating apoptosis, cell proliferation, tumor suppressor genes and various molecular pathways. [21] Neem contains flavonoids and various other ingredients that play major role in inhibition of cancer development.

Effect of neem as anti-inflammatory agent:

Neem leaves, bark and seeds having anti-inflammatory activity. Leaf extract showed significant anti-inflammatory effects but it is less efficacious than dexamethasone [22]. Certain study resultsuggest that nimbidin suppresses the functions of macrophags relevant to inflammation.

Wound healing effect:

Various plants and their constituents play an important role in wound healing. Neem leaves promote wound heling activity through increased inflammatory response and neovascularization [23].

Antimicrobial effects:

Neem and its ingredients play role in inhibition of growth of numerous microbessuch as bacteria, viruses and phytopathogenic fungi.

Antibacterial activity:

Leaf extract of neem contains certain compound having antibacterial properties that can potentially be used to control food borne pathogens and spoilage organisms. Bark, leaves, fruit extract, seed of neem having antibacterial activity.

Antifungal activity:

According to experimental studies neem leaf extract is used to inhibit and control seed born fungi like Aspergillus and Rhizopus. Another finding shows that the antimicrobial role of aqueous extracts of neem cake in inhibition of spore germination fungi such as *C. lunata, H.*

pennisetti and C. gloeosvorioides mangifera [24] and recent study revealed that methanol and ethanol extract of A.indica showed growth inhibition against *Aspergillus flavus*, *Alternaria solani* and *Cladosporium* [21].

Various clinical trials based studies show that herbal products and their derivatives play a vital role in disease control and treatment. Neem bark extract have antisecretory and antiulcer effect in human. Clinical study of 6 week was made to check the efficacy of neem extract in dental gels with chlorohexidine gluconate mouth wash has positive control and significantly reduced plaque index and bacterial count [22]

Conclusion:

Herbal medication has received worldwide attention for its multiple health benefits and minimum side effects. Treating a particular disease by using pharmaceutical products like chemotherapy in case of cancer has a lot of side effects. The main moto behind the use of herbal medicines is to minimize these side effects or adverse effects along with that use of herbal medicines enhance general health and wellbeing.

References:

1. Fades M. C., Cecilia Lau, jail Bhikhchandani, H.T.Lynch Curcumin: An age-old anti-inflammatory and anti-neoplastic agents.
2. Jagentia Ganesh Chandra and B.B. Aggrawal " Spice up " of immune system by curcumin . Jan 2007
3. Hewlings S. J.and D.S. Kalman Curcumin:A review of it's effects on human health . Oct 2017
4. Catanzaro M., E. Cousins, M. Rosini, M. Racchi and C. Lanni. Immunomodulators inspired by nature. Oct 2018
5. Rahman A. H., M.A. Alsahli, S.M.Aly, M. A.Khan, Y.H. Aldebasi. Role of curcumin in disease prevention and treatment. Feb 2018.
6. Akrom M., Shahab-uddin, A. Ahemd, K.Usmanghani, A.Hannan, E. Mohiuddin, A.Asif. Curcuma longa and curcumin. Jan 2010
7. Yallapu M.M., P.K.Bhusetty Naresh, M. Jaggi and S.C. Chauhan. Therapeutic application of Curcumin Nanoformulations.
8. Cohen M.M. Tulsi- Ocimum sanctum: A herb for all reasons.2014
9. Bhateja sumit, Arora Geetika. Therapeutic benefits of holy basil(tulsi) in general and oral medicine.

10. Sampath Kumar KP, Debjit Bhowmik, Biswajit, Chiranjib, Pankaj AND KK Tripathi Margret Chandira. Traditional Indian herbal plants tulsi AND it's medicinal importance .2010
11. Maiti Manoj, Effect of tulsi (Ocimum sanctum) in human as a medicinal plant .2020
12. Gupta SK, Prakash J, Shrivastav S. Validation of traditional claim of tulsi as a medicinal plant 2002.
13. Singh Narendra, Mohit bhalla, Prashanti de Jager AND marilena gilca. Ashwagandha: A rasayan(rejuvenator) of Ayurveda. 3 july 2011.
14. Joshi Krutika, Kalpesh Panara, Swagara D Tavhare, Pravin Kumar. Studies of ashwagandha (Withania somnifera Dunal) Jan 2016
15. Zinhiruddin Sultan, Parakh basist , Abida Paraveen, Rabea Parveen, Washim Khan, Gaurav, Sayeed Ahmad. Ashwagandha in brain disorders 2020.
16. Sitansu Kumar Verma AND Ajay Kumar. Therapeutic uses of Withania somnifera with a note on withanolides AND its pharmacological actions 2011
17. Singh G, P.K.Sharma , R Dudhe AND S. Singh. Biological activities of Withania somnifera.
18. Biswal Biswa Mohan, Siti Amrah Sulaiman, Hasanah che lsmail. Effects of W. somnifera on development of chemotherapy-Induced fatigue AND quality of life in breast cancer patients 2011.
19. Jose Francisco islas, Ezeiza Acosta, Buruno Escalanta, Jorge E Morenocuvas . An overview on neem AND it's potential impact on health.2020
20. Mohammad A. Alzohairy. Therapeutics role of Azadirachta indica (neem) AND their active constituents in disease prevention AND treatment.
21. Shrivastava D.K, Swarnkar k. Antifungal activitiy of leaf extract of neem (Azadirachta indica linn)
22. Anjali K, Ritesh K, Sudarshan M, Japan S.C, Kumar S. Antifungal efficacy of aqueous extracts of neem cake, karanj cake AND vermicompost against some phytopathogenic fungi.
23. Osunwoke Emeka A, Olotu Emamoke J, Allison Theodore A, Onyekwere Julius C. The wound healing effects of aqueous leave extract of neem on wistar rat. Journal of natural science AND research.
24. Cohen E Quisted G.B. Casida J.E. Cytotoxicity of nimbolide, epoxyazadione and other limonoids for neem extract.

METHYLENE BLUE AS AN ADJUVANT THERAPY AGAINST COVID-19

Sunil Kumar Patidar[1] and Satish Piplode[2]

[1]Department of Chemistry,

S. N. Govt. P. G. College Khandwa, Dist. Khandwa, M. P. India - 450001

[2]Department of Chemistry,

S. B. S. Govt. P. G. College Pipariya, Dist. Hoshangabad, M. P. India - 461775

Corresponding author E-mail: sunil87patidar@gmail.com, satish.piplode@gmail.com

Abstract:

Methylene blue (MB) is an oldest synthetic substance in medicine and it claims as a promising candidate for an active treatment of SARS-CoV-2 infection. MB is a multipurpose drug and it is being used for the treatment of various diseases such as malaria, methemoglobinemia, urinary tract infection, fungal infection etc. MB is a powerful antidote against CO and CN poisoning.The rationale for considering MB for treatment was due the following properties: (1) anti-viral activity against the SARS-CoV-2 virus (2) anti-hypoxemia activity by changing oxidation state of iron from +3 oxidation state (ferric) to the +2-oxidation state (ferrous) (an approved medicine for methemoglobinemia) (3) inhibitor of nitrite production (nitrite converts ferrous iron to ferric iron in hemoglobin) (4) inhibitor of reactive oxygen species (superoxide anion and hydrogen peroxide scavenger) (5) inhibits caspase activation (6) antifungal agent (7) anti-inflammatory agent. Recent clinical trials against SARS-CoV-2 infection, suggest that, MB is an excellent supplementary therapy againstCOVID-19.

Keywords: Methylene blue, COVID-19, SARS-CoV-2, corona treatment.

Introduction:

On 25th December 2019, the first case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) called coronavirus diseases 2019 (COVID-19), was appears in Wuhan, China (Wu et al 2020). Suddenly the cases of this diseases increase worldwide. More than 150 countries were affected by COVID -19. In March 2020, the World Health Organization (WHO) has been declared COVID-19 is a pandemic (Matheson and Lehner 2020). Various problems has been emerged due to pandemic situation and it exposed the weakness of the health system worldwide. Alpha (α) coronavirus, beta (β) coronavirus, gamma (γ) coronavirus, and delta (δ) are the common classification of the coronavirus. Among them, alpha- and beta-coronaviruses genus generally infects mammals while the type of gamma and delta-coronaviruses infect birds. The

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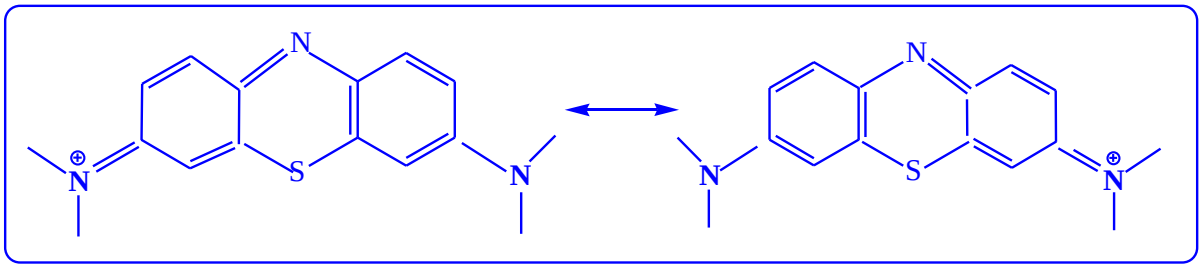
COVID-19 is a novel coronavirus of the β genus and according to electron microscopic observations its diameter is ≈ 60-140 nm in size and it is round or crown-shaped structure (Zhu *et al.*, 2020).

Till date there is no proper medications were available for the treatment of the COVID-19. Some scientists say that the vaccination is only ultimate solution to handle the COVID-19. A little bit knowledge of possible preventives or therapeutic treatments for COVID-19 was discussed by various scientists (Guy *et al.*, 2020, Jean *et al.*, 2020, Kandeel and Al-Nazawi, 2020, Li and De Clercq, 2020, Serafin *et al.*, 2020). The drug development is a costly and very time-consuming process (Lythgoe and Middleton, 2020). Therefore, drugs repurposing seems to play an important role to provide medications in minimum time duration. The number of different drugs, including repurposed antivirals, antibiotics, anti-inflammatory, antioxidants and immunomodulators were tested against COVID-19 (Lythgoe and Middleton, 2020, Ottaviani and Stebbing, 2020).

Methylene blue (MB) is a salt of the formula $[(C_{16}H_{18}N_3S)^+ Cl^-]$ [IUPAC name: 3,7-bis(dimethylamino)- phenothiazin-5-ium chloride]. It was first synthesized as a dye in the end of 19th century, and Guttmann and Paul Erlich reported antimalarial properties of the MB dye (Guttmann and Erlich, 1891). The MB^+ cation consists of three condensed six-membered rings with two heteroatoms in the central one, and two coplanar NMe_2 substituents. The MB^+ exhibits typical intense blue colour in many solvents. The presence of total 18 P_π electron in the resonating structure of MB^+ suggest the aromatic nature of compound (Scheme 1).



Scheme 1: The resonating structures of aromatic MB+

In 20th century lot of work have been done for the therapeutic use of MB dye. Some researcher also successfully applied MB dye for various treatment, including methemoglobinemia, urinary tract infections, vasoplegic syndrome, carbon monoxide (CO) and cyanide (CN) poisoning (Brooks, 1933, Draize, 1933, Mansouri and Lurie, 1993, Gurr, 1962, Evora *et al.*, 1997). It is also used to distinguish between cancerous and normal tissues (Gurr, 1962). Currently methylene blue has been used for the treatment of malaria (Dicko *et al.*, 2018, Mendes *et al.*, 2019). The utility of MB against SARS-CoV-2 has been extensively reviewed by

Andreu (Andreu, 2021). Hamidi Alamdari *et al.,* successfully completed the phase I, phase II and phase III trial to determine the efficiency of MB dye against SARS-CoV-2 infection (Hamidi Alamdari *et al.,* 2020, Hamidi Alamdari *et al.,* 2021a, Hamidi Alamdari *et al.,* 2021b).The results revel that, MB is an excellent supplementary choice for the treatment of hypoxemia in COVID-19 patients.

Methylene blue (MB) is an oldest synthetic substance in medicine and it claims as a promising candidate for an active treatment against SARS-CoV-2 infected peoples and for COVID-19 patients. The safe use of methylene blue in humans for a long period and its multifunctionality prompt us to illustrate the features of MB as an adjuvant therapy against COVID-19. The present chapter deals the key role of MB against COVID-19 and we would like to illustrate the following features of MB (i) antiviral (ii) anti-inflammatory (iii) caspase activation inhibitor (iv) anti-hypoxemia activity (v) antifungal agent.

Anti-viral activity:

MB is a phenothiazine drug, which consist planarity in its tricyclic structure. This structural property of the drug helps to intercalates within nucleic acid strands, which could inhibit the viral replications (Tuite and Kellt, 1993). The presence of positive charge on MB enhances its binding affinity towards negatively charged RNA (Floyd *et al.,* 2004). Lozano and co-workers proved that MB can produce highly reactive singlet oxygen species in the presence of light radiations and it is able to break the nucleic acid strands (Lozano *et al.,* 2013). Therefore, MB shows a broad-spectrum of virucidal property and it can inactivate the viruses in blood before the transfusion.

The methylene blue is an oxidized form and shows blue colour, however the leuco-MB is a reduced form of MB and it is colourless (Fig. 1). The leuco-MB can easily penetrateinto the lysosomal membranes and it can increase the pH upon deprotonation (Wainwright and Amaral, 2005). MB can stop the endosome maturation and it could help to reduce the import of virions into the cytosol. Alamdari and co-workers reported in their clinical trials that the leuco-MB form works effectively in treatment of SARS-CoV-2 infection (Hamidi Alamdari *et al.,* 2021a, Hamidi Alamdari *et al.,* 2021b).

MB has a 11 mV redox potential and in the presence of suitable redox centres in mitochondria it could efficiently cyclize in its oxidized (MB) and reduced (leuco-MB) forms. It produces H_2O_2 upon regeneration from leuco-MB to MB. The increased concentration of H_2O_2 in phagocytes, enhance the formation of HOCl and it could provide the biocidal action against SARS-CoV-2 (Ramalingam *et al.,* 2018).

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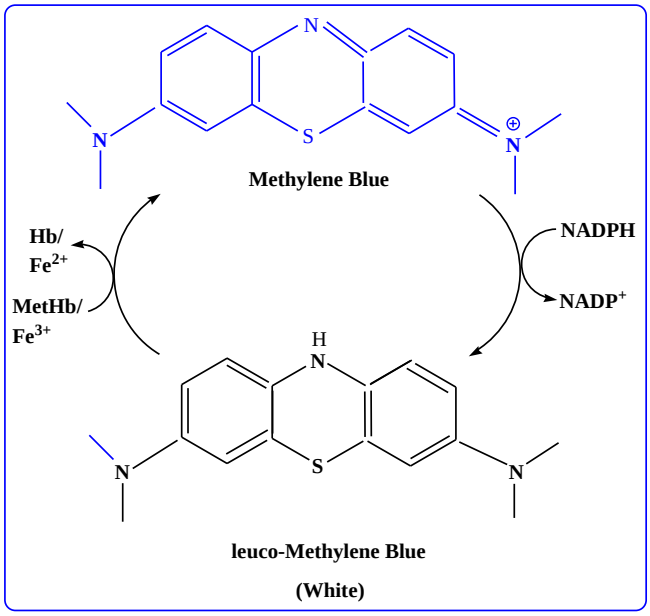

Figure 1: Structure of methylene blue (blue colour/oxidized form) and leuco-methylene blue (white colour/reduced form)

It has been proven that the MB can shows antiviral property without any support of light radiations (Cango *et al.,* 2020, Gendrot *et al.,* 2020, Danir *et al.,* 2021). Cango et al, reported that MB shows antiviral activity even at low micromolar concentrations (Cango *et al.,* 2020). The in vitro studies carried out by Gendrot and co-workers suggest that the non-photoactivated MB could also inhibit SARS-CoV-2 viral replication (Gendrot *et al.,* 2020). Buchwald and co-workers investigate that MB inhibits the binding interaction betweenSARS-CoV-2 spike protein and ACE2 receptor (Bojadzic *et al.,* 2021). Therefore, above mentioned evidences support the prominent role of MB against viral activity and it is the best choice for the treatment of SARS-CoV-2 infection.

Anti-inflammatory:

In severe COVID-19 patients pro-inflammatory cytokine counts such as IL-1β, IL-2, IL-6, IL-8, IL-17, G- CSF, GM- CSF, IP10, MCP1 and CCL3 enhanced rapidly in blood level. This phenomenon is known as cytokine release syndrome or “cytokine storm” (Xu *et al.,* 2020). This cytokine storm causes the immune mediated damage of multiple organs.

The NLRP3 inflammasome plays an important role in regulating inflammation (Wang *et al.,* 2020). During the SARS-CoV-2 infection, NLRP3 inflammasome activated by viroporin 3a and it also promotes the secretion of IL-1β (Chen *et al.,* 2019). Recent studies show that MB

inhibits the assembly of the NLRP3 inflammasome and it could reduce the secretion of IL-1β and caspase-1 (Ahn *et al.*, 2017).

During virus induced pneumonia, nitric oxide (NO) plays a pivotal role (Perrone *et al.*, 2013). In COVID-19 patients, the blood nitrites and nitrates level which may reflects the NO level, have increases significantly (Hamidi Alamdari *et al.*, 2020). It has been reported in various studies, MB inhibits the nitric oxide (NO) action on vasculature (Oz *et al.*, 2011, Lomniczi *et al.*, 2008). Hence, MB showshigh efficacy against the inflammations and it could be used in treatment of SARS-CoV-2 infection.

Caspase activation inhibitor:

In lymphopenia condition the reduction of CD4T and CD8T lymphocytes occurs and it indicates severity of inflammation in COVID-19 patients (Liu *et al.*, 2020). The uncontrolled cytokines release increases the level of TNF-α and IL-6, which causes the T-cell apoptosis and it contributes to lymphopenia (Wan *et al.*, 2020). In COVID-19 patients, the inflammatory cell death known as pyroptosis occur. The non-apoptotic programmed cell death leads to caspse-1 activation. The drug which leads to protect from regular cell death (apoptosis) could be the best choice for the treatment of COVID-19 patients. It has been proven that the MB has potential to inhibit caspase activation in human cancer cells (Zhou *et al.*, 2019). Therefore, MB has a capability to inhibit the caspase activation and results to reduce the blood inflammation and it can protect from uncontrolled cytokine release. These features support the significant role of MB against SARS-CoV-2 infection.

Anti-hypoxemia activity/ischemic tissue damage:

MB has a capacity to protect tissues and organs against pathogens, inflammation, cell death and damages due to hypoxia. It works well in hypoxic/ischemic tissue damage condition. MB can be used to protect the severe lungs infection in COVID-19 patients. Tian and co-workers claimed that MB can protect isolated rat lungs infection (Tian *et al.*, 2018). MB shows anti-hypoxemia activity by changing oxidation state of Fe from +3 oxidation state (ferric) to the +2-oxidationstate (ferrous) (an approved medicine for methemoglobinemia) (Smith *et al.*, 2020). MB helps to prevent the multiorgan tissue damage and it could protect against ischemic tissue damage. The hypoxemic condition in severe COVID-19 patients is responsible for the multiorgan failure and it is a one of the biggest caused of the death of the patients. Therefore, MB can be used to protect the multiorgan involvements in SARS-CoV-2 infection.

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Antifungal agent:

MB has a great potential to use as antifungal agent.The antifungal activity of methylene blue has been explored in many articles (Teichert *et al.,* 2002, Machdo-de-Sena *et al.,* 2014, Thomas *et al.,* 2013, Moiz *et al.,* 2016). Methylene blue work as antifungal agentagainst fungal pathogens such as *Candida albicans*. Its antifungal activity might be due to the mitochondrial dysfunctioning of pathogens. In post COVID-19 patients, especially in India, mucormycosisis a serious problem. Mucormycosis is also a type of fungal infection and it caused by fungi of the class Zygomycetes. Therefore,MB could be a promising drug in the treatment of fungal infections and it can reduce serious issues caused by SARS-CoV-2 infections.

Conclusions:

The best adjuvant therapy against COVID-19 would be those that includes antivirals, anti-inflammatory, immunomodulators, anticoagulants and antioxidants properties into one drug. MB is a low-cost and FDA-approved drug for methemoglobinemia. In India, Golwalkar initiate the use of MB for the treatment of pneumonia in SARS-CoV-2 infected patients with some success (Golwalkar 2021). The property of MB has also been explored in phase I, phase II and phase III clinical trials (NCT04370288) for the treatment of SARS-CoV-2 infected patients in Iran. The results of these trials suggest that, MB is an excellent supplementary choice for the treatment of SARS-CoV-2 infected patients.

Additionally, the antiviral, anti-inflammatory, anti-hypoxemia and anti-fungal property of a single drug might be helpful in prevention and treatment ofSARS-CoV-2 infection. It could inhibit the viral replication, reduce the binding interaction between SARS-CoV-2 spike protein and its receptor(ACE2), control the cytokine storm, reduce the fungal growth and prevent from hypoxemia condition, all these properties of MB strongly support its effectiveness in prevention as well as in the treatment of critically ill COVID-19 patients.

The economically favourable, easy availability and its high safety profile, all these features of MB could also help to overcome from present pandemic situation. Therefore, we conclude that MB is an excellent supplementary choice against SARS-CoV-2 infection and it can be used along with standard care protocols for the treatment of COVID-19 patients.

References

1. Ahn H, Kang SG, Yoon SI, Ko HJ, Kim PH, Hong EJ, An BS, Lee E, Lee GS (2017) Methylene blue inhibits NLRP3, NLRC4, AIM2, and non-canonical inflammasome activation. Sci Rep 7(1): 12409
2. Andreu G. L. P., the rational for methylene blue utility against SARS-CoV-2 infection complications, J Pharm Pharmacogn Res (2021) 9(3): 380.
3. Bojadzic D., Oscar Alcazar and Peter Buchwald (2021); Methylene blue inhibits SARS-CoV-2 spike-ACE2 protein-protein interaction-a mechanism that can contribute to its antiviral activity against COVID-19, frontiers in pharmacology, 11, 600372.
4. Brooks MM (1933) Methylene blue as antidote for cyanide and carbon monoxide poisoning. JAMA 100: 59
5. Cagno, V., Medaglia, C., Cerny, A., Cerny, T., Tapparel, C., and Cerny, E. (2020). Methylene blue has a potent antiviral activity against SARS-CoV-2 in the absence of UV-activation in vitro. Available at: https://www.biorxiv.org/ content/10.1101/2020.08.14.251090v1.
6. Chen IY, Moriyama M, Chang MF, Ichinohe T (2019) Severe acute respiratory syndrome coronavirus Viroporin 3a activates the NLRP3 inflammasome. Front Microbiol 2019;10: 50.
7. Dicko A, Roh ME, Diawara H, Mahamar A, Soumare HM, Lanke K, Bradley J, Sanogo K, Kone DT, Diarra K, Keita S, Issiaka D, Traore SF, McCulloch C, Stone WJR, Hwang J, Müller O, Brown JM, Srinivasan V, Drakeley C, Gosling R, Chen I, Bousema T (2018) Efficacy and safety of primaquine and methylene blue for prevention of Plasmodium falciparum transmission in Mali: a phase 2, single-blind, randomised controlled trial. Lancet Infect Dis 18(6): 627–639.
8. Draize JH (1933) Sodium tetrathionate and methylene blue in cyanide and carbon monoxide poisoning. Science 78: 145.
9. Evora PR, Ribeiro PJ, Andrade JC (1997) Methylene blue administration in SIRS after cardiac operations. Ann Thorac Surg 63(4): 1212–1213.
10. Floyd RA, Schneider JE, Dittmer DP (2004) Methylene blue photoinactivation of RNA viruses. Antivir Res 61: 141– 151.
11. Gendrot, M., Andreani, J., Duflot, I., Boxberger, M., Bideau, M. L., Mosnier, J., et al. (2020). Methylene blue inhibits replication of SARS-CoV-2 in vitro. Int. J. Antimicrob. Agents. 56, 106202.

12. Golwalkar, D. (2021). Treatment for COVID-19 using methylene blue. Medium. Available at: https://medium.com/@dr.deepak.golwalkar/treatment-for-covid19-using-methylene-blue-d23fc5a31a4d (Accessed May 20, 2021).
13. Gurr E., (1962) Staining-Practical and Theoretical, 2nd ed., Williams and Warkins, Baltimore, MD, (p. 303).
14. Guttmann P, Ehrlich P (1891) Ueber die wirkung des methylenblau bei malaria. Berl Klin Wochenschr 28: 953– 956.
15. Guy, R. K., DiPaola, R. S., Romanelli, F., and Dutch, R. E. (2020). Rapid repurposing of drugs for COVID-19. Science. 368 (6493), 829–830.
16. Hamidi-Alamdari D., Bagheri-Moghaddam A., Amini S., Reza Keramati M., Moradi Zarmehri A., Hamidi Alamdari A., Damsaz M., Banpour H., Yarahmadi A., and Koliakos G. (2020) Application of methylene bluee-vitamin C-N-acetyl cysteine for tratment of critically ill COVID-19 patients, report of a phase-I clinical trial. European Journal of Pharmacology 885 173494.
17. Hamidi-Alamdari D., Hafizi-Lotfabadi S., Bagheri-Moghaddam A., Safari H. , Mozdourian M., Javidarabshahi Z., Peivandi-Yazdi A., Ali-Zeraati A., Sedaghat A., Poursadegh F., Barazandeh-Ahmadabadi F., Agheli-Rad M., Tavousi S. M., VojouhiS. Amini S., Amini M., Majid-Hosseini S., Tavanaee-Sani A., Ghiabi A., Nabavi-Mahalli S., Morovatdar N., Rajabi O., and Koliakos G. (2021a), methylene blue for treatment of hospitalized COVID-19 patient: a randomized, controlled, open-label clinical trial, phase-2, Rev Invest Clin. ;73(3):190-8
18. Hamidi-Alamdari D., Hafizi-Lotfabadi S., Mavaji Darban B., Agheli-Rad M., Saadatian S., Hadi Hashemi S., Barazandeh Ahmadabadi F., Morovatdar N., Arastoo M., Bhushan B., (2021b) methylene blue for treatment of hospitalized COVID-19 patient: a randomized, controlled, open-label clinical trial, phase-3, Aristotle Biomedical Journal, Vol 3, No 2 e-ISSN: 2653-9748
19. Jean SS, Lee PI, Hsueh PR (2020) Treatment options for COVID-19: The reality and challenges. J Microbiol Immunol Infect 53(3): 436–443.
20. Kandeel M, Al-Nazawi M (2020) Virtual screening and repurposing of FDA approved drugs against COVID-19 main protease. Life Sci 251: 117627
21. Li G, De Clercq E (2020) Therapeutic options for the 2019 novel coronavirus (2019-nCoV). Nat Rev Drug Discov 19: 149–150.

22. Liu K, Fang YY, Deng Y, Liu W, Wang MF, Ma JP, Xiao W, Wang YN, Zhong MH, Li CH, Li GC, Liu HG (2020b) Clinical characteristics of novel coronavirus cases in tertiary hospitals in Hubei Province. Chin Med J (Engl) 133(9): 1025–1031.
23. Lomniczi A, Cebral E, Canteros G, McCann SM, Rettori V (2008) Methylene blue inhibits the increase of inducible nitric oxide synthase activity induced by stress and lipopolysaccharide in the medial basal hypothalamus of rats. Neuroimmunomodulation 8(3): 122–127.
24. Lozano, M., Cid, J., and Muller, T. H. (2013). Plasma treated with methylene blue and light: clinical efficacy and safety profile. Transfus. Med. Rev. 27 (4), 235–240.
25. Lythgoe MP, Middleton P (2020) Ongoing clinical trials for the management of the COVID-19 pandemic. Trends Pharmacol Sci 41(6): 363–382.
26. Lythgoe MP, Middleton P (2020) Ongoing clinical trials for the management of the COVID-19 pandemic. Trends Pharmacol Sci 41(6): 363–382.
27. Machado-de-Sena RM, Corrêa L, Kato IT, et al. Photodynamic therapy has antifungal effect and reduces inflammatory signals in Candida albicans-induced murine vaginitis. Photodiagn Photodyn Ther 2014; 11(3): 275-82.
28. Mansouri A, Lurie AA (1993) Concise review: methemoglobinemia. Am J Hematol 42: 7-12.
29. Matheson, N. J., and Lehner, P. J. (2020). How does SARS-CoV-2 cause COVID-19?. Science. 369 (6503), 510–511.
30. Mendes JM, Ouermi L, Meissner P, Compaoré G, Coulibaly B, Nebie E, Krisam J, Klose C, Kieser M, Jahn A, Lu GD, Alessandro U, Sié A, Mockenhaupt FP, Müller O (2019) Safety and efficacy of artesunate-amodiaquine combined with either methylene blue or primaquine in children with falciparum malaria in Burkina Faso: A randomized controlled trial. PLoS One 14(10): e0222993.
31. Moiz A. Ansari , Zeeshan Fatima and Saif Hameed, antifungal action of methylene blue involves mitochondrial dysfunctions and disruption of redox and membrane homeostasis in *C. albicans*, The Open Microbiology Journal, 2016, 10, 12-22
32. Ottaviani S, Stebbing J (2020) What is the best drug to treat COVID-19? The need for randomized controlled trials. Med (NY) 1: 9–10.
33. Oz M, Lorke DE, Hasan M, Petroianu GA (2011) Cellular and molecular actions of methylene blue in the nervous system. Med Res Rev 31(1): 93–117

34. Perrone LA, Belser JA, Wadford DA, Katz JM, Tumpey TM (2013) Inducible nitric oxide contributes to viral pathogenesis following highly pathogenic influenza virus infection in mice. J Infect Dis 207(10): 1576–1584.
35. Ramalingam S, Cai B, Wong J, Twomey M, Chen R, Fu MR, Boote T, McCaughan H, Griffiths SJ, Haas JG (2018) Antiviral innate immune response in non-myeloid cells is augmented by chloride ions via an increase in intracellular hypochlorous acid levels. Sci Rep 8(1): 13630.
36. Serafin MB, Bottega A, Foletto VS, da Rosa TF, Horner A, Horner R (2020) Drug repositioning an alternative for the treatment of coronavirus COVID-19. Int J Antimicrob Agents 55(6): 105969
37. Smith, T., Bushek, J., LeClaire, A. and Prosser, T., (2020). COVID-19 drug therapy. Elsevier.
38. Teichert MC, Jones JW, Usacheva MN, Biel MA. Treatment of oral candidiasis with methylene blue-mediated photodynamic therapy in an immunodeficient murine model. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93(2): 155-60.
39. Thomas E, Roman E, Claypool S, Manzoor N, Pla J, Panwar SL. Mitochondria influence CDR1 efflux pump activity, Hog1-mediated oxidative stress pathway, iron homeostasis, and ergosterol levels in Candida albicans. Antimicrob Agents Chemother 2013; 57(11): 5580-99.
40. Tian WF, Zeng S, Sheng Q, Chen JL, Weng P, Zhang XT, Yuan JJ, Pang QF, Wang ZQ (2018) Methylene blue protects the isolated rat lungs from ischemia-reperfusion injury by attenuating mitochondrial oxidative damage. Lung 196(1): 73–82.
41. Tuite EM, Kelly JM (1993) Photochemical interactions of methylene blue and analogues with DNA and other biological substrates. J Photochem Photobiol B Biol 21: 103–124.
42. Wainwright M (2000) Methylene blue derivatives- suitable photoantimicrobials for blood product disinfection? Int J Antimicrob Agents 16(4): 381–394.
43. Wainwright M, Amaral L (2005) The phenothiazinium chromophore and the evolution of antimalarial drugs. Trop Med Int Health 10(6): 501–511.
44. Wan S, Yi Q, Fan S, Lv J, Zhang X, Guo L, Lang C, Xiao Q, Xiao K, Yi Z, Qiang M, Xiang J, Zhang B, Chen Y, Gao C (2020) Relationships among lymphocyte subsets, cytokines, and the pulmonary inflammation index in coronavirus (COVID-19) infected patients. Br J Haematol 189(3): 428–437.

45. Wang Z, Zhang S, Xiao Y, Zhang W, Wu S, Qin T, Yue Y, Qian W, Li L (2020b) NLRP3 inflammasome and inflammatory diseases. Oxid Med Cell Longev 2020: 4063562
46. Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. (2020): A new coronavirus associated with human respiratory disease in China, Nature, 579:365-9.
47. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Liu S, Zhao P, Liu H, Zhu L, Tai Y, Bai C, Gao T, Song J, Xia P, Dong J, Zhao J, Wang FS (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8: 420–422.
48. Zhou L, Flores J, Noël A, Beauchet O, Sjöström PJ, LeBlanc AC (2019) Methylene blue inhibits Caspase-6 activity, and reverses Caspase-6-induced cognitive impairment and neuroinflammation in aged mice. Acta Neuropathol Comm 7(1): 210.
49. Zhu, N.; Zhang, D.; Wang, W.; Li, X.; Yang, B.; Song, J.; Zhao, X.; Huang, B.; Shi, W.; Lu, R. China Novel Coronavirus I, Research T. (2019) A novel coronavirus from patients with pneumonia in China, . N. Engl. J. Med. 382, 727–733.

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CULTURE OPTIMIZATION FOR BACITRACIN PRODUCTION IN *BACILLUS SUBTILIS*

Naveen Kumar Kudure Jayanna[1] and Venkatesh[2]*

[1]Department of Studies in Microbiology,

[2]Department of Studies in Food Technology,

Davangere University, Shivagangotri campus,

Davanagere-577007, Karnataka, India

*Corresponding author E-mail: venka.biotech@gmail.com

Abstract:

Bacitracin is a mixture of related cyclic peptides produced by organisms of the licheniformis group of Bacillus subtilis. Bacitracin is one of the most important polypeptide antibiotics; it is produced by some strains of *Bacillus licheniformis* and *Bacillus subtilis* and functions as an inhibitor of the cell wall biosynthesis. It is a potent antibiotic used clinically in combination with other antimicrobial drugs. The present study is concerned with the biosynthesis and optimization of antibiotic bacitracin by *Bacillus subtilis* on laboratory scale. The 40°C was the optimum temperature for bacitracin production. In average the optimum pH for bacitracin production was in the range of 7 and 9. The optimum time for bacitracin production was found to be 24 hrs. Bacitracin is used to help prevent minor skin injuries such as cuts, scrapes, and burns from becoming infected.

Keywords: Bacitracin, *Bacillus subtilis*, Culture optimization, Polypeptide antibiotics

Introduction:

One of the major scientific achievements of the early 20th century was discovery of antibiotics, which are produced by microorganisms. The microorganism produce antibiotics have pharmaceutically important. Especially, *Bacillus* species gain an attention for production of economically important peptide antibiotics such as bacitracin, bacilysin, tyrocidine etc. (Hammes and Frank, 1979; Chopra *et al.*, 1996; Singh *et al.*, 2004; Trookman *et al.*, 2011). The peptide produced by this bacillus species showed significant activity on gram positive bacteria than gram negative bacteria (Ming and Epperson, 2002; Ohki *et al.*, 2003; Jyothi *et al.*, 2010). The *Streptomyces* species also produces antibiotics (Pavithra *et al.*, 2009). Bacitracin produced by the bacillus species are composed of one or more polypeptide chains (Ohki *et al.*, 2003; Neeraj and

Behal, 2010). The function of the bacitracin interferes protein synthesis, which halt the cell biosynthesis. The scientist and researcher discovered different types of bacitracin (A, A1, B, C, E, F, F1, F2, F3 and G). Among them, Bacitracin A showed promising antibacterial activity (Meshcer *et al.,* 1979; Arky, 1997; Schallmey *et al.*, 2004).

Bacillus licheniformis is a bacitracin generating microorganism. Bacitracin includes one or greater of the antimicrobial polypeptides produced through positive traces of Bacillus licheniformis. The reasonably-priced uncooked cloth for bacitracin manufacturing need to be with no trouble to be had and reasonably-priced including wheat bran, rice hulls, soyabean meal, etc. Thus improvement of this era in our us of a might bring about saving reasonable quantity of forex through exploiting indigenous resources. The goal of the present examine changed into to optimize the manufacturing of bacitracin through the usage of *Bacillus licheniformis* NCIM 2536 from the reasonably-priced uncooked cloth including wheat bran, rice hulls and soyabean meal (Phillips, 1999; Rajan and Kannabiran, 2014).

In the present study, the soil samples collected from Bhadra reserve forest area of shimoga district, Karnataka. This region contains rich biodiversity of plants and microorganisms. The production of secondary metabolite or peptide varies significantly from one biodiversity to other biodiversity. The microbes isolated from this region have a potent to produce pharmaceutical important metabolite or peptides. In the present study culture condition are optimized for the production of Bacitracin**.**

Materials and Methods:

Sample collection

Soil sample was collected from Bhadra reserve forest area of shimoga district, Karnataka. This region contains vast number of medicinal plants. The traditional practitioners used various parts of medicinal plants to treat many diseases.

Isolation of Bacitracin producing strains

Soil samples were collected and subjected to serially dilute up to 10^{-9} and aliquots (0.1 ml) was plated on sterilized Nutrient agar plates and incubated for 24 hours at 37°C After incubation colonies of organism are obtained. Pick the colony from the plate and streak it on the sterilized agar plate. This culture was maintained in nutrient agar slants and broth.

Identification and screening of Bacitracin producing strains

The isolated strains were subjected for identification by using standard manuals and biochemical tests such as Indole production test, Methyl-red and Voges-proskauer tests, Citrate utilization test, Starch hydrolysis test, Glucose fermentation, Gelatin hydrolysis, Catalase test

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and Urease production. The isolated strains were screened for Bacitracin production on test organism such as *Streptococci sp., Staphylococci sp., and E. coli.* Isolated strains are streaked on nutrient media containing test organisms, then indcube for 24 hrs at 37°C.

Optimization for Bacitracin production

To study the effect of Bacitracin production, the culture conditions and media were optimized. The isolated strains were cultured in different carbon sources (glucose, fructose, lactose, sucrose), nitrogen sources (peptone, tryptone, beef extract, yeast extract). Once the culture media source was optimized, the changing the cultural condition like pH 4, 5, 6, 7, and 8 and incubation temperature such as 4 ºC, 27 ºC, 37 ºC, and 40 ºC. At the end of each experiment, the isolated strains were centrifuge at 5000 rpm for 15 min at 25ºC. Discard the pallets and take supernatant in to test tubes. Take separating funnel and keep it on the stand, then add 5ml of supernatant and 5ml of butanol to it and mix well. After mixing, two layers separate out, collect it separately in to different test tubes. Prepare nutrient agar media, sterilized it and inoculate test organism (*E. coli*) and pour it in to petriplates. Make the wells in to the nutrient agar plate using cork borer. Inoculate cell debris in one well in another inoculate extract by using micropipette. Incubate the plates for 24 hours in the incubator at 37ºC. After incubation observe the zone of inhibition and measure its millimeter.

Production and purification of Bacitracin

The production of Bacitracin was carried out in submerged fermentation method. The inioculums were inoculated to autoclaved and cooled, optimized nutrient broth (autoclaved for 15 minutes at 121 ºC) and incubated at 37 °C for 48 hr in an orbital shaker. Then fermentation broth was centrifuged at 5,000 rpm for 15 minutes and supernatants were subjected to n-butanol extraction for Bacitracin production. The purification was done by salting out (Ammonium sulphate precipitation) method (Jadwiga, 1998).

Agar well diffusion assay

Antibacterial activity was determined to check the Bacitracin production in culture media by using agar diffusion method. The culture plates were prepared with sterile nutrient agar media. 100 µl of bacterial culture (105 cells/ ml) was inoculated on to the culture plate using sterile L-shaped glass rod to get uniform distribution of bacteria. Add one hundred µl of tested bacteria culture (10^5 cells/ ml) to the sterile medium media and spread the inoculums by using L-shaped glass rod. Then wells are created using sterile stainless steel cork borer (6.0 mm) under aseptic condition. Add 100 µl into wells. The ciprofloxacin standard drug was used as reference. Then, the cultured plates were incubated for 24 h at 37 ºC. After incubation, the zone of

inhibition in mm was measured. The experiments were repeated thrice and the values are statistical analyzed by using ezANOVA software.

Results:

Isolation and characterization of Bacitracin producing organism

Soil sample was serially diluted and spreaders on the nutrient agar media colonies of organisms are obtained. The colonies are streaked on the agar media and broth, pure culture was obtained and maintained. Confirmatory tests were carried out by Gram's staining. Morphology of the organism was identified. The organism was gram positive, Bacilli. Biochemical Tests showed in table 1. The organism was identified and confirmed by Gram's staining and Biochemical tests as *Bacillus subtilis*.

Table 1: Biochemical Tests for *Bacillus* species

Sr. No.	Biochemical Tests	Results
1	Citrate utilization Test	Positive
2	Glucose utilization Test	Positive
3	Catalase test	Positive
4	Starch Hydrolysis	Positive
5	Gelatin Hydrolysis	Positive
6	Voges Proskar Test	Positive
7	Methyl Red Test	Negative
8	Indole	Negative
9	Urease production	Negative

The optimized culture condition for Bacitracin production showed Maximum activity was observed at pH 7 = 18.67±0.88, temperature 37ºC = 25.67±0.88, Glucose =16.33±0.88 (Fig 2a & b), Tryptone = 19.33±1.2 (Fig 2c). In the present showed that Bacitracin isolated from *Bacillus subtilis*. Murphy *et al.,* (2007) isolated Bacitracin was isolated by *B. licheniformis* organism. Recently many more available for isolation and production of Bacitracin from various bacterial species were found.

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Table 2: Optimization of Bacitracin producing organism for various culture conditions

Physical Parameters		**Zone of inhibition**
pH	pH - 4	No zone
	pH - 5	11.67±0.88
	pH - 7	18.67±0.88
	pH - 8	15.67±0.88
Temperature	4º C	No zone
	27º C	10.67±1.2
	37ºC	25.67±0.88
	40º C	24.33±0.88
Chemical Parameters		**Zone of inhibition**
Carbon Source	Fructose	No zone
	Glucose	16.33±0.88
	Sucrose	12.33±1.2
	Lactose	10.33±0.88
Nitrogen Source	Peptone	18.67±0.88
	Tryptone	19.33±1.2
	Beef extract	16.33±0.88
	Yeast extract	11±1.15

Figure 1: (a and b) Bacitracin (carbon source) showed zone of inhibition. C) Bacitracin (Nitrogen source) showed zone of inhibition

Production and antibacterial activity of Bacitracin

The production media was devised and fermentation was carried out in fermentor, in which maximum growth of organism and yield of antibiotic was observed. From the fermented media antibiotic extract was separated using physical separation method and tested for activity. Maximum zone of inhibition was obtained against *Staphylococci sp.*, *Streptococci* sp. and less zone of inhibition was obtained against *E. coli* indicates that the Bacitracin antibiotic inhibits the growth of Gram positive organisms (Table 3 and Figure 3).

The present study also showed maximum inhibition against Gram positive organisms like *Staphylococci sp.*, *Streptococci sp.* and little less against Gram negative, *E.coli.*

Table 3: Antibacterial activity of Bacitracin

Sr. No.	Organism	Zone of inhibition (mm)
1	*Staphylococci* sp.	27.33±1.2
2	*Streptococci* sp.	22.67±0.88
3	*E. coli*	18±0.58

Figure 2: Bacitracin showed Zone of inhibition on *Staphylococci* sp., *Streptococci* sp. *and E. coli*

Discussion:

The use of antibiotics in clinical biology is a common phenomenon and their uses are rapidly increasing day by day. Most of the antibiotics have been obtained from microorganisms (Harvel *et al.*, 1999; Das and Kumar, 2012). Antibiotics which have been used today are either natural or semi synthetic or synthetic by nature. Semi synthetic antibiotics are prepared by modifying the natural antibiotic, either chemically or enzymatically with an aim to enhance the efficiency of original antibiotics. Antibiotics used in medical practices have been obtained from

few groups of microorganisms only. The two genera of bacteria *Streptomyces* and *Bacillus* are the maxi-mum contributor of antibiotics. The antibiotics produced by Bacillus species are being recommended for infection caused by gram positive bacteria (Murphy *et al.*, 2007; Sharma and Das, 2010; Trookman *et al.*, 2011).

Bacitracin produced by different Bacillus species such as *Bacillus subtilis, Bacillus cereus*, and *Bacillus licheniformis* etc. The Bacitracin produced by bacillus species showed significant activity on gram positive bacteria than gram negative bacteria. The Yousaf *et al.* (1997) and Azevedo *et al.* (1993) showed effectively used against gram positive organism. Hussein *et al.* (2006) reported that *Bacillus licheniformis* was isolated from soil of different house gardens, which produces Bacitracin that showed antibacterial activity against some species of Gram positive and some extent to Gram. Bacillus species also produces other antibiotics such as bacilysin, lipooligopeptides and subsporins A–C etc (Priest, 1992). Mendo *et al.* (2004) reported that Bacitracin isolated from *Bacillus licheniformis* strain, 189 showed strongly inhibit the growth of gram positive bacteria. In the present study was showed maximum antibacterial activity at pH – 7 (18.67±0.88) and maximum at 37oC (25.67±0.88). Haavik (1975) reported that bacitracin production by *Bacillus licheniformis* ATCC 14580 was observed after 48-72 hour incubation. Yousaf (1997) reported that 20 hours old vegetative inoculums of *B. licheniformis* gave the maximum yield of bacitracin. In the present study maximum zone of inhibition was observed in *Staphylococci sp.*

Conclusion:

Most of the peptide antibiotics are produced by bacilli that are active against gram-positive bacteria; however, compounds such as polymyxin, colistin and circulin exhibit activity almost exclusively upon gram-negative bacteria, whereas bacillomycin, mycobacillin and fungistatin are effective against molds and yeasts. Bacitracin is derived from cultures of Bacillus subtilis. It is a white to pale buff, hygroscopic powder, odorless or having a slight odor. The Bacitracin produced by culture optimization in Bacillus species. Maximum activity was showed against gram-positive bacteria. Further precursors are necessary to feed in culture media for enhanced productions.

Acknowledgements:

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References:

1. Arky, R. (1997): Bacitracin is metal dependant peptide antibiotic from cultures of *Bacillus*, Journal of Serbian Society, 69:883-886.
2. Azevedo, E.C., Rios, E.M., Fukushima K., and Campos-Takaki G. M. (1993): Bacitracin production by a new strain of *Bacillus subtilis*, Bacteriology, 94:248-35.
3. Chopra, M., Smith, K.N., Weinberg, H.R. (1996): Bacitracin induced cell lysis, Journal for Biological Sciences, 23:564-575.
4. Das, M. and Kumar, S. (2012): Characterization of lactamase producing bacterium from soil of Mahendragiri region, Orissa, Asian Journal of Microbiology, Biotechnology & Environmental Sciences, 14 (2):229- 232.
5. Farzana, K., Shah, S. N. H., Butt, F. B., and Bukhsh, S. (2004): Biosynthesis of bacitracin in stirred fermenter by *Bacillus licheniformis* using defatted oil seed cakes as substrate. Journal of Research (Science), 15(3):285-290.
6. Hammes, E.J., Frank, M.P. (1979): Bacitracin Chemistry, Metabolism, dietary sources, Proceedings of the National Academy of Sciences, 17:405-417.
7. Harvel, Y.M., A. Bailone and Bibi E. (1999): Resistance to bacitracin as modulated by an Escherichia coli homologue of the bacitracin ABC transporter Bcr C subunit from *Bacillus licheniformis*, Journal of Bacteriology, 181:6176-6178.
8. Hussein, A.A., and Al-Janabi, S. (2006). Identification of bacitracin produced by local isolate of *Bacillus licheniformis*. African journal of Biotechnology, *5*(18).
9. Jyothi, P., Kiran, K.J., Radhika, M. (2010): Antimicrobial studies of selected antibiotics and their combination with enzymes, International Journal of Pharmacy and Pharmaceutical Sciences, 2(3): 25-33.
10. Meshcer, R., Hummel, J.P., Dreyer, W.J. (1974): Bacteriocidal activity of Bacitracin. Journal of Biochemistry, 92:97-299.
11. Ming, L.J., and Epperson, J.D. (2002). Metal binding and structure–activity relationship of the metalloantibiotic peptide bacitracin, Journal of inorganic biochemistry, 91(1):46-58.
12. Murphy, T., Roy, I., Harrop, A., Dixon K. and Keshavarz, T. (2007): Effect of oligosaccharide elicitors on bacitracin a production and evidence of transcriptional level control. Journal of Biotechnology, 131:397-403.
13. Murphy, T., Roy, I., Harrop, A., Dixon, K., and Keshavarz, T. (2007): Effect of oligosaccharide elicitors on bacitracin A production and evidence of transcriptional level control, Journal of Biotechnology, 131(4):397-403.

14. Neeraj, M., Behal, K.K. (2010): Antimicrobial activity of some species against selected microbes, International Journal of Pharmacy and Pharmaceutical Sciences, 2(3):35-42.
15. Ohki, R.A. (2003): Bacitracin – Resistant *Bacillus subtilis* Gene Encodes a Homologue of the Membrane-spanning subunit of the *Bacillus licheniformis* ABC Transporter, Journal of Bacteriology, 185(1):51-59.
16. Ohki, R., Tateno, K., Okada, Y., Okajima, H., Asai, K., Sadaie, Y., and Aiso, T. (2003): A bacitracin-resistant *Bacillus subtilis* gene encodes a homologue of the membrane-spanning subunit of the *Bacillus licheniformis* ABC transporter. Journal of Bacteriology, 185(1):51-59.
17. Pavithra, P.S., Sreevidya, N., and Verma, R.S. (2009): Antibacterial activity and chemical composition of essential oil of *Pamburus missionis*. Journal of Ethnopharmacology, 124(1):151-153.
18. Phillips, I. (1999): The use of bacitracin as a growth promoter in animals produces no risk to human health. Journal of Antimicrobial Chemotherapy, 44(6):725-728.
19. Rajan, B.M., and Kannabiran, K. (2014): Extraction and identify cation of antibacterial secondary metabolites from marine Streptomyces sp. VITBRK2, International Journal of Molecular and Cellular Medicine, 3:130-137.
20. Schallmey, M., Singh, A., and Ward, O.P. (2004): Developments in the use of Bacillus species for industrial production, Canadian journal of microbiology, 50(1):1-17.
21. Sharma, D. and Das, M. (2010): Efficacy of antimicrobial metabolites of *Pseudomonas fl uroscenes* PFG against phytopathogenic fungi, Proceedings of the National Academy of Sciences, 1:68-71.
22. Singh, S.H., and Owen, A.P. (2004): Ward. Developments in the use of Bacillus species for industrial production, Canadian Journal of Microbiology, 50:117.
23. Trookman, N.S., Rizer, R.L., and Weber, T. (2011): Treatment of minor wounds from dermatologic procedures: a comparison of three topical wound care ointments using a laser wound model, Journal of the American Academy of Dermatology, 64(3):S8-S15.
24. Trookman, N.S., Rizer, R.L. and Weber, T. (2011): Treatment of minor wounds from dermatologic procedures: A comparison of three topical wound care ointments using a laser wound model, Journal of the American Academy of Dermatology, 64:08-15.
25. Yousaf, M. (1997): Studies on the cultural production of antibiotic bacitracin by *Bacillus licheniformis* (Doctoral dissertation, Islamia University, Bahawalpur).

VALUE ADDED HOME MADE NATURAL SQUASH PREPARED FROM THREE UNDERUTILISED FLOWERS

S. M. Prasad*, A. S. Mohamed Rilwan and S. Mohamed Yousuf

Post Graduate Department of Nutrition and Dietetics,

SadakathullahAppa College (Autonomous),

Rahmath Nagar, Tirunelveli, Pin-627 011 Tamil Nadu, India

***Corresponding author E-mail:** prasadsm33@gmail.com

Abstract:

Flowers are of different types. Some may be edible, others are non-edible and some may go unnoticed. Mostly all flowers are rich in colour and contain antioxidant properties as well as disease curing properties. In Ayurveda flowers occupy a good position in medicine preparation. Some flowers are also used in the treatment of cancer. So keeping three underutilised flowers *(Ixora coccinea, Clitoria ternatea, Hibiscus rosasinensis)* in mind, squash is prepared by allotting codes namely *Ixora coccinea* (ICS), *Clitoria ternatea* (CTS), *Hibiscus rosasinensis* (HRS), These products are also standardized by using 5 point hedonic scales.10 trained panel members evaluated the products during sensory evaluation. From the result the present study shows the product HRS shows good quality, sensory and over all acceptability.

Keywords: Underutilised Flowers, Squash, Shelf Life, Storage Sensory Evaluation

Introduction:

Like fruits and vegetables, spices, leaves and nuts, flowers too are a part of nature roster of nutrition providers to us. India's culinary history shows us that flowers have been used for therapeutic purposes for balancing of tastes, fragrance and even improve the aesthetic appearance of a dish (Kumud Dadlani, 2020). Natural drinks provide some Vitamins, Minerals, Energy and especially β-Carotene, Vitamin C, and Potassium Rekha Sharma (2004). Flowers of *Ioxra coccinea* have been used to treat hypertension, amenorrhea and irregular menstruation, hemoptysis and catarrhal bronchitis and dysentery (http://www.healthbenefittimes.com). A decoction of the flower or the bark is employed as a lotion against eye troubles, sores and ulcers (http://proseanet.org/prosea/).

In India the *Clitoria ternatea* (Butterflypea flower) is a holy flower in rituals and can also be used as food colour (World Crop Data Base). The flower looks like a shape of a conch. Butterfly pea is abundantly found in south India (Balasubramaiam, 2018). In South East Asia the

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blue flowers are used as a natural food colouring agent and added to rice,tea, fruit juices or cock tails. The butterfly peas are also known to contain natural antioxidants (Nazareth, 2015).

Hibiscus rosasinensis is often used as a salad garnish, are also used to brew tea. They are known to contain anthocyanin and antioxidants. They low blood pressure and cholesterol (Times of India, 2016). The flower can be dried and stored and used in jams, baking cakes, muffins and breads (Srinivasan, 2017). In the light of the above facts realizing the importance of the flowers the study "Value Added Home Made Natural Squash Prepared from Three Underutilised Flowers" were under taken with the following objectives.

Objectives of the study:

1. To identify the underutilised flowers such as *Ixora coccinea* (Chethi flower) *Clitoria ternatea* (Butterfly Pea) and *Hibiscus rosasinensis* (Shoe flower)
2. To prepare natural squash from the above said flowers
3. To assess the sensory evaluation of the prepared squash product
4. To know the shelf life, storage quality of the prepared product under room temperature and refrigerated temperature

Methodology:

These trials for the research work entitled "Value Added Home Made Natural Squash Prepared from Three Underutilised Flowers" were carried out in the Department of Nutrition and Dietetics, Sadakathullah Appa College (Autonomous) Rahmath Nagar, Tirunelveli, Tamil Nadu, during the academic year from December 2018- April 2019.

Selection of Raw Material:

The main raw material *Ixora coccinea* (Chethi flower) *Clitoria ternatea* (Butterfly Pea Flower) *Hibiscus rosasinensis* (Shoe flower) needed for the study was collected from the house premises of the investigator. The samples have been collected as per the requirement. It was collected during the month of January 2019.

Identification of the Sample:

The flowers were identified with a help of a botanist

Selection of Other Ingredients:

The Ingredients like Sugar, Cardamom, Lemon and Ginger were purchased from a local super market near Tirunelveli and stored for research purpose.

Figure 1: ***Ixora coccinea***

Figure 2: ***Clitoria ternatea***

Figure 3: ***Hibiscus rosasinensis***

Pretreatment Employed:

The samples after collecting were cleaned by removing all the dust particles, stigma, calyx, and dead flowers attached to it. Then the samples are thoroughly washed in running water 2-3 times. With careful attention the samples were packed in polyethylene bags and stored in refrigeration for further analysis as per **Prasad et al., (2020).** More over the flowers must not soak over a very long time since butterfly pea flower may drain its colour very lightly.

Material used for the study:

Electronic weighing balance:

Electronic weighing balance of AY-220 model was used to weigh the samples in termsof grams and milligrams.

Refrigerator:

Refrigerator (Kelvinator) with a temperature range of 6-20^{0}C was used to store the samples.

Mixer:

Mixer model of 2k-458 was used to grind ginger.

Strainer:

Stainless steel strainer were used for the extraction of the sample

Other Minor Equipment:

Other materials like stainless steel cups, mugs, plates, knives, bottles, utensils, were used for the preparation of the product.

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Formulation of the product:

Preparation of *Ixora coccinea* Squash (ICS):

Table 1: Ingredients used for making ICS squash

Sr. No.	Ingredients Used	Amount
1	*Ixora coccinea*	250 g
2	Sugar	250 g
3	Lemon Juice	50 ml
4	Ginger Juice	50 ml
5	Cardamom	2 Nos
6	Water	700 ml

Ixora coccinea squash were prepared from 250 g of fresh flowers, 250 g of sugar, 50ml of lemon juice, 50 ml of ginger juice, cardamom 2 Nos and 700 ml of water.

Preparation of *Clitoria ternatea* Squash (CTS):

Table 2: Ingredients used for making CTS squash

Sr. No.	Ingredients Used	Amount
1	*Clitoria ternatea*	250 g
2	Sugar	250 g
3	Lemon Juice	50 ml
4	Ginger Juice	50 ml
5	Cardamom	2 Nos
6	Water	700 ml

Clitoria ternatea squash were prepared from 250 g of fresh flowers, 250 g of sugar,50 ml of lemon juice, 50 ml of ginger juice, cardamom 2 Nos and 700 ml of water.

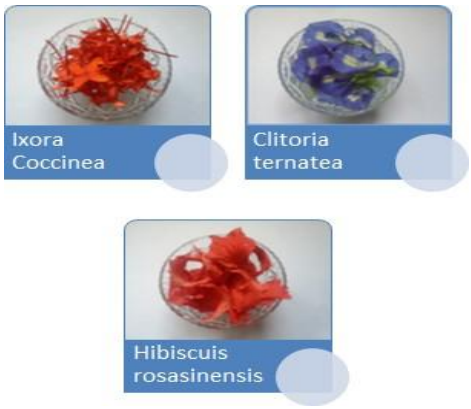
Preparation of *Hibiscus rosasinensis* Squash (HRS):

Hibiscus rosasinenisis squash were prepared from 250 g of fresh flowers, 250 g of sugar, 50 ml of lemon juice, 50 ml of ginger juice and 700 ml of water. Soft drink constitutes one of the largest food industries in the world today. In India in the organized sector alone, annual production of soft beverages is about 45 million cases (Shankuntalamanay and Shadakshraswamy, 20007).

General methodology for the preparation of the squash from underutilised flower is shown in the flow chart given below

Table 3: Ingredients used for making HRS squash

Sr. No.	Ingredients Used	Amount
1	*Hibiscus rosasinensis*	250 g
2	Sugar	250 g
3	Lemon Juice	50 ml
4	Ginger Juice	50 ml
5	Cardamom	2 Nos
6	Water	700 ml



Sample selection (250 g) and sorting

↓

Soaking in water (700 ml)

↓

Boiling at 100^{o} C

↓

Straining through a muslin cloth

↓

Collect the clear liquid

↓

Add Sugar (250 g) and dissolve it

↓

Add ginger juice, lemon juice and cardamom

↓

Boil for 5-10 minutes

↓

Allow it to cool

↓

Store it in sterile air tight bottles and refrigerate

Figure 4: Methodology for preparing squash from flowers

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Figure 5: ***Ixora Coccinea Squash***

Figure 6: ***Clitoria ternatea Squash***

Figure 7: ***Hibiscus rosasinensis Squash***

Sensory evaluation:

The prepared squash products were subjected to 10 trained panel members for a five point hedonic rating scale and results were recorded. Each and every product must possess a good shelf life study. For this the prepared products were carefully observed in refrigerated and in room temperature for about 28 days.

Results and Discussion:

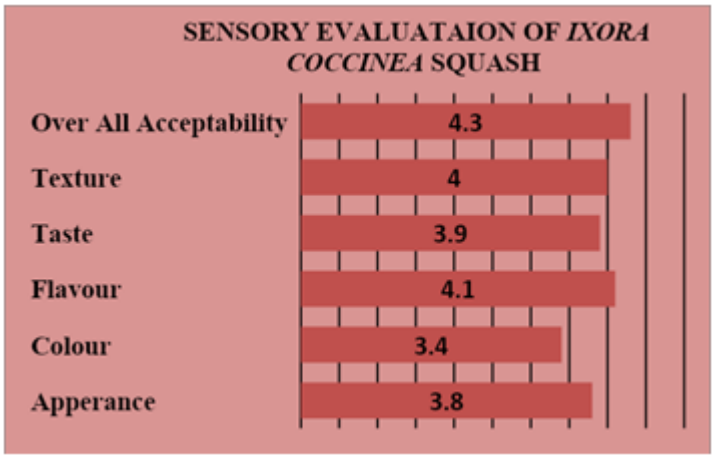
The results of the study "Value Added Home Made Natural Squash Prepared from Three Underutilized Flowers" are consolidated, analyzed and systematically presented under the following headings.

Sensory evaluation of the prepared product:

The formulated product was organoleptically evaluated by using numerical card to estimate the acceptance by 10 trained panel members. The panel members were asked to evaluate the product for appearance, color, flavor, taste, texture and overall acceptability.

Sensory Evaluation of *Ixora coccinea* Squash (ICS):

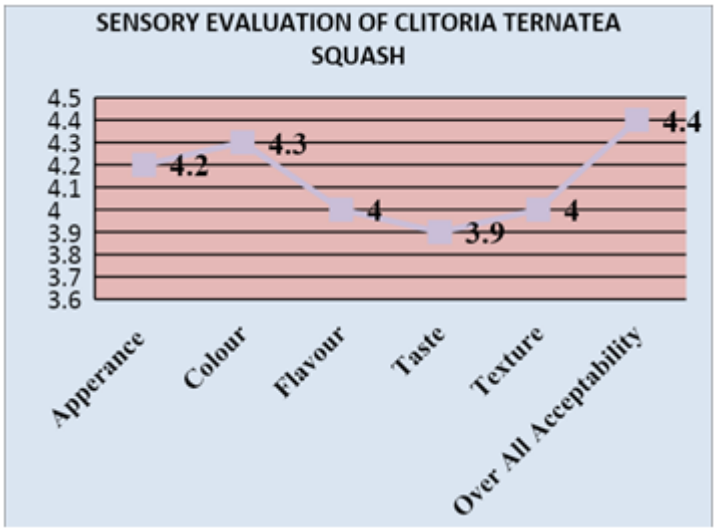
Table 4: Sensory Evaluation of *Ixora coccinea* Squash



From table no 4, it is clearly evident that for appearances the values goes to 3.8, colour 3.4, flavour 4.1, taste 3.9, texture 4.0, and over all acceptability 4.3

Sensory Evaluation of *Clitoria ternatea* Squash (CTS):

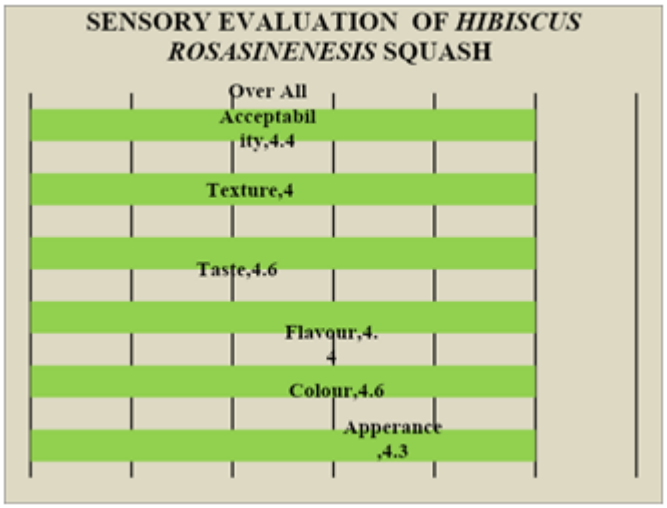
Table 5: Sensory Evaluation of *Clitoria ternatea* Squash



From table no 5, it is clearly evident that for appearances the values goes to 4.2, colour 4.3, flavour 4.0, taste 3.9, texture 4.0, and over all acceptability 4.4

Sensory Evaluation of Hibiscus *rosasinensis* Squash (HRS):

Table 6: Sensory Evaluation of *Hibiscus rosasinensis* Squash



From table no 6, it is clearly understood that for appearances the values goes to 4.3, colour 4.6, flavour 4.4, taste 4.6, texture 4.0, and over all acceptability 4.4

Shelf life study:

The selected squash products were stored in airtight glass bottles still analyzing various parameters. Care was taken to see that they were stored in clean and dry place where it was away from sunlight and pests. Prepared squash were stored in normal room temperature and in

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refrigerated to assess their storage pattern. Every day the containers were checked to see any microbial growth and the development of off flavor. Prepared squash products were kept for nearly 28 days to find out the production of gases also.

Table No 7: Shelf-Life study of the Prepared Squash Products

Sr. No.	Days	SQUASH ICS		SQUASH CTS		SQUASH HRS	
		RT	RS	RT	RS	RT	RS
1	1st to 6th day	NC	NC	NC	NC	NC	NC
2	7th to 13th day	NC	NC	NC	NC	NC	NC
3	14th to 20st day	NC	NC	NC	NC	NC	NC
4	21nd to 27th day	SFC	NC	SFC	NC	NC	NC
5	28th day	SFC	NC	SFC	NC	NC	NC

RT-Room Temperature, RS-Refrigeration Storage, NC-No Change, SFC-Slight Flavour Change

Regarding refrigeration storage no changes were observed from 1st to 28th day in the squashes (ICS, CTS, HRS) but in room temperature storage slightly flavour change occurred only in ICS and CTS from 21st to 28th day in the prepared squashes. From this study HRS showed a good overall acceptability.

Summary and conclusion:

Most of the commercial foods that we purchase may contain preservatives added beyond its limit but when we don't add preservatives or any other harmful substances the particular food can be consume by all people without any hesitation not only that preservatives can even destroy the natural components like antioxidants and phytochemicals in the food. This study shows the natural homemade squash prepared from flowers can be used as natural drink for all age groups.

Recommendation:

1. Supplementation of squash to various degenerative diseases can also be taken as a part of future research
2. Formulation of new product like cakes, biscuits, bread can be tried
3. Therapeutic diet planning by using the plant for various clinical conditions can be noticed
4. Antioxidant level of squash can be studied

Acknowledgement:

The authors sincerely thank Dr. Cissie Theeblyn David, Assistant Professor, Department of Food Science and Nutrition ICAR-KVK, Tirupathisaram, Kanniyakumari District, Tamil Nadu, India for her timely help, support and providing valuable suggestions.

Refernces:

1. Chitra Balasubramaniyam (2018). Food: Sip those Blue Away. Down to Earth. https://www.downtoearth.org.in/news/food/sip-those-blues-away-60302
2. Kumud Dalani (2020). 12 Edible Flowers of India. https://www.thursd.com/articles/12-edible-flowers-of-india/
3. Marianne De Nazareth., (2015). The Hindu. E-Paper. A brilliant Flash of Blue. https://www.thehindu.com/features/metroplus/a-brilliant-flash-of-blue/article7706830.ece
4. Pankaja Srinivasan (2017). The Hindu.E-Paper.https://www.thehindu.com/life-and-style/food/five-flowers-that-you-can-eat/ar ticle17558205.ece
5. Prasad, S.M., Vijayashree V., Janaki Raman, P., Cissie Theeblyn David and Magreswari, P., (2020).Value Added Products from Yam (Dioscorea Spp.).Shanlax International Journal of Arts Science and Humanities, 8(1), P 207-212. https://doi.org/10.34293/sijash.v8i1.2424
6. Rekha Sharma (2004). Diet Management. Third Edition. Reed Elsiever India Private Limited.ISBN:81-8147-545-3.P-14
7. http://proseanet.org/prosea/
8. https://www.healthbenefitstimes.com/jungle-geranium/. Herbs and Spices : Benefits of Jungle Geranium
9. Shankuntalamany. N., Shadaksharaswamy. M., (2007). Foods Facts and Principles. New Age International Publishers.
10. Times of India (2016). E-Paper. Healthy Living.25 Edible Flowers that will Add Colour, Flavor, and Nutrition to your Food. https://www.indiatimes.com/health/healthyliving/25-edible-flowers-that-will-add-colour-flavo ur-and-nutrition-to-your-food-249224.html
11. World Crop Data Base. Retrieved from https://world-crops.com/asian-pigeonwings/#:~:text=Butterfly%20pea%20Asian%20pigeonwings%20is,holy%20flower%2C%20used%20in%20rituals.

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IMPORTANCE OF HETEROCYCLES IN PHARMACEUTICAL INDUSTRIES

Jyoti Agashe
Department of Chemistry,
HPT Arts and RYK Science College,
Nashik, M.S., India
Corresponding author E-mail: jyotiagashe@gmail.com

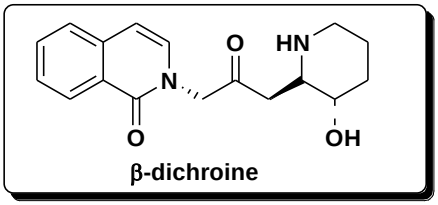
Heterocycles in medicine:

Heterocycles are the foundation of most medicines. According to a review of the best selling brand name pharmaceuticals in 2007, heterocycles were found in 8 of the top 10 and 71 of the top 100 [1]. This is hardly surprising given that heterocycles have always dominated pharmaceutical chemistry. Many pharmaceutical companies' patents in the United States are for heterocyclic chemicals, which reflect their prominence. A study of the patent literature from 1976 to September 2008, for example, shows that the word “pyridine” appears in 1729 patents awarded to Pfizer as a representative business. The word pyridine appears in 3504 Merck patents in the US.

This isn't exclusive to pyridine; other heterocycles in medicine include indoles, quinolines, azepines, and pyrimidine in a wide range of pharmaceutically active substances. The selection of these five groups is arbitrary, and it ignores a variety of additional heterocycles, but it is intended to provide instances of heterocycles' application in medicine. This categorization is also oversimplified. Several types of ring systems can be found in medicinal substances. The pyridine molecules utilised as proton pump inhibitors, for example, also have a benzimidazole structure. The indole ring is also present in dimebon, which is addressed in the section on pyridines.

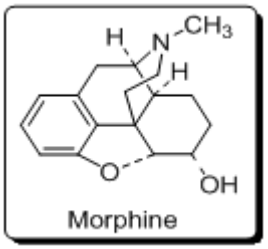
This is also arbitrary, and it is not intended to infer that the pyridine structure is more relevant for Alzheimer's treatment than the indole structure. The United States Adopted Names Council assists health care providers by selecting nonproprietary names for novel medications based on pharmacological and/or chemical correlations. These new medications are organised by chemical structure in one monograph, and heterocycles are discussed extensively [2]. 5-membered heterocycles, 6-membered heterocycles, 5-membered heterocycles fused to one benzene ring, 6-membered heterocycles fused to one benzene ring, bicyclic-fused heterocycles, and polycyclic-fused heterocycles are all examples of heterocycles with five or six members.

Heterocycles have been used for medical purposes from the dawn of time. Shen Nung, a Chinese scholar-emperor who flourished in 2735 B.C., recommended the plant Ch'ang Shan for fever treatment [3]. Dichroins [4], such as –dichroine [5] were later discovered in Ch'ang Shan.



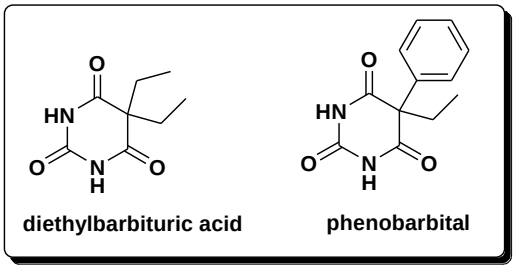
β-dichroine

Opium is another example of an old heterocyclic compound in use. Opium, which contains multiple alkaloids including morphine, was introduced from Greece by the Egyptians prior to the Trojan War in 1200 B.C. [6]. The Sumerians (Babylonians) carved tablets featuring images of the opium plant long before the ancient Greeks. Opium was used in some of the early drug trials on animals. Robert Whytt, for example, utilised frogs to research the effects of opium on the heart in the 1700s [7].



Morphine

Antipyrine appears to be the first synthesised heterocyclic medication. Antipyrine, like aspirin, is a pyrazole analgesic and antipyretic. Ludwig Knorr used Emil Fischer's discovery of phenyl hydrazine to make antipyrine, and he received a patent for the process in 1883. One year after the market opened, about 6 metric tonnes were sold, and by 1899, sales had risen to nearly 800 metric tonnes [8]. Antipyrine has lately been used to treat ear pain and swelling in a solution containing benzocaine. Another early drug family is based on malonylurea, which was discovered by Von Baeyer in 1864. [9,10] Knorr's synthesis. Von Baeyer named malonylureabarbituric acid because it is made up of urea and malonic acid [11].



diethylbarbituric acid phenobarbital

Barbituric acid comes in a variety of forms. Diethylbarbituric acid, commonly known as barbital, malonal, or gardenal [12], was the first to be commercialised. Bayer Pharmaceuticals first introduced phenobarbital in 1912, and it is being used to treat epilepsy today [13]. The impact of phenobarbital on cerebral circulation was investigated in 1926. More than 2500

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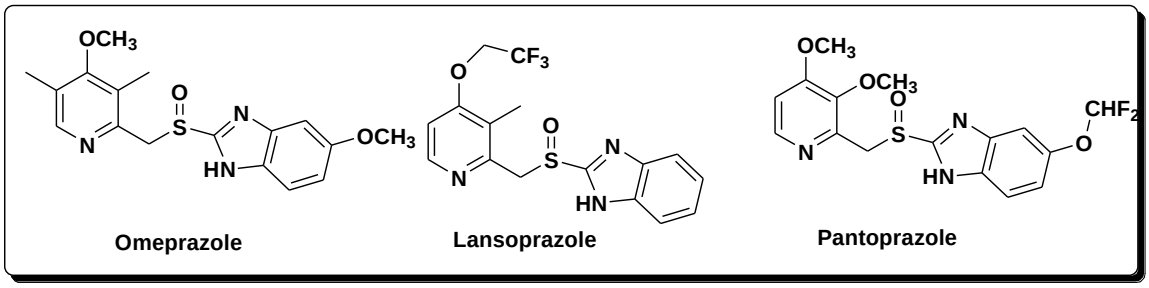
barbiturates were synthesised during the twentieth century, with 50 of them being used in clinical trials.

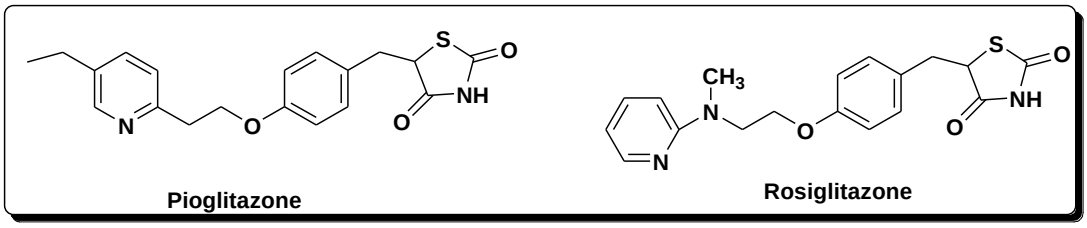
There are many heterocyclic compounds shows pharmaceutical importance such as

Pyridine:

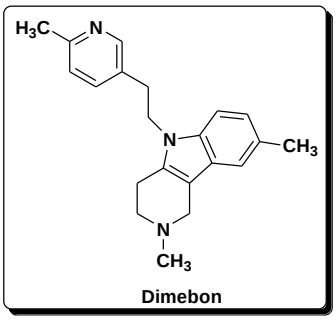
Many modern medications contain the pyridine ring. Some proton pump inhibitors, which are intended to reduce the quantity of acid produced by the stomach, contain it. Reflux illness, ulcers, and heartburn can all be treated with these medications. Some examples include omeprazole, lansoprazole, and pantoprazole.



Pioglitazone and rosiglitazone are two thiazolidinedione chemicals that contain the pyridine ring and are used to treat diabetes.

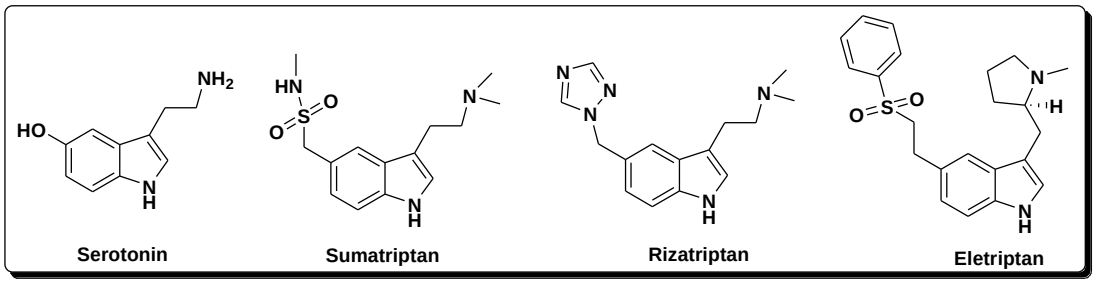


Dimebon, which has both a pyridine and an indole ring, is being developed jointly by Pfizer Inc. and Medivation Inc. For Alzheimer's disease, Dimebon is in Phase III clinical studies [14].



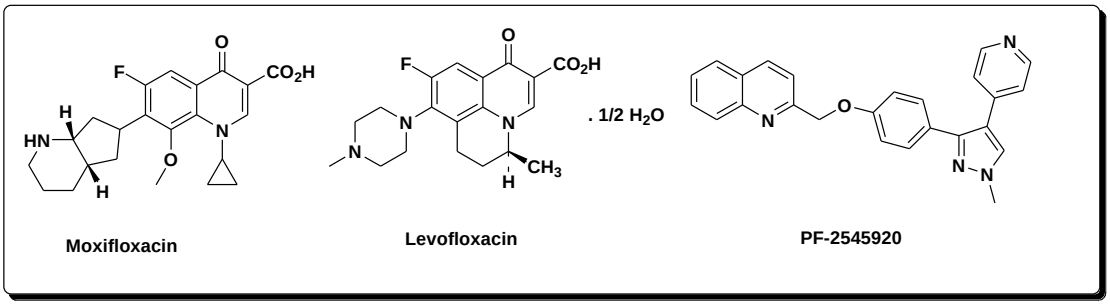
Indoles:

Serotonin, an indole, is produced naturally in the body. Serotonin levels drop in the majority of migraine cases. The indole structure is used in several migraine medicines.

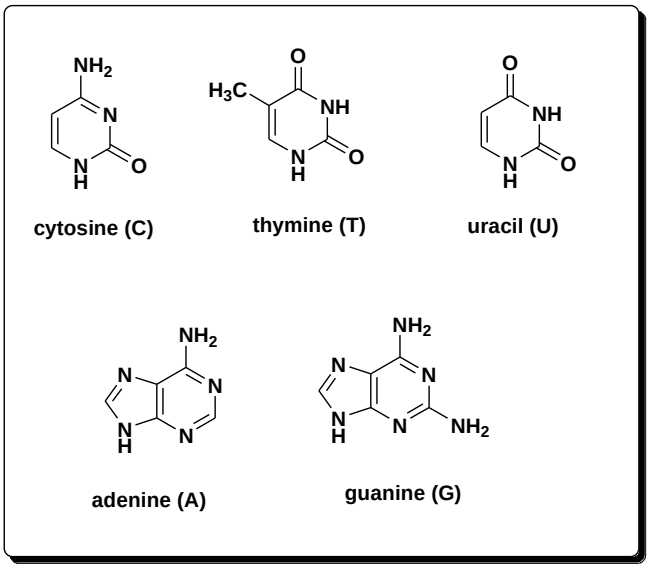


Quinoline:

Quinolone antibiotics, particularly fluoroquinolone antibiotics, are a class of medicines that include the quinoline ring. In the early 1960s, the first quinolone antibiotic was discovered by chance [15]. Second-generation antibacterials are fluoroquinolones. Ciprofloxacin and moxifloxacin kill sensitive bacteria by preventing them from producing vital proteins that they need to survive. In a sterile ophthalmic solution, moxifloxacin is employed. Pfizer had initiated phase II clinical studies for PF-2545920, a quinoline-based molecule, for the treatment of schizophrenia in 2008 [16].



Pyrimidines:



The pyrimidine ring is found in cytosine, thymine, and uracil, while the purine ring is found in adenine and guanine. Because the pyrimidine/purine ring is present in all five nucleic acid bases, it's no surprise that pyrimidines are prevalent in pharmaceutically active ingredients

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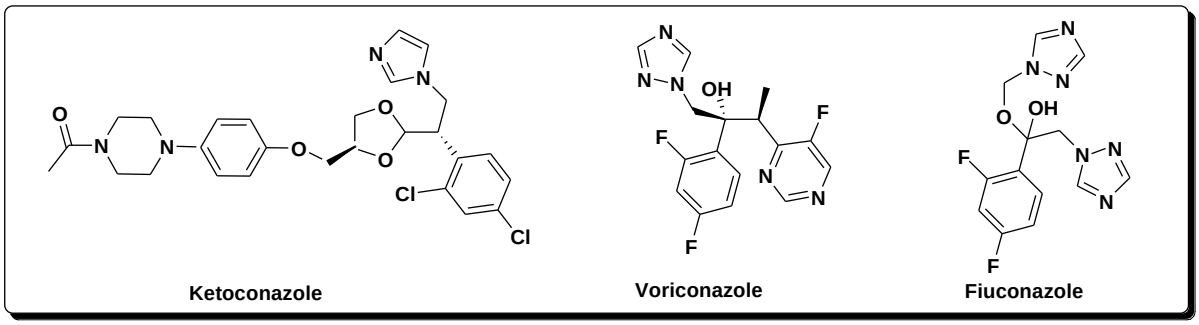
used in a wide range of therapies, including antipsychotics, cholesterol reduction, cancer, erectile dysfunction, antivirals, and human immunodeficiency virus (HIV).

Imidazole:

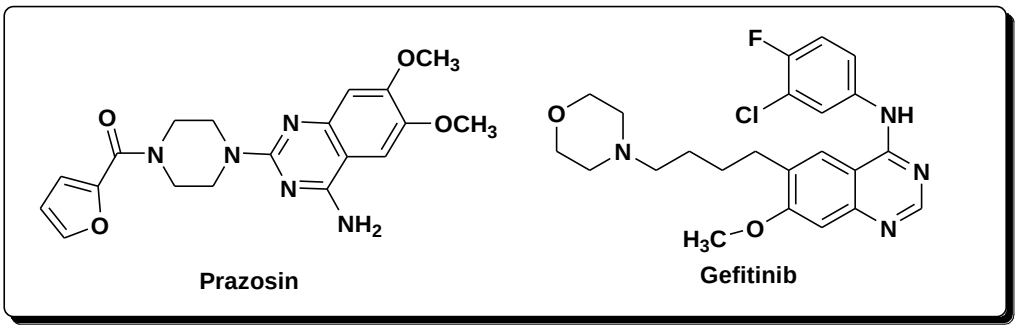
Many systemic fungal diseases benefit from the use of substituted imidazole derivatives. Ketoconazole, miconazole, voriconazole, and fluconazole are among the azole antifungals of the imidazole class [17].



Quinazoline containing drugs:

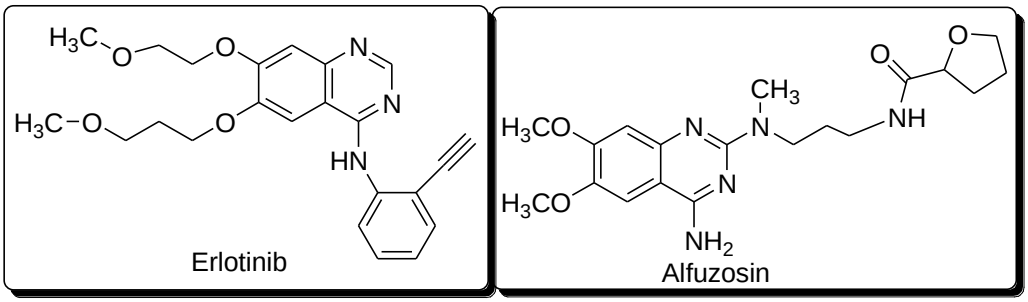
2-[4-(2-furoyl)piperazin-1-yl]-6,7-dimethoxy quinazo line-4-amine [18] is the chemical name for prazosin. It's a sympatholytic medication that's used to lower blood pressure. It belongs to the -adrenergic blocker family, which works by relaxing blood arteries to reduce blood pressure. Prazosin is specific for the -1 receptors found in vascular smooth muscle. The vasoconstrictive action of norepinephrine, which would typically elevate blood pressure, is mediated by these receptors. Prazosin lowers blood pressure by inhibiting these receptors. Minipress, vasoflex, pressin, and hypovase are some of the other names for it.

Gefitinib, commonly known as Iressa and marketed by Astra Zeneca and Teva, is a medication used to treat certain cancers. Gefitinib is an EGFR (epidermal growth factor receptor) inhibitor that prevents target cells from signalling through the epidermal growth factor receptor. Although gefitinib has yet to be demonstrated successful in additional malignancies, it has the potential to be used in the treatment of tumours involving EGFR overexpression. On the basis of the most recent scientific evidence, applications to expand its designation as a first-line treatment in patients with EGFR mutations are currently in the works. N-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy) quinazolin-4-amine [19] is the chemical name for it.

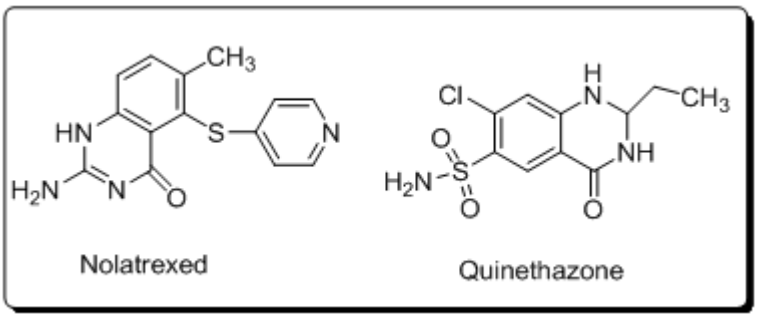


Tarceva is the brand name for Erlotinib, which is also known as N-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy)quinazolin-4-amine in the chemical world [20]. It is a cancer treatment that is used to treat non-small cell lung cancer, pancreatic cancer, and a variety of other malignancies. It works on the epidermal growth factor receptor as a tyrosine kinase inhibitor (EGFR). Erlotinib is an anti-EGFR drug. The medicine comes after Iressagefitinib, which was the first of its kind. Erlotinib is a tyrosine kinase inhibitor that targets the epidermal growth factor receptor (EGFR), which is widely expressed and occasionally mutated in cancer. It binds to the receptor's adenosine triphosphate (ATP) binding site in a reversible manner. It's a tyrosine kinase inhibitor for the epidermal growth factor receptor.

UroXatral, urion, xatral, and alfetim are brand names for alfuzosin, which is chemically known as N-[3-[(4-amino-6,7-dimethoxy-quinazolin-2-yl)-methyl-amino]propyl] tetrahydrfuran-2-carboxamide [21]. It's an antagonist for the -1 receptor that's used to treat benign prostatic hyperplasia (BPH). It makes it easier to urinate by relaxing the muscles in the prostate and bladder neck. In patients with severe renal insufficiency, alfuzosin should be taken with caution, and it should not be recommended to individuals who have a history of QT prolongation or who are taking drugs that extend the QT interval.



2-Amino-6-methyl-5-(4-pyridylthio)-1H-quinazolin-4-one is the chemical name for Nolatrexed. Thymidylate synthase inhibitor Nolatrexed is a drug. Hydromox is a brand name for 7-chloro-2-ethyl-4-oxo-1,2,3,4-tetrahydro quinazoline-6-sulfonamide [23], which has a chemical name of 7-chloro-2-ethyl-4-oxo-1,2,3,4-tetrahydro quinazoline-6-sulfonamide. Quinathazone is a type of thiazide diuretic that is used to treat high blood pressure. Dizziness, dry mouth, nausea, and potassium deficiency are all common adverse effects.



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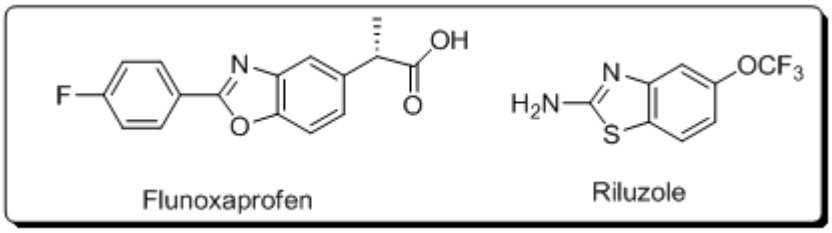
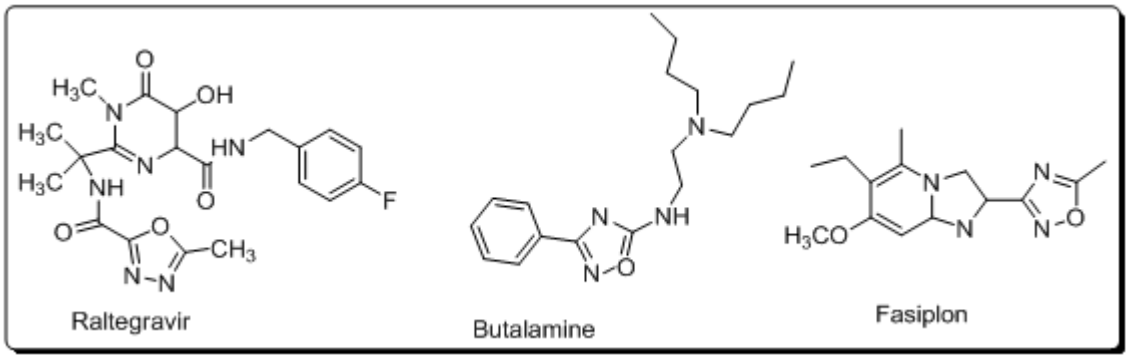
Benzoxazole and benzothiazole:

Flunoxaprofen, also known as priaxim, is a non-steroidal anti-inflammatory medication that is chiral in nature. The medication riluzole is used to treat amyotrophic lateral sclerosis (ALS).



Oxadiazole:

Raltegravir is an antiretroviral medication that is used to treat HIV. Butalamine is a vasodilator, while Fasiplon is a nonbenzodiazepine anxiolytic from the imidazo pyrimidine family. "2-(oxadiazolyl)- and 2-(thiazolyl)imidazo[1,2-a] pyridines as agonists and inverse agonists at benzodiazepine receptors [24].



The heterocyclic nucleus is one of the most significant components of a wide range of natural products and pharmaceuticals. Antimicrobial, anti-inflammatory, analgesic, antiepileptic, antiviral, antineoplastic, antihypertensive, antimalarial, local anaesthetic, antianxiety, antidepressant, antihistaminic, antioxidant, antitubercular, antiparkinson's, antidiabetic, antiobesity, and immunomodulatory agents, among others, contain a heterocyclic nucleus as a core structural component

This chapter reflects the role of heterocycles in the biological evolution of society, as well as in the understanding of life processes and efforts to improve people's quality of life. Researchers have been fascinated by sulphur, oxygen, and nitrogen-containing heterocyclic molecules for decades, thanks to the historical development of organic synthesis. Their biological activity and unusual architectures sparked curiosity, leading to a variety of applications in pharmacological, agrochemical, and, more recently, material sciences research.

References:

1. Lednicer, D. In The Organic Chemistry of Drug Synthesis, Vols. 1-7, Wiley- Interscience, New York.
2. Lednicer, D. In Strategies for Organic Drug Synthesis and Design, Wiley- Interscience, New York, 1998, 190.
3. Walker, W. G. U. S. Pat. No. 4,256,759, 1981.
4. Chem. Abst. 1981. 95, 7046.
5. Katritzky, A. R.; Rees, C. W.; Scriven, E. F. V. In Comprehensive HeterocyclicChemistry II, Vol. 2, Pergamon, Oxford, 1996.
6. Bradshaw, J. In Chronicles of Drug Discovery, Lednicer, D. Editor, American Chemical Society, Washington, DC, 1993, p. 45.
7. McIntosh, J. M.; Khalil, H. Can. J. Chem. 1975,53, 209.
8. Yoshida, C.; Hori, T.; Momonoi, K.; Nagumo, K.; Nakano, J.; Kitani, T. Fukuoaka, Y. Saikawa, I. J. Antibiot. 1985, 38, 1536.
9. Doyle, F. P.; Long, A. A. W.; Nayler, J. H. C.; Stove E. R. Nature1961,192, 1183.
10. Self, C. R.; Barber, W. E.; Machin, P. J.; Osbond, J. M.; Smithen, C. E.; Tong, B. P.; Wickens, J. C.; Bloxham, D. P.; Bradshaw. D. J. Med.Chem. 1991, 34, 772.
11. White, R. L.; Wessels, F. L.; Schwan, T. J.; Ellis, K. O. J. Med. Chem. 1987, 30, 263.
12. Lancini, G. C.; Lazari, E. Experentia1965, 21, 83.
13. Budowski, M. Angew. Chem. Int. Ed. 1968, 7, 827.
14. Ames, M. M.; Kovach, J. S.; Rubin, J. Cancer Res. 1984, 44, 4151.
15. Piml, J.; Sorm, F. Coll. Czech. Chem. Commun. 1964, 29, 2576.
16. Yang, J.; Teng, Y.; Ara, S.; Rallipalli, S.; Cook, J. M. Synthesis2009, 1036.
17. Arduengo, A. J. US patent 6,177,575.
18. Shen, H. In Illustrated Pharmacology Memory Cards: PharMnemonics. Minireview2008, 13.
19. Pao. W.; Mille,r V.; Zakowski, M. E. G. F. Proceedings of the National Academy of Sciences of the United States of America 2004, 101, 13306.
20. Raymond, E.; Faivre, S.; Armand, J. Drugs 60 Suppl.2000, 1, 15.
21. Hwang, D. F.; Noguchi T. Adv. Food Nutr. Res. 2007, 52, 141.
22. Hughes, A. N.; Rafi, I.; Griffin, M. J. Clin.Cancer Res. 1999, 5, 111.
23. Cohen, E.; Klarberg, B.; Vaughan, J. R. J. Am. Chem. Soc.1960,82, 2731.
24. Tully, W. R.; Garder, C. R.; Gillespie, R. J.; Westwood, R. J. Med. Chem.1991, 34, 2060.

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RESEARCH AND DEVELOPMENT IN PHARMACOLOGY AND TOXICOLOGY

Ramandeep Kaur, Rishabh chalotra and Neeru

M. Pharmacy (Pharmacology), M.M.C.P,

Maharishi Markandeshwar (Deemed to be University)

Mullana, 133203, Ambala (Haryana), India

Corresponding author E-mail: ramandeepk0013@gmail.com, rishabhchalotra5@gmail.com

Abstract:

Research and development is the backbone of modern discoveries of drugs, devices, as well as concepts with proper evidences. As we know a lot of researchers, organisations are trying best efforts to make a formulation, drugs that would be more effective or having less side-effects than already existing in market but unless and until we don't check them or check their efficacy on living system, we can't be sure about the results. Pharmacology plays a great role in this. It helps in the testing of compounds or test drugs on the particular animal, which resembles the human most with following proper ethical guidelines. So that we could understand the Pharmacokinetic as well as Pharmacodynamic of the drug more efficiently. The toxic effects of the drug are also determined in pre-clinical as well as in clinical phases. Because drugs sometimes have very small therapeutic index, if dose get increase it could cause serious harm, similarly if dose is less, there would be no therapeutic effect. Optimum dose is also determined with these testing. All the studies are performed by following proper guidelines, so that all the data should be internationally accepted without any malpractice, or unethical behaviour.

Research and development:

Research and development is the process which is intended to create something new or improve the existing, that can give advantage at business, national or even to whole world. Research means you try to study about new ideas and information and try to discover facts about it and whereas development means to give the perfect shape to the ideas it can be product or process. Research and development as whole means, to identify and search new ideas, methods or knowledge and turning these ideas to reality.

It is also known as a systematic and creative work, the main purpose of Research and development is increasing the stock of knowledge, along with that it also increases the

knowledge of man, culture and society. Research and development is the most important factor for the development for a country.The main use of this knowledge is to desire new innovations.In Healthcare Rand D includes the Basic research of compounds (synthetic as well as Natural origin), equipment's and instruments etc. [1]. We apply these researches and experiments to get evidences or proof of their actionsand to make new drugs, vaccines and a lot of experimental development in research. The goal is to compete with whole world by improving the existing systems or innovate something new to serve others.

As we know today everything is updating or mutating even the diseases are getting modified, like microorganisms are getting resistant to the existing medications or antibiotics, so only by modifying new drugs or creating other alternates could help us. [2] The most common example is Covid-19, whose variants are getting more and more mutations like B.1.1.7, B.1.351, B.1.617.2 etc. making the disease more severe and difficult to cure. [19]

Pharmacology:

It is defined as a branch of science which deals with the study of drugs or compounds that can be from natural origin, synthetic as well as endogenous (within own body) on living system (cell, tissue, organ, or whole organism), i.e., the pharmacokinetics, and pharmacodynamics of the drug which is how a body react to the drug and what drug does to the body. The study can be performed *in vivo* or *in vitro* including the toxicological studies. In basic, it's the branch of science which determines the drug effect on living system.

History:

Research and development was originated in the 19th century theirefforts makes drug discovery anddevelopment possible. Many early medicinal treatments were discovered in antient timeslike Chinese herbal medicines, Egyptian medicines, Ayurvedic medicines, etc, but there was not a scientific evidence to prove their efficacy. The extraction of morphine from opium by Friedrich Serturner in the year 1806 launched the 1st generation of true drugs and he was also the first to study their pharmacological effects in animals. A lot of drugs were discovered using Serturner's experiment method. [3]

Pharmacology is one of the keystones of the drug discovery process. The medicinal chemist may create the candidate compound, but the pharmacologist is the one who will test their pharmacological activity. Pharmacology study helps to find the effect of drug and also tell us their exact effects that are its Pharmacokinetics as well as Pharmacodynamics.

The birth of Pharmacology is not clearly known, but the word Pharmacology is derived from Greek word Pharmakon which mean "Poison" in classical Greek, whereas "Drug" in Modern Greek. It has both meaning Remedy and poison. Specifically, pharmacology is a

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biomedical science primarily dealing with research, discovery and characterization of compounds and their biological effects on cellular and biological functions. In the early 19th century, Physiologists performed many Pharmacological studies. In the United States, the first chair in pharmacology was established at the University of Michigan in 1890. Today there is a Pharmacology department in every college of medicine of Pharmacy. [4]

Pharmacology is considered to have emerged as a separate science only when the first chair in the university was established according to Walter Sneader, this occurred in 1847.In this year, Rudolf Buchheim was appointed as thefirst Professor of pharmacology at university of Dorpat in Estonea (Russia). He also built alaboratory at his own expense in the basement of his home. [5]

The Process:

Analysis across all therapeutic area indicates that the development of new medicines from target identification through approval for getting medicine to the market, takes about 12 years and often much longer. [6] It is also estimated that cost of developing a new medicine ranges from $314 million to $2.8 billion.[7] It is also estimated that from more than 5000 compounds there is only 1 compound which reaches to final stage, rest of all compounds get rejected. According to WHO more than 60,000 clinical trials were registered worldwide in 2018.

Why Research and development is important?

Research and development is a main component in the successful discovery and development of new drugs and development of new drugs and medical devices getting approved for market, it's also an area of big businesses, with investment in Rand D experiencing historic growth year after year. Research and development is known by its innovation and obtaining new knowledge, before introducing new products and services into the marketplace or improving the existing similar systems.

Types of Rand D:

There are mainly three types of research and development as were explained by the National Science Foundation:**[15]**

1. **Basic Research:** It is a broad approach to the research, which may not have specific end goals in mind. The main focus is on gaining knowledge, which can then be linked to a company's stated goals.
2. **Applied Research**: It is a more defined approach with a particular goal. A company may have identified a specific target and is now looking for ways or compounds to act on that target.

3. **Development:** It is defined to be as research gathered in new innovations or in improving existing products and services, it can also lead to additional paths for research.

Animals in research:

As we know, animals been a companion to humans since thousands of years, they have helped us a lot like hunting, protection, milk, meat etc.Similarly,when a new drug and surgical technique is developed, we cannot use them directly on humans, because they could seriously harm us rather than being good and there are several guidelines too which prevent us from doing so, like Declaration of Helsinki, which was adopted by the World Medical Association (WMA). These drugs are tested in animals, to make sure that it is safe and effective. Selection of animals is also very major concern, selection is based on the characters of animal, how much animal is similar to human being, on that basis animal models are selected. For example, as we know a lot of our experiments are performed on rats and mouse, but if we want to induce vomiting to our animal, rats would not be suitable, due to lack of Chemoreceptor Trigger Zone (CTZ) in that case we use other animals like ferrets, dogs, cats, etc. [13] One or more species can be used to determine the drug response, which is dependent on ADME[16]

Authorities for using animals for experimentation:

Table 1: Animals with some of their research applications [14]

Sr. No.	Animal	Some uses
1	Rats	Ageing, cancer, arthritis, infectious diseases, hypertension, surgical induced diseases, genetic principles.
2	Mice	Ageing, cancer, arthritis, infectious diseases, hypertension, surgical induced diseases, genetic principles.
3	Dogs	Cardiology, endocrinology, orthopedics, prostatic devices, surgical techniques, product safety.
4	Cats	Neuroscience, consequences of ageing, certain inherited diseases, infectious diseases.
5	Hamster	Obesity, Prostatic disease, toxicity, induced carcinogenesis, dental caries, chronic bronchitis, teratogenesis.
6	Aquatic species	Cancer, tissue healing studies, developmental studies, gene functions.

CPCSEA (The **C**ommittee for the **P**urpose of **C**ontrol and **S**upervision of **E**xperiments on **A**nimals) is a committee for the prevention of Cruelty to animals enacted in 1960. India is one

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of the few countries to introduce this law, whereas such laws were instituted in France in 1963 and in USA in 1966. **[18]**

There main roles are to give humane care to animals, like proper Food, water, temperature, Rh, storage facility, well trained staff, maintenance of record, Anaesthesia, Euthanasia, and Disposal, etc.

Similarly on institutional levels **IAEC** (**I**nstitutional **A**nimal **E**thical **C**ommittee)are formed to monitor the animals well-being.

Toxicology:

Toxicity testing of new medicines or compounds is very crucial for drug development process. Toxicology is defined as a branch of science which deals with how natural as well as man made compounds or substances have undesirable effects on living organisms (Humans, animals, and environment). Whereas some also refer it as "science of safety". In these studies, we predict, how different compounds or chemicals could harm and then shares that information to protect public health. There should be few things which we need to look when we study toxicology.

Every person will not respond to toxicity as same way, there are a lot of factors responsible like-

- The amount and duration of exposure to the compound.
- Persons susceptibility to a substance.
- Empty stomach or with meal.
- Condition of the patient.
- Route of administration.
- Ageand Sex of person.Etc.**[8,9]**

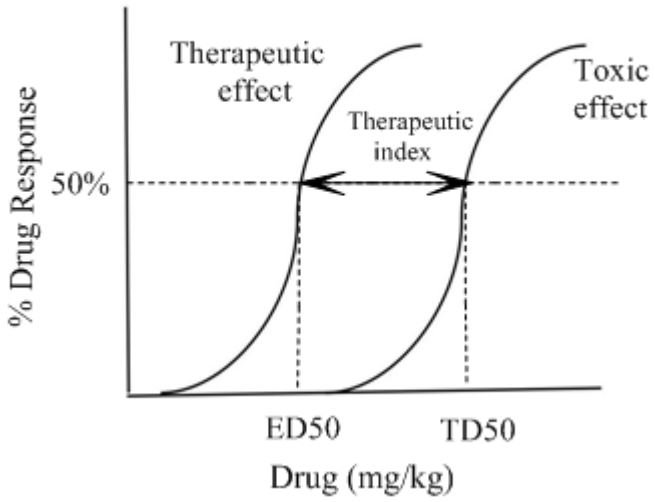
R and D in toxicology:

The use of animals as in toxicology studies began in 1920. J.W, Trevan proposed the use of LD_{50} i.e., lethal dose at which 50% population dies, the tests is to determine the lethal dose of individual chemicals. After that, a FDA scientist John Draize developed a method for testing eye and skin irritation using rabbits, this method was widely accepted for testing the effects of compounds. Later the US National Cancer Institute (NCI) developed tests to identify carcinogenic compounds, by daily dosing of rats and mice for 2 years.

The most famous example of toxicity was Thalidomide tragedy, which was used for sedative and tranquiliser, but later on it was being used off label against morning sickness, nausea, by pregnant women, which was very widely used drug in 1960's, but later on when these females gave birth to children, thousands of babies were born with birth defects caused by thalidomide.After this tragedy, all regulatory agencies concentrated on determining the toxicity studies, and made them mandatory for Investigational New Drug (IND), In the late 1980's the Organisation for Economic Co-operation and development (OECD) and the International Conference of Harmonisation (ICH) brought out the guidelines for toxicity study of compounds, or pharmaceutical drugs.[12]

OECD-The Organisation for Economic Cooperation and development is an intergovernmental economic organisation, founded in 1961 to seek answers for common problems, coordinate domestic and international policies for members. It has 38 members. These countries follow same standards, which are accepted by all members for quality products like GLP. GLP (Good Laboratory practice) are the rules and regulations that are required for quality management system to ensure the validity, integrity and reliability of non-clinical safety for regulatory evaluation and approval. GLP believes everything should be well documented and essentially follow SOP's [17].

This is a graphical representation of therapeutic effect vs toxic effect, in some compounds or drugs the gap between therapeutic effect vs toxic effect is very small, in those cases, we have to be extra cautious regarding the dose. These things can only be discovered when we test them on living system, so animals are used to determine these studies.



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In **OECD ICH** guidelines there is proper information about the study of followings:

Acute, sub-acute and chronic toxicity by Oral, Dermal, and Inhalational.

Acute eye irritation, skin sensitization, dermal irritation and toxicity studies.

Reproductive toxicology studies, Genotoxicity studies, carcinogenic studies. Etc.

These guidelines are represented by specific guideline number some examples of which are enlisted below.

Table 2: Toxicity guidelines with their number's

Sr. No.	Toxicity Guideline	Guideline No.
1	Acute Dermal toxicity: Fixed dose	402
2	Acute Dermal Irritation/Corrosion	404
3	Acute Eye irritation/Corrosion	405
4	Repeated dose 28 days Oral toxicity study in Rodents	407
5	Repeated Dose Dermal Toxicity: 21/28days study	410
6	Acute Oral Toxicity	423
7	Carcinogenicity studies	451
8	Chronic Toxicity studies	452

These guidelines tell us about the following-

1. Procedure of the study
2. Housing and feeding of animals
3. Species of animal
4. Administration of dose
5. Number and sex of animals
6. Duration of study
7. Physical parameters (body weight, food/water intake, inflammation)
8. Haematology and clinical biochemistry
9. Histopathology
10. Data management and reporting, etc.

Toxicity is also determined in clinical trials (Phase I, II, III) as well as Phase IV clinical trials (Post market surveillance) because as we perform tests on limited people and a particular class of people, there could be less chances, that the compound could show toxic effect or

adverse effect, but as the number of people increases with different ethnic backgrounds the chances of toxic effect or adverse effect also increases. That is why a lot of drugs are withdraw from market after successfully completing clinical trials till phase III.

Table 3: Signs and symptoms of toxicity and their effector organ [20]

Sr. No.	Category	Affected system	Signs and Symptoms
1	Dermatological	Skin	Itching, Redness, Dermatitis, Burning
2	Endocrine	Glands	Hormone imbalance
3	Gastrointestinal	Intestine, Stomach	Nausea, Vomiting, Stomatitis, Diarrhea
4	Hematological	Blood	Anemia, Polycythemia, Thrombocytopenia
5	Nervous	Brain and spinal cord	Confusion, dizziness, convulsion, coma
6	Renal	Kidney	Urinary frequency, Quantity, Color, and smell
7	Reproductive	Testis, Ovaries	Miscarriage, Stillbirth, Infertility, Birth defects
8	Respiratory	Nose, Lung, Trachea	Coughing, Irritation, Choking, Tight chest

Table 4: Things which are kept in mind, while testing compounds [10]

Sr. No.	Studies
1	**Good Laboratory Practice**
2	**Test Animals**
A	Care, maintenance, and housing
B	Selection of species, strains, and sex
C	Age
D	Number and Sex
E	Infected Animals
F	Animal identification
G	Caging
H	Diet
I	Assignment of control and compound treated animals
J	Mortality

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K	Autolysis
L	Necropsy
3	**Test Substance**
A	Identity
B	Composition/ Purity
C	Conditions of storage
D	Expiry date
4	**Experimental design**
A	Duration of testing
B	Route of Administration
C	Dose groups
I	Selection of treated doses
II	Controls
D	Computerized systems
5	**Observations and clinical Testss**
A	Observations of test animals
B	Body weight and Feed intake Data
C	Clinical testing
I	Opthalmological Examinations
II	Hematology
III	Clinical chemistry
IV	Urinalyses
V	Neurotoxicity Screening/ Testing
VI	Immunotoxicity
6	**Necropsy and Microscopic Examination**
A	Gross Necropsy
B	Organ weight
C	Preparation of tissues for microscopic Examination
D	Microscopic Evaluation
E	Histopathology of Lymphoid Organs

References:

1. Aghion P. and Howitt, 1997: endogenous Growth theory the MIT Press, Cambridge Massachusetts.
2. Odonkor Stephen T,2011: Bacteria Resistance to Antibiotics: Recent Trends and Challenges, International Journal of Biological and Medical Research, https://www.researchgate.net/publication/256734983.
3. The history of Therapeutic Drug Development,2017: Twist Bioscience, https://www.twistbioscience.com/blog/perspectives/history-therapeutic-drug-development.
4. Oldham F.K, Kelsey F.E, Geiking E.M.K,1955: Essential of Pharmacology, Lippincott, Philadelphia.
5. Sneader w,1985: Drug Discovey, The Evolution of Modern Medicines, Wiley, New York.
6. Dimasi J.A, Feldman L, Seckler A, Wilson A, 2010:Trends in risks associated wwith new drug development, success rates for investigational drug, clin pharmacol thesis, 87:272-277 [pubmed] [google scholar]
7. Wouters Olivier J., Estimated Research and Development Investment Needed to Bring a New Medicine to Market, 2009-2018.
8. Toxicology, National Institute of Environment Health Sciences, https://www.niehs.nih.gov/health/topics/science/toxicology/index.cfm.
9. Toxicology curriculum for Communities Trainer's Manual, Agency for Toxic Substances and Disease Registry, https://www.atsdr.cdc.gov/training/toxmanual/modules/1/lecturenotes.html.
10. Redbook 2000: IV.B.1. General Guidelines for Designing and Conducting Toxicity Studies, Center for Food Safety and Applied Nutrition, Office of Food Additive Safety, April 2007, https://www.fda.gov/regulatory-information/search-fda-guidance-documents/redbook-2000-ivb1-general-guidelines-designing-and-conducting-toxicity-studies.
11. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127354/ Important
12. Parasuraman S, Toxicology screening, Journal of pharmacology and Pharmacotherapeutics, PMID: 21772764, doi: 10.4103/0976-500X.81895, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3127354/

13. Isselbacher Kurt J.,1991: Why Are Animals Used in Research, SCIENCE, MEDICINE, AND ANIMALS, National Academy Press Washington, D.C., Page No. 4, https://www.nap.edu/read/10089/chapter/1.
14. Huerkamp Michael J., 2021: Animals used in Research, MSD MANUAL Veterinary Manual, https://www.msdvetmanual.com/exotic-and-laboratory-animals/laboratory-animals/animals-used-in-research.
15. Research and Development is More Important Than You Think, 2021:Treximo, https://treximo.com/blog/importance-of-research-and-development/
16. Steinmetz Karen L., 2009: The basics of preclinical drug development for neurodegenerative disease indications, BMC Neurology, https://bmcneurol.biomedcentral.com/articles/10.1186/1471-2377-9-S1-S2.
17. OECD.org, https://www.oecd.org/chemicalsafety/testing/good-laboratory-practiceglp.htm
18. Committee for the purpose of control and Supervision of Experiments on Animals, Ministry of Fisheries, Animal Husbandry and Dairying Department of Animal Husbandry and Dairying, http://cpcsea.nic.in/Auth/index.aspx
19. Tracking SARS-COV-2 variants, World Health Organization, https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/
20. Deshmukh Chinmay Devidas, 2020, General Principles, Types, Diagnosis and Management of Poisoning, Scholars Academic Journal of Pharmacy, DOI: 10.36347/sajp.2020.v09i05.001, https://www.researchgate.net/publication/341609961.

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About Editors

Prof. R. Uma Maheshwari is working as faculty of Pharmacy at Ultra College of Pharmacy, Madurai, Tamilnadu, India. She has more than 22 years of teaching experience. She is M. Pharm; M.B.A and completed P.G. Diploma in Hospital Management from Madurai Kamaraj University, Tamilnadu. She has guided fifteen U.G. projects and 4 P.G. projects. She worked as examiner and evaluator for Diploma, Degree and P.G exams of different universities and board exams. Her research areas of interest are the development of oral formulation as controlled drug delivery systems, and developing phyto-formulas. Prof. R. Uma Maheshwari have published more than 25 papers as review and research articles in National and International publications and presented more than 30 papers in national seminars.

Dr. K.V. Madhusudhan is Assistant Professor of Botany and Vice Principal, Government College, Kurnool, AP. He obtained his PhD in Botany from S.K.University, Anantapur, AP. He was nominated for Russian Government Scholarships. He has 20 years of Teaching and Research experience. He has published 2 books and 37 research articles in Indian and International Journals. He has presented more than 70 research papers in International and National Seminars/Conferences. He was Principal Investigator of a Research Project funded by UGC. He is working as Editorial Board member for several Journals. He organized 2 International Seminars and 5 National Seminars. He is Fellow and life member of various research and academic associations. His area of research interests includes Stress Physiology and Medicinal Botany. He held several academic and administrative positions in the College. He is recipient of Best Teacher Award from Science Tech Foundation, Bengaluru and received Certificate's of Commendation in recognition of outstanding contribution from Department of Youth Services, Govt. of AP

Dr. Narayan Dattatraya Totewad is currently working as Assistant Professor in Microbiology at B. K. Birla College of Arts, Science & Commerce (Autonomous), Kalyan, Dist. Thane, Maharashtra. He has eight years of Teaching and 14 years of Research Experience. He completed Ph. D. in Biotechnology on topic "Probiotics as feed supplement for growth and development of potential aquatic organisms under the guidance of Dr. G. Gyananath in 2013, from SRTUM, Nanded, M.S. He also completed post-graduate diploma in Bio-nanotechnology (2020). He published 2 books, written 6 book chapters and 10 research papers in National and International Journals of well repute. He was honoured with Excellence and Innovation Award in Microbiology (2020) from Pearl Foundation. Two Australian Innovation Patents granted and five Indian Patents published in Indian Patent Journal, India. He selected as Mentor by CSIR-Summer Research Training Programme-2020 conducted by CSIR Jorhat, Assam, India and Guided 11 students across India. His area of research interest is Basic and Applied Microbiology, Bio-nanotechnology, Environmental Science, Food Microbiology and Biomedical Sciences.

Mr. Umesh D. Laddha is working as Assistant Professor at MET's Institute of Pharmacy, Nasik. He has total Six years experience in the field of teaching and research. Within short interval of time he has added many feathers in his hat. He is author of 6 books. He has been awarded with title Best Teacher Award by Institute of Scholars, Best Researcher of the Year Award by Operant Pharmacy Federation, Innovative Research Award by Sakal Media Groups, Young Scientist and Bronze medal at SIPRA Innovative Pharmaceutical Research Awards-2016, Hyderabad. He has represented Savitribai Phule Pune University at State level Avishkar-2019 organized by Gondwana University, Gadchiroli. He has published 18 papers in well reputed National and International journals. One patent is also on his name which is granted by Indian Patent Office. He has presented more than thirty papers in National and International conferences.

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