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Advances in Plant Science

Volume II

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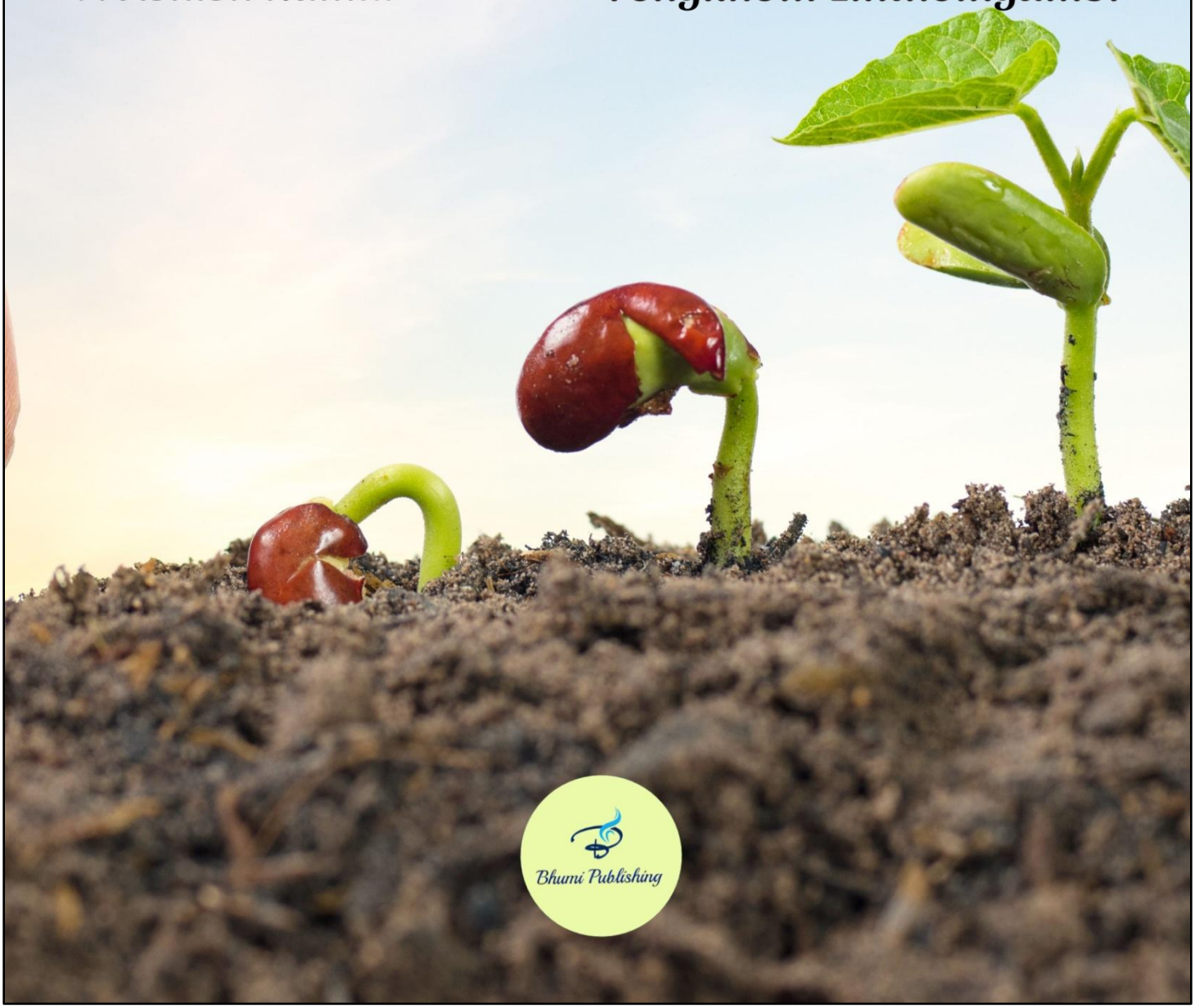

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PREFACE

We are delighted to publish our book entitled ***"Advances in Plant Science Volume II"****. This book is the compilation of esteemed articles of acknowledged experts in the fields of plant science providing a sufficient depth of the subject to satisfy the need of a level which will be comprehensive and interesting. It is an assemblage of variety of information about advances and developments in plant science. With its application oriented and interdisciplinary approach, we hope that the students, teachers, researchers, scientists and policy makers will find this book much more useful.*

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, India for compilation of such nice data in the form of this 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.

- Editors

Advances in Plant Science Volume II

CONTENTS

A COMPARATIVE STUDY ON HEAVY METAL ANALYSIS OF *SENNA AURICULATA* (L.) ROXB. OBTAINED FROM NATURAL AND POLLUTED SOURCES BY ATOMIC ABSORPTION SPECTROSCOPY

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Abstract:

Heavy metals are the most harmful contaminants because of their persistence and proclivity to accumulate in biota. The objective of this study was to compare the heavy metal concentrations of *Senna auriculata* (L.) Roxb. with soil samples collected from natural and polluted sources. Heavy metals such asPb, Cr, Cd, Zn, Cu, and Fe were determined by using atomic absorption spectroscopy. The results revealed that the majority of the heavy metal levels of the analyzed soil samples were below the limit values. However, The Cd level exceeded the standard in polluted areas such as site-3 soil sample (9.65mg/kg) and site-4 soil sample (6.74 mg/kg). The Zn level exceeded the permissible limit in the site-3 soil sample (115.84 mg/kg). Analytical results of *S. Auriculata* show that samples A and B (natural sources) had heavy metal contents below the permissible level except for cadmium. Cadmium is not detected in sample B. while the samples from polluted sources had heavy metal contents higher than the permissible level except cadmium and copper. The levels of lead, and zinc in sample C, as well as iron and chromium in sample D, of both leaves and floral extract, exceeded the standard limit.These results showed that the majority of heavy metals have been accumulated above the standard levels in the plant parts of *S.auriculata* collected from the polluted sources when compared to the natural sources. This could be the result of Heavy metal pollution from industrial sources navigating their way from soil to plants. These exceeded levels of these heavy metals become toxic to humans on consumption.Therefore, to utilize and gain the values of the pharmacological effects of this experimental plant, the source obtained from the unpolluted natural environment is preferable.

Keywords: Heavy metals, natural source, permissible limit, polluted source, *Senna auriculata*

Introduction:

Environmental pollution is a significant ecological issue since it directly or indirectly impacts people. The main causes of environmental pollution are urbanization, industrialization, and the growth of contemporary technologies. Many organic and inorganic substances that are discharged into the environment can be dangerous. When a chemical exceeds its tolerated limit, it is classified as contamination. Heavy metals are the most harmful contaminants because of their persistence and proclivity to accumulate in biota. The topic of heavy metal pollution in the soil is gaining attention in most major cities throughout the world. As a result, the environment may experience geo accumulation, bioaccumulation, and bio-magnification. Rapid industrialization and disorderly urbanization, together with the ongoing use of enormous quantities of fertilizers and pesticides, resulting in the accumulation of toxic compounds in soil, water, and air (Kumar *et al*., 2015, Rodriguesa *et al.,* 2017). Exhaust emissions and fossil fuel combustion have also been recognized as the primary sources of metallic pollution, particularly in roadside soil. Arsenic, cadmium, lead, and mercury is among the most dangerous elements in the environment, according to the Environmental Protection Agency (EPA) by Goyer (2004). Among different heavy metals lead, cadmium, copper, zinc, nickel, iron, and chromium are the major metallic pollutants of the roadside soil (Silva *et al*., 2005). Even in low concentrations, lead, and cadmium is harmful. Therefore, Lead, Cadmium, Chromium, Zinc, Iron, and Copper are focused in this study.

Heavy metals can be absorbed by plants from the soil. Plant consumption by humans and animals can be ascribed to heavy metal uptake by plant roots from polluted soil as well as direct deposition of pollutants from the atmosphere on plant surfaces (Zhuang *et al*., 2009). The interaction of soil-plant roots is crucial in regulating the transit of heavy metals from the soil to plant components (Sharma *et al*., 2009). Heavy metals' toxicity to humans is determined by their dosage, rate of emission, and duration of exposure. In low amounts, copper and zinc are vital trace metals for living organisms; yet, in excessive concentrations, they can cause toxicity. Heavy metal excesses in plants and animals can have a wide range of acute and chronic consequences on a wide range of organisms in various habitats.With the commercialization of herbal medicine, ensuring the safety, uniqueness, and efficacy of medicinal plants and herbal products has become critical. The safety of a medicine is defined by its pharmacological-toxicological profile and the adverse effects induced by contaminants in bulk and dosage forms, therefore toxic metal content should always be kept to a minimum. The detection of highly hazardous heavy metals in plant extracts is critical for food safety and quality management. The reason for this is, of course, that pharmaceuticals grown in the wild are more difficult to control

for all potential sources of pollution. As expected, the possibility of the deposition of toxic pollutants on plants cannot be disregarded. Little is known about the harmful metal status of these herbal medicinal plants produced in contaminated areas. The danger is that using herbs cultivated in polluted places could expose humans and animals to hazardous contaminants. The detection of heavy metals in plants is critical. Since the absorption of harmful trace metals by humans, this, even in moderate doses over time, can cause organ failure and prolonged arrhythmia. Several attempts have been made to estimate heavy metals and harmful trace elements in plants and medicinal formulations (Kishan *et al.*, 2014) but none have been made in *Senna auriculata* (L.) Roxb. *Senna auriculata* leaves and flowers are used to make a variety of herbal products such as senna tablets, pickles, tea, idly powder, tonic, avaram poo soornam, soup powder, diabetic food supplement, etc. Therefore, it was imperative to analyze the heavy metals (Pb, Cd, Cr, Zn, Fe, and Cu) in *Senna auriculata* leaves and flowersobtained from natural and polluted sources.

Materials and Methods:

Study species

In the present study, we chose one of the most important medicinal plants *Senna auriculata* (L.) Roxb. is a member of the Fabaceae family. It is an evergreen shrub commonly known as Tanner's cassia. The flower, buds, leaves, stem, root, and unripe fruit are used for treatments in the Ayurvedic and Siddha systems of medicine. It is frequently used in the treatment of rheumatoid arthritis, conjunctivitis, and diabetes.

***Senna auriculata* (L.) Roxb.**

Study sites

Soil and plant samples were collected from both natural and polluted sources for this investigation. Two samples (samples A and B) were taken from unpolluted areas of site 1 (Pachaimalai hills) and site 2 (Kolli hills) for natural sources.Two samples (Sample C and D) were obtained from the roadside in industrial areas of the Tiruchirappalli district. Site 3 is on the avur road, and site 4 is on the samayapuramkariyamanickam road. Many industries are located within 500 meters of this region.

Collection and identification

Senna auriculata leaves and flowers, as well as soil samples, were collected from the four study locations in June 2021. The plant species were identified and authenticated by the Botanical Survey of India, Southern Regional Centre,and Coimbatore (BSI/SRC/5/23/2021/Tech-166).

Heavy metal analysis

Apparatus

Heavy metal concentrations in powdered samples were evaluated by Atomic Absorption Spectroscopy (THERMO SCIENTIFIC- ice 3000) at the National College Instrumentation Facility (NCIF), National College, Trichy.

Working Principle and Instrumentation of AAS

Atomic-absorption spectroscopy measures the absorption of ground-state atoms in a gaseous state. Atoms absorb ultraviolet or visible light and progress to higher levels of electronic energy. The absorbance of the analyte determines its concentration. After calibrating the equipment with a known concentration of a standard substance, the concentration is usually measured using the working curve. An AAS is made up of the following functional components: the light source: A 'hollow cathode lamp,' which has a tungsten anode and a barrel-shaped hollow cathode constructed of the component to be resolved, is the most frequent source of light. These are placed in a glass tube that is filled with an inert gas (neon or argon). An atomizer, which can be a flame or an electrothermal or cold vapor/hydride producing framework, is used to atomize the sample, and a monochromator is used to choose the specific wavelength of light (spectral line) that is absorbed by the sample. The use of a specific light allows for the assurance of the selected element in the presence of others. The monochromator transmits the light onto a detector, which is typically a photomultiplier tube. This calculates the amount of light absorbed and a recorder to record and produce an electrical sign according to the light intensity.

Chemicals

All chemicals and reagents used in this study were of analytical grades. Nitric acid (HNO_3), Hydrochloric acid (HCl), Perchloric acid ($HClO_4$).Stock standard solutions for each metal lead (Pb), cadmium (Cd), zinc (Zn), iron (Fe), and copper (Cu) with a concentration of 1000 ppm. The standard solutions for all the heavy metals under study were prepared in deionized water.

Preparation of soil samples

Each soil sample was homogenized and air-dried in an oven at 105°C. The sieved material was then placed in a 300ml beaker and heated at 230°C with 15ml of nitric acid (HNO_3, 69%) and 25ml of perchloric acid ($HClO_4$,58%). The digested solution was filtered using Whatman No. 42 filter paper after being changed to ash, and the volume was increased to 50ml in a volumetric flask. An atomic absorption spectrophotometer is used to determine the metal concentration (Mehmet 2008).

Preparation of plant samples

The plant samples were air-dried at room temperature for 5-7 days before being ground into a fine powder. Uddin *et al*. (2016) stated that the most efficient digesting procedure for plant material was the wet digestion method. A total of 2.0 g of each sample was weighed and placed in a conical flask. The conical flask was then filled with a 9 ml combination of nitric-hydrochloric acids HNO_3 (65%) andHCl (37%) in a ratio of 1:3. The mixture was then gently heated over a water bath at 95 °C for 4–5 hours, or until the sample was completely dissolved. The digest was allowed to cool at room temperature before being filtered through a Whatman No. 42 filter paper and diluted with deionized water to a final volume of 50 ml. The experiment was done 3 times with each sample being aspirated twice.

Analytical procedure

Heavy metals such as lead, chromium, cadmium, zinc, copper, and iron were evaluated quantitatively on digested samples using atomic absorption spectroscopy (THERMO SCIENTIFIC- ice 3000). Table 1 lists the instrumental conditions used during heavy metal analysis. All measurements on samples and standard solutions are done in triplicate. The mean and standard deviation of the heavy metal values from three replicates were calculated using Microsoft Excel.

Table 1: Instrumental condition of AAS for heavy metal analysis

AAS Parameter	Elements					
	Pb	**Cr**	**Cd**	**Zn**	**Cu**	**Fe**
Wavelength (nm)	283.3	357.9	228.8	213.9	324.8	248.3
Slit width (nm)	0.7	0.7	0.7	0.7	0.7	0.2
Current (A)	10	12	3.5	5.0	15.0	30
Flame	AA	AA	AA	AA	AA	AA

AA - Air-acetylene

Results and Discussion:

In the present study Heavy metalslike Pb, Cr, Cd, Zn, Cu, and Fe were determined in the plant parts (leaves and flowers) of *Senna auriculata* and the soil samples from natural and polluted sources. The contents of these elements in the samples as a means oftriplicate.

Heavy metal contents in the soil samples

The mean concentrations of heavy metals in the soil samples that were collected from 4 study sites are presented in Table 2. The results revealed that the majority of the heavy metal levels of the analyzed soil samples were below the limit values given in Table 4. However, the Cd and Zn levels of some soil samples were above the standard levels. The Cd level exceeded the standard in polluted areas such as site-3 soil sample (9.65mg/kg) and site-4 soil sample (6.74 mg/kg). The Zn level exceeded the permissible limit in the site-3 soil sample (115.84 mg/kg).

Table 2: Heavy Metal concentrations in soil samples from natural and polluted sources

Elements	Natural sources		Polluted sources	
	Site - 1	Site - 2	Site - 3	Site- 4
Pb	3.09±0.28	0.07±0.21	27.44±0.02	23.95±0.30
Cr	0.97±0.02	2.73±0.04	15.82±0.07	27.69±0.15
Cd	0.28±0.37	0.04±0.02	9.65±3.21	6.74±0.02
Zn	10.6±0.01	9.02±2.37	115.84±0.25	89.35±0.29
Cu	8.02±0.62	9.76±0.01	25.89±0.03	13.32±0.03
Fe	10.23±0.03	17.8±0.21	54.03±0.74	98.46±0.42

Concentrations (Mean ± SD) (mg/kg),SD – Standard deviation; ND – not detected.

Site 1- Pachaimalai hills,Site 2 – Kollihills, Site 3 - Avur road,

Site 4 - Samayapuramkariyamanickam road.

The high Cd and Zn value of the samples from the polluted sources are attributable to the paint, chemicals, and electrical industries and some cement factories within 300m distance from the sample collection site. The dumping of industrial wastes or the deposition of pollutants into the atmosphere raises the total concentration of Cd in soils (Weggler et al., 2004). In developed countries, there is concern about anthropogenic Cd accumulations in the environment, and it is categorized as a potentially hazardous element in terms of soil biological activity, plant metabolism, and human and ecosystem health (Kabata-Pendias 2001).Zinc occurs naturally in soil (about 70mg kg1 in crustal rocks), however, Zn concentrations are increasing unnaturally as a result of anthropogenic inputs. Most of the Zn is added during industrial processes such as mining, coal and waste incineration, and steel production. Heavy metal pollution from industrial sources navigates its way to plants via soil and water.

Heavy metal contents in *Senna auriculata*

The mean concentrations of metals in leaves and flowers of*Senna auriculata*have been presented in Table 3.

Table 3: Heavy metal concentrations in *S. auriculata* from natural and polluted sources

Elements	Plant parts	Natural sources		Polluted sources	
		Sample - A	Sample - B	Sample - C	Sample - D
Pb	Leaves	1.86±0.03	0.01±0.01	13.46±0.02	7.43±0.26
	Flowers	1.02±0.01	ND	12.78±0.03	7.38±0.37
Cr	Leaves	0.63±0.01	1.07±0.03	1.89±0.01	3.84±0.01
	Flowers	0.42±0.01	0.91±0.26	1.65±0.04	3.06±0.02
Cd	Leaves	0.05±0.03	ND	0.32±0.01	0.21±0.02
	Flowers	0.03±0.01	ND	0.27±0.03	0.19±0.02
Zn	Leaves	2.63±0.02	1.56±0.01	59.05±0.12	39.24±0.01
	Flowers	0.87±0.02	1.38±0.04	56.12±0.65	38.96±0.03
Cu	Leaves	1.98±0.01	3.48±0.02	9.52±0.03	6.02±0.01
	Flowers	1.46±0.02	3.35±0.01	9.46 ±0.02	5.73±0.01
Fe	Leaves	4.87±0.27	5.48±0.03	11.92±0.01	19.28±0.22
	Flowers	3.84±0.43	5.07±0.02	10.72±0.01	18.02±0.24

All data are the mean ± SD of three replicates (mg/kg).

SD – Standard deviation; ND – not detected.

Table 4: Permissible limits for heavy metals

Sources	Elements(mg/kg)					
	Pb	**Cr**	**Cd**	**Zn**	**Cu**	**Fe**
Soil	85	100	0.8	100	36	150
Plants	10	2	0.3	50	10	15

According to WHO, (1996; 2005; 2007)

Analytical results show that samples A and B (natural sources) had heavy metal contents below the permissible level except for cadmium. Cadmium is not detected in sample B. while the samples from polluted sources had heavy metal contents higher than the permissible level except cadmium and copper. The levels of lead, and zinc in sample C, as well as iron and chromium in sample D, of both leaves and floral extract, exceeded the standard limit (Fig. 1). This could be due to the presence of paint, battery, cement, electrical, chemical, and steel manufacturing companies on site3 and site 4.Similar results were reported by Hussain and Khan (2010) in *Taraxacum officinale*, Ghani (2010) in *Zea mays*, andJuan *et al.* (2020) in *Jatropha curcas L.*

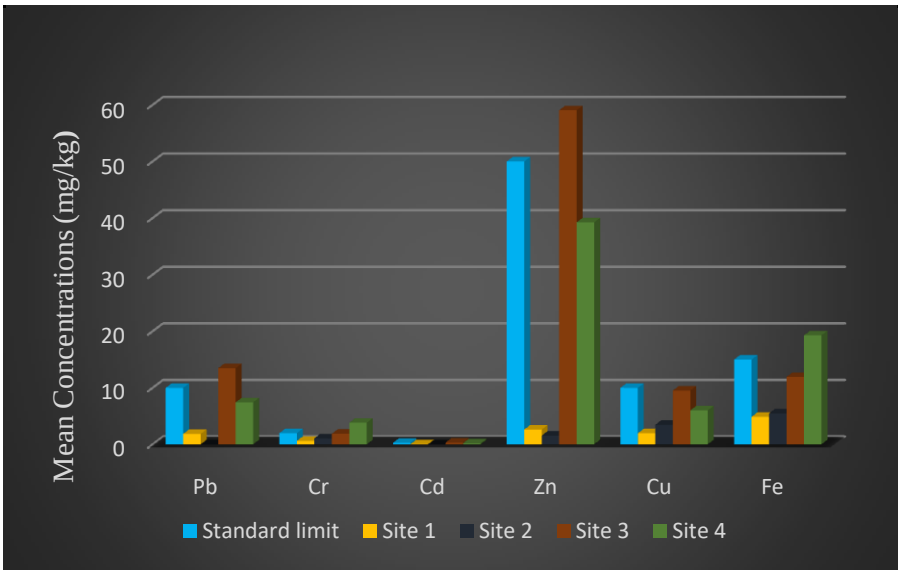

Figure 1: Heavy metal contents in *Senna auriculata* from natural and polluted sources

Lead (Pb) is a non-essential toxic heavy metal that is widespread and causes a variety of negative effects in living organisms. Although it is a natural element, anthropogenic activities such as mining, fossil fuel burning, and manufacturing, paints, batteries contribute due to its higher concentration in the soils (Tchounwou *et al*., 2012). Pb is carcinogenic, harms the respiratory and digestive systems, and inhibits the immune system. This metal is especially dangerous to children since it harms their intelligence and nervous systems (Borges *et al*., 2003).

Due to immunomodulatory, oxidative, and inflammatory pathways, Pb exposure can cause urinary tract and cardiovascular illnesses. Zinc functions as a cofactor in several enzymes involved in macronutrient metabolism and cell reproduction. Blast furnace slag and sewage, mining wastes, soil coal and fly ash, and the usage of commercial products such as zinc-containing fertilizers, fungicides, and wood preservatives, Galvanizing, plating iron and steel, luminous paints, batteries, deodorants, flame retardants, and other zinc industrial effluents are the most prominent sources of anthropogenic zinc in the soil. Excessive intake can result in arteriosclerosis, stomach cramps, pancreatic damage, and caustic effects on the skin, liver failure, renal failure, and anemia (Duruibe *et al*., 2007). Chromium is essential for metabolic operations in the body, however, the hexavalent form is toxic to humans (Khurshid and Iqbal, 1984). Because of its anti-corrosive properties, it is widely utilized in industries such as metal surface plating, leather tanning, glassware cleaning, textile production, and so on. Acute chromium poisoning results in nausea, vomiting, acute renal failure, irritation, contact dermatitis, eczema, allergies, contact dermatitis, eczema, and reproductive damage, among other things.

Iron (Fe) is a vital element. It is thought to exist in soils mostly as oxides and hydroxides as minute particles or in association with the surfaces of other minerals.Iron is a necessary component of haemoglobin, the red pigment in our blood that transports oxygen throughout our bodies.It can cause conjunctivitis, choroiditis, and retinitis. Chronic inhalation of high amounts of iron oxide fumes orspecks of dust can cause siderosis, a type of pneumoconiosis. Excessive iron oxide inhalation can raise the risk of lung cancer. The iron and steel industry, sewage, and iron mine dust, iron sulphate fertilizer, and herbicide are all anthropogenic sources of iron. The accumulation of heavy metals in medicinal plants has been reported to be based on various factors such as climatic factors, plant species, and soil pollution, apart from other environmental factors (Sovljanski *et al*., 1989). Heavy metal deposition in edible and medicinal plants required careful examination to prevent higher heavy metal concentrations from reaching the customer (Steenkamp *et al.,* 2000). The morphology of medicinal plants does not ensure their safety from contamination, especially when plants are harvested from contaminated environments (Olowoyo *et al*., 2012). According to Chaiyarat *et al*. (2011) selective site selection, combined with appropriate soil management, can assist to reduce heavy metal accumulation by medicinal plants.

Conclusion:

These results showed that the majority of heavy metals have been accumulated above the standard levels in the leaves and flowers of *Senna auriculata* collected from the polluted sources

when compared to the natural sources. This could be the result of Heavy metal pollution from industrial sources navigating their way from soil to plants. These exceeded levels of these heavy metals become toxic to humans on consumption. Based on this finding, it is suggested that medicinal plants used for human consumption or to manufacture herbal products and standardized extracts should be collected from an unpolluted natural environment is preferable.

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TREE CROWN EXTRACTION FROM AERIAL IMAGERY USING DEEP LEARNING

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Abstract:

This study presents an application of deep learning in very high-resolution, remotely sensed data. The study highlights pixel-wise segmentation using deep learning to extract tree crown features from UAV imagery. UAV imagery object detection or segmentation is always challenging because of very different object views, i.e., top view, lack of available data, and minimal object size. We choose this task to demonstrate automatic tree crown extraction in very high resolution to bypass manual digitization tasks requiring a massive workforce and time and are primarily unsuitable for high-resolution imagery such as UAV. The canopy mapping will give us the idea of vegetation coverage over a space. However, also it can help us derive the biomass of an area with further insight into its biodiversity. The vast size of imagery in terms of dimensions and size also creates a problem for segmentation tasks. In this study, we discuss all the issues and challenges that the aerial imagery segmentation task has and highlight possible solutions and approaches. The objective of our study is to accurately extract canopy cover and analyze its efficiency, which is not possible by other classification or segmentation methods. Due to UAV's high temporal and spatial resolution, forest health study and canopy closure monitoring can be done.

Keywords: Deep Learning, Segmentation, Aerial images, UAV, Remote Sensing.

Introduction:

UAV remote sensing and deep learning

Aerial photography and its use is not a new science. UAV-RS surveys help us overcome traditional fieldwork methods, which are time consuming, tedious, exhaustive, and impractical for carrying out small periodic surveys. It has been used for decades; however, the advancement made in its use and practical applications in almost all possible fields has been recent. These

lightweight UAVs can be directly controlled from the ground and fly below the cloud cover. UAVs are also known as remotely piloted vehicles (RPV), Remote controlled (RC) helicopters, remotely operated aircraft (ROA), unmanned vehicle systems (UVS), which are designed to operate without humans on board. UAV was started as a goal in military surveillance of hostile areas. UAV can be used for precision farming in agriculture to get accurate damages or problems in the field. The UAV flight is widely recognized nowadays due to its ability to characterize landscape at any elevation and slope.

Further, a low weight UAV gives us an alternative platform through which remote sensing technology can be applied effectively over an area of a tiny scale. UAV provides us the platform to study the structural properties of land use features. It also helps us know the changes in an area over a short period, and the study can be done effectively within a brief period. UAVs can be of two types fixed wing and rotary wings. The following study has been done using a rotary type of UAV. It has wide applications in the field of forestry as well. The payload capacity of the UAV is 1.5-2 kg, and various sensors like multispectral, thermal, and hyper spectral, optical, or LIDAR can be used in flight as per user requirements. UAV's can be used for both manuals as well as autonomous flights. UAV remote sensing (UAV-RS) is widely used for large scale monitoring, vegetation structure, to urban modeling.

Deep Learning also has enormous potential in various applications in remotely sensed data for classification, segmentation, and object detection. Early methods relied on Object based Image Analysis (OBIA). However, we have focused on segmentation using deep learning to generate tree crowns from high resolution aerial images. Deep learning has been used in object based tree crown delineation from high resolution imagery (Whiteside et al., 2011). Also, an index based approach for vegetation extraction from satellite images can be made (Hebbar et al., 2018). We have explored various deep architecture for this task and found that deep Learning based architecture is most suitable for semantic segmentation on VHR aerial data. This is motivated to use the advantage that deep learning has over OBIA for segmentation tasks on aerial data. Texture algorithms have been applied to extract tree crown (Gomes and Maillard, 2015), watershed segmentation, marked point process, and template matching. Generated map is precious as this gives the capability to generate a very high resolution map without manual digitization. Support vector machine and principal component analysis for individual tree crown extraction from hyper spectral imagery (La *et al.*, 2015). It is very significant for remote sensing as these tasks require massive person hour efforts.

Currently, most case studies focus on CCTV data or moving vehicles with standard RGB and RGB-D cameras. The study through UAV helps us to overcome the labor cost. Also, it can

be flown with a minimum interval of time or as per user requirements. Point cloud based segmentation (Disney and Calders, 2018), an object based image analysis tool (Sara *et al.,* 2012) for canopy extraction. A deep convolution neural network for image net classification can be done (Krizhevsky *et al.,* 2012). From a computation perspective, our inference should be efficient and feasible regarding time and memory requirements for training and testing. The ability to train end to end using stochastic gradient descent is also beneficial, more manageable, and repeatable. Long et al. has extended this work to semantic segmentation using a convolution neural network. Further, encoder-decoder based architecture for medical image segmentation has been proposed (Ronneberger *et al.,* 2015).

Materials and Methods:

Study area

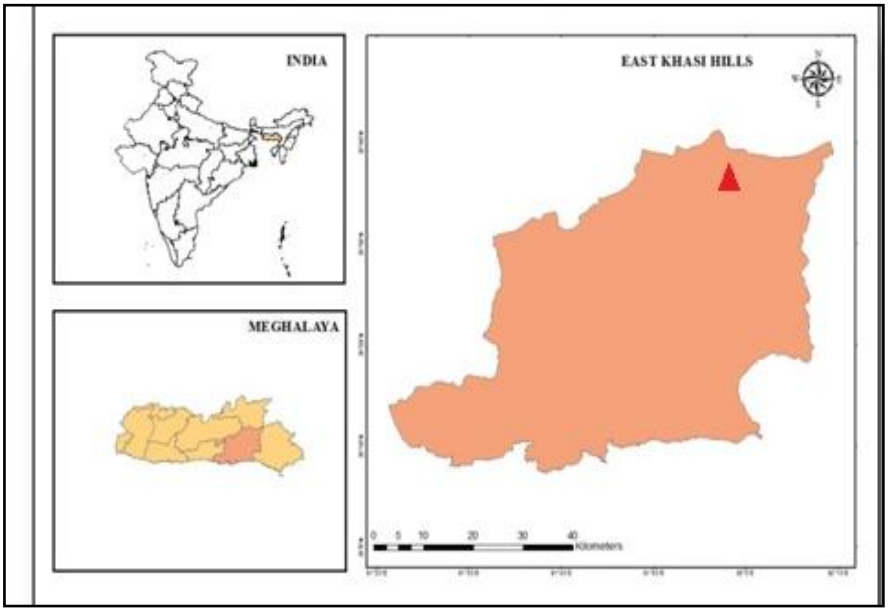

Figure 1: Map showing location of the study area

The study has been done in an area located in East Khasi Hills of Meghalaya (Fig.1). The state is characterized by a tropical climate and can provide us with our desired features of canopy cover. The state's geographical area is around 22,429 sq. km, and the total forest cover is 8510 sq. km. Meghalaya (the abode of clouds) is situated between Bangladesh in the south and the Brahmaputra valley in the north, a North-Eastern region of India. The state includes Khasi, Garo, Jaintia hills, Assam ranges at its border and lies between the latitudes of 25°02' and 26°07' N and longitudes 89°49'and 92°50'E. The state has Tropical wet evergreen, Tropical Semi-evergreen, Tropical moist deciduous, Subtropical broadleaved hill, and Subtropical pine forests.

Pines, Bamboo, Sal, are pretty rampant in this region. The soil type is sandy loamy, reddish-brown in color, and the soil pH ranges from 4.6 to 5.8. The maximum temperature is 25°C during summers while 16°C in winters. The minimum temperature of Meghalaya is 15°C during summers, while the same in winters is 4°C. The annual rainfall in the state varies from 4000mm to 11,436mm. Mawsynram and Cherrapunji, located in the southern part, are the world's rainiest spots. The study area covers varied land use features. The area has sparse forest cover with agricultural and human settlements. The region's climate was characterized by average annual precipitation of 2000 mm.

UAV data aquisition

A hex copter UAV has been used with a resolution of 5 cm. The targeted areas were captured in multiple UAV flights of 40 minutes each. The UAV flight was done in areas having spatial variability so that the results could be validated much more accurately. The UAV flight requires an area of interest (AOI) with required ground sample distance (GSD), and the platform for control stations is provided in the ground with the help of a remote controller showing real-time data such as position waypoints, altitude, speed, and distances. DJI Matrice 600 hex copter UAV is chosen for the study having flying endurance of 50-60 minutes with a range of 5-10 km. The UAV flight was done at an altitude of 100 m, covering a range of 5-6 km. A low weight UAV of 5 kg is used in the following study with a multispectral sensor named Parrot sequoia. A multispectral sensor with four 1.2 panchromatic bands such as Green (550 nm), Red (660nm), Red edge (735nm), and NIR (790nm) is used. Absolute GPS coordinates were utilized in this study to obtain mosaic. A tiff file has been utilized to process Arcgis and further, Pix 4Dsoftware was used to transform the multispectral images into ortho mosaic data and 3D map. However, in our study, we have directly used the RGB images obtained from UAV. More than 200 images have been used resulting from UAV flight. These RGB images form the basis of our study in canopy extraction.

Data pre-processing and dataset preparation

In the UAV survey, we have captured very high resolution (4000×3000 pixels) images of various locations. Each survey results in 100-150 such images. These images are used to generate orthomosaic of the areas as there is no public dataset available for drone imagery with ground truth suitable for our task, so we have generated our dataset. Our training dataset consists of 800 images and manually annotated ground truth. We divided them into two sets: The training and validation sets. The training set consists of 500 images, and the validation set consists of 300

images. We intentionally choose dissimilar validation set images. These results in more generalized learning of trained models and avoid overfitting. As processing on that significant scale is complicated, we divided every image into patches of 512×512 pixels. We trained our model with these patches. For the testing dataset, we selected our study area orthomosaic image other than the training dataset and divided it into patches of 512×512 pixels. In this way, we got 200 images in our test dataset.

We trained our model without using any pertained weight. Our earlier approaches to use transfer learning with Resnet [10] pre-trained weight failed as it always results in non-convergence of learning. So we initialize our network weight randomly. We train our network for 500 epochs. We trained our network on a 6 GB Nvidia graphics card. In training to avoid our model to overfit, we use a drop out of 20% in each layer. We have used data augmentation like rotation and brightness change in the original image to generalize learning.

Model Architecture

We have used CNN architecture influenced by the Encoder-Decoder network. Each encoder has a corresponding decoder in this network and is followed by the final pixel-wise classification layer. Our network uses a VGG style encoder-decoder (Simonyan at al., 2014) where upsampling in the decoder network has been done using transposed convolutions. In addition, it employs additive skip connections from the encoder to the decoder.

In the encoder path, we perform the down sampling of the image using the max pooling operation. Decreasing the size of the image is required as we have a limited capacity of GPU resources. This operation also creates multi-scale features of the image.

There is a hierarchy of decoders for each encoder in a decoder network. Decoder used transposed convolution for upsampling the image, followed by a series of convolution operations and non-linearity. There are several interpolation methods for upsampling, e.g., nearest neighbor or bilinear. However, we have used transposed convolution to have learnable parameters instead of predefined parameters. For training, we have used manually generated ground truth on aerial images.

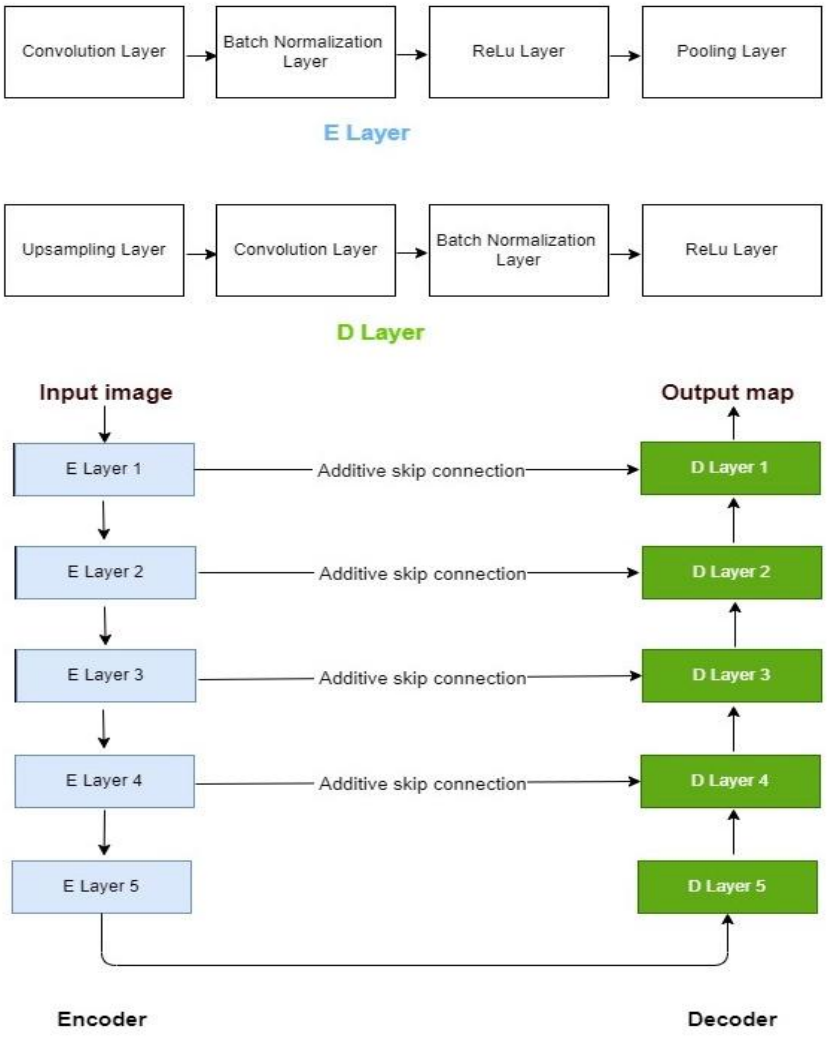

Figure 2: A figure showing network architecture

Results and Discussion:

We have used two matrices-Accuracy and Intersection over union (IOU) to easure the performance of our model.

The matrices calculated are as such:

$$\text{Accuracy} = \frac{(\text{TP} + \text{TN})}{(\text{TP} + \text{TN} + \text{FP} + \text{FN})}$$

TP, TN, FP, and FN represent several true positives, true negatives, false positives, and false negatives

$$IOU = \frac{\text{Area of overlap}}{\text{Area of union}}$$

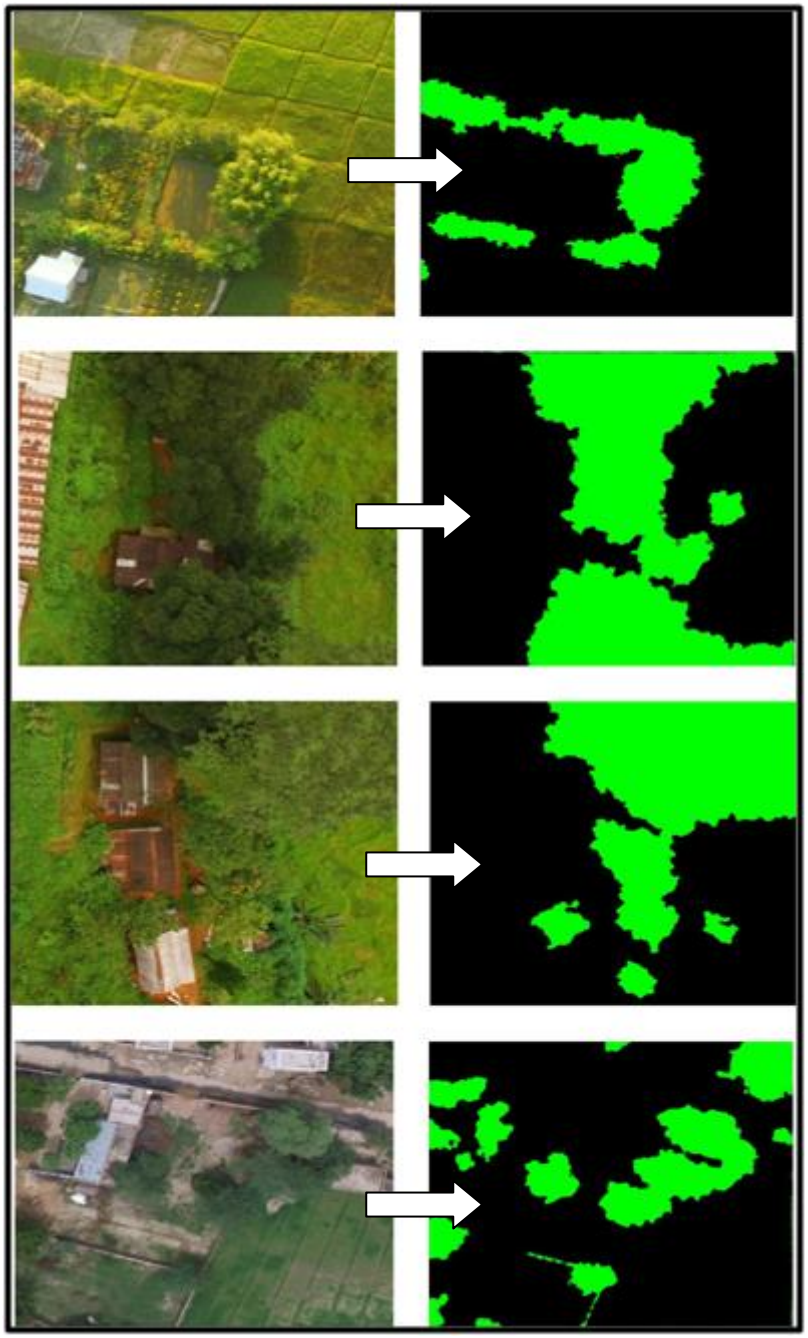

Figure 3: Input RGB (left) and generated output (right) showing canopy extraction

Our model has achieved 86.02 % accuracy and 77.3% IOU on training datasets. This study shows how efficiently green cover can be discriminated against and tree canopy cover extracted. The algorithm used in the study enables us to do large-scale forest canopy mapping and to use it in forest monitoring applications further. Various studies have been done regarding the use of UAVs in various fields. The use of unmanned aerial vehicles (UAV) has been rapidly increasing reliability, flexibility, low-cost maintenance, and accessible flight handling. The data

generated from UAVs help study various features due to their high-resolution data. Also, the time taken to generate the data is significantly less and user friendly. UAV's have applications ranging from aerial imaging to research and development. It covers almost all fields ranging from surveying and mapping to precision agriculture. The main drawback of satellite imagery is that it lacks coarse ground resolution. Nevertheless, UAV imagery allows us to overcome the drawback of resolution and provides an excellent resolution for ground study.

Conclusion:

The objective to extract canopy cover of a varied range and differentiate it effectively through deep learning algorithms has been achieved. UAV survey in a region like northeast India having difficult terrain accessibilities is greatly improved by UAV-RS. Detailed canopy structure analysis can lead us to know the structural dynamics of forest cover and help us evaluate forest loss. Canopy cover gives us estimates of stock in an area. Further tree density can also be determined and its changes due to various natural and anthropogenic events. Deep learning has been used in one way or another in various fields of object identification. However, it has been limited in use in the field of forestry.

Further, only a few data have been documented on tree crown extraction using deep learning. The periodic acquisitions of UAV imagery are used to estimate the forest's biophysical properties. Remote sensing has high potential in forestry, and it has been widely used over the last few decades. The study can further be enhanced by including the NIR band for model training.

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COELASTRACEAE AND *SCENEDESMACEAE* FROM BANGANGA WATER RESERVOIR

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Abstract:

Osmanabad district is part of the Godavari River Basin in Maharashtra, whereas Manjra, Sina, Terna, Bori, Benitura and Banganga are the main rivers which are flowing through the district. The author has earlier described the algal taxa belonging to Chlorophyceae, and cyanophyaceae from the Banganga project of Osmanabad district in Maharashtra. Present paper deals with Coelastraceae and Scenedesmaceaeasthe addition of taxa from the study area. During the present study, the algal members of Chlorophyaceae were noted from the study area and presented in the present communication.

Keywords: Chlorophyaceae, Algae, Flora, Banganga.

Introduction:

The Banganga Project is built up on Banganga River near Songiri and Bhongiri village of Bhoomtehsil in Osmanabad district. It is mainly used for irrigation and drinking water purpose. The study of the algal flora of the water reservoir was carried out to explore the diversity of algae of the water reservoir. The algal structure ranges from unicellular mucilaginous colonies to multicellular compact forms which show considerable diversity in form and adaptation to their distinctive environment (Krishnamurthy, 2000). During the present study, the Coelastraceae and Scenedesmaceae algal taxa were observed.

Materials and Methods:

The random sampling technique has been used for the collection of algal samples at the Banganga Project. Collections were made for 2 consecutive years (2016-2018), from October to

January. The algal samples were preserved in 4% formalin. Identification of taxa was carried out by using Prescott (1951), Pal *et al.* (1962), Philipose (1967), Patel and Satyanarayan (1976), Patel and Satyanarayan (1976), Prasad and Misra (1992), Hajarika and Datta (1994), Krishnamurthy (2000), Hosmani (2010) and other relevant literature.

Results and Discussion:

During the study, 10 taxa of Coelastraceae and Scenedesmaceae were observed which are described as under.

Family: Coelastraceae

Genus: *Coelastrum* Naegeli

The colonyis usually a hollow sphere, rarely polygonal to pyramidal, and of 4 to 32 or more cells. Cells spherical, ovoid or pyramidal are closely adjoined and compressed or interconnected by narrow processes to form small or large- intercellular spaces. A cell wall is composed of an inner cellulose layer and an outer pectic layer which is often locally thickened to form polar outgrowths or lateral processes that connect the cells. Chloroplasts arecup-shaped to diffuse and with a pyrenoid.

1) *Coelastrum cambricum* Archer var. ***intermedium*** (Bohlin) G.S.West

Prasad and Misra, 1992, p 30, pl 4, f 5

Colonies spherical, consisting of 32 cells; cells spherical in middle and sub- spherical at periphery with slightly thick, blunt and rounded projections, intercellular spaces more or less triangular; chloroplast parietal with one pyrenoid. . Colony 62.5 µ in diameter, cells 15 µ.

2) *C. microporum* Naegeli

Philipose, 1967, p 228, f 135 (a)

Colonies more or less spherical and of 8-16 cells with small intercellular spaces. Cells soherical to ovoid, enclosed by delicate gelatinous sheaths and interconnected by almost imperceptible gelatinous processes. Cells with sheath 6.25 µ in diameter. Colonies 35 µ in diameter.

3) *C. proboscideum* Bohlin.

Prasad and Misra, 1992, p 31, pl 4, f 7

Colonies pyramidal or cubical consisting of 16 cells; cells truncatel conical, enclosed by a gelatinous sheath and joined along lower lateral walls, cell wall slightly thickened at poles; Chloroplast single, parietal with one pyrenoid. Colony 25 µ in diameter, cells 10 µ.

4) *C. scabrum* Reinsch

Philipose, 1967, p 231, f 140 (a)

Colony 16 celled, less spherical. Cells angular globose with three or morewart-like truncate processes from the outer surface. Cells 10 µ in diameter, colony 27.5 µ.

Family: Scenedesmaceae

Genus: *Crucigenia* Morren

Colony free-floating, consisting of a plate of 4 to 16 trapezoid or rhomboid cells lying in one plane about a small or large central space; chloroplast a parietal plate with a one pyrenoid in each cell; colony enclosed by a thin, inconspicuous gelatinous envelope which often causes the families to adhereto one another and to form colonial complexes.

5) *Crucigenia tetrapedia* (Kirchner) W.et G.S.West

Philipose, 1967, p 240, f 151 (a)

Colony 4 celled, quadrate with a minute rectangular space at centre. Cells flattened and triangular with rounded ends. Outer side of cells always concave. Cells 10 µ.

Genus: *Scenedesmus* Meyen

Colony flat (rarely curved) plate of usually 2 to 8 (rarely 16 to 32) cells which are always in multiples of two. Cells acicular, ellipsoid, ovoid or cyllendrical, arranged in one or two rows and in lateral contact. Cell wall smooth or granulate, with or without lateral ridges, lateral teeth or spines. Chloroplast single and parietal and often filling the cell, and with a single pyrenoid.

6) *Scenedesmus acutiformis* Schroeder

Philipose, 1967, p 260, f 169 (a)

Colony 4 celled. Cells cylindrical fusiform and arranged in a single linear series. Cell wall smooth. Median cells with a lateral longitudinal ridge extending from pole to pole on each

side. Terminal cells with two or four ridges. Poles of cells acute and without teeth or spines, but sometimes with a minute papilla. Cells 7.5 µ broad, 15 µ long.

7) *S. arcuatus* (Lammermann) Lammermann

Philipose, 1967, p 256, f 166 (d)

Colonies 8 celled, curved and with small intercellular spaces.Cells in eight celled colonies in two series, ablong avoid, sometimes slightly angular at the base due to mutual pressure. Cell wall smooth, without teeth or spines. Cells 7.5 µ broad, 17.5 µ long.

8) *S. armatus* (Chodat) G.M.Smith var. ***major*** G.M.Smith

Philipose, 1967, p 266, f 171 (k)

Colonies Four celled. Cells oblong, ellipsoid and arranged in linear series. Terminal cells with a single long spine from each pole. All cells with a median lateral longitudinal rib which is sometimes indistinct. Four celled colony 10 µ broad, 20 µ long. Cells 7.5 µ broad, 25 µ long.

9) *S. armatus* (Chodat) G.M.Smith var. ***bicaudatus*** (Guglielmetti) Chodat

Philipose, 1967, p 262, f 171 (e)

Colony 2 celled. Differs from the type in having a long spine from one of the poles of the terminal cell only, the spines of the two terminal cells alternating with each other. Cells 5 µ broad, 12.5 µ long, and spine 7.5 µ long.

10) *S. bijuga* (Turp.) Lagerheim

Prescott, 1951, p 276, pl 63, f 2

Colony composed of 4 cells in a single flat series; cells ovate or oblong, without teeth or spine, cells 5 µ in diameter, 12.5 µ long.

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PHYTOCHEMICAL AND ANTIMICROBIAL ANALYSIS OF *TECOMA STANS* FROM PARBHANI DISTRICT

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Abstract:

Tecoma Stan is a well-known medicinal plant. The Plant metabolites are the promising choice over modern synthetic drugs for its minimum side effects and remedy for several diseases. The medicinal values of the phytochemicals have remarkable biological activities such as antimicrobial, antioxidant, anti-inflammatory, antidiabetic and wound healing etc. *Tecoma stans* is a flowering perennial shrub belonging to the family Bignoniaceae was subjected to phytochemical screening.The present investigation revealed that the methanolic and ethanolic extract has maximum amount of phytochemical and proved to be highly antimicrobial therapeutic activity.

Keywords: *Tecom stans*, phytochemical screening, therapeutic activity, biological activities

Introduction:

Natural phytochemicals from plant sources either as pure compound or as standardized extracts provide unlimited availability of new drug leading to challenges in the medical field [1]. According to the World Health Organization (WHO) nearly 20,000 medicinal plants exist in 91 countries [2], antifertility [3], preparation of dyes [4] etc. *Woodfordia fruticosa* is known for its medicinal value and used extensively in Atavas and Arishtas for self-generation of alcohol [5] and they are commonly used for treatment of rheumatism, leucorrhea, liver disorder, asthma and inflammatory action [6]. Traditionally, the crude extracts of various parts of the plant have taken a momentum since 1990s [7], food and feed [8] and curative agents [9, 10]. *Tecoma stans* or yellow bells from family Bignoneacea. It is a semi evergreen ornamental tropical shrub which is used traditionally for reducing blood glucose. Almost all the parts of *Tecoma stans* are of medicinal importance and used traditionally for the treatment of various ailments. The plants leave, bark and root have been used as a muscle relaxant mild cardio tonic and of chloretic

activities. As pharmacological uses *Tecoma stans* have been used as herbal medicine treatment for diabetes [11], Digestive problems, controls of yeast infection as powerful diuretic, vermifuge and tonic [12]. Flower and leaves have some medicinal value for the treatment of various cancer [13]. Its leaves are used traditionally in Mexico to control Diabetes [14]. Hence an attempt is made in the present study to analyze the functional group of phytoactive compounds present in the flower extract in ethanol of *Tecoma stans.*

Pharmacological activities:

Antioxidant activity:

Springob *et al.* reported Antioxidant activity and both phenolic compound and flavonoid total content were determined for callus tissue of *T. stans* cultured in either a set photoperiod or in darkness [15].

Anti-Inflammatory activity:

Sawapna Chaudhary *et al* evaluated the anti-inflammatory activity of chloroform root extract of *Tecoma stans*. Chloroform extract was analyzed for anti-inflammatory activity against carrageenan-induced paw edema method in Wistar albino rats [16].

Antidiabetic Activity:

Tecoma stans aqueous extract (TAE) of leaves is widely used as a traditional antidiabetic remedy in Mexico. Tecomine was shown to be one of the compounds responsible for the hypoglycemic action. Aguilar-Santamaria et al evaluated in vivo and in vitro intestinal α-glycosidase inhibition as the possible mode of action of TAE on type 2 diabetes mellitus (DM2) animal models [17].

Wound healing activity:

Das *et al.* evaluated the methanolic bark extract of Tecoma stan linn for wound healing activity in albino rats.Administration of methanol extract of the bark has shown more significant wound healing activity in excision and incision wound models and support the popular use of plant to open wound in folk medicine [18].

Materials and Methods:

Tecoma stans is a herbal medicine used for treatment of diabetes, digestive problems, control of yeast infections, as powerful diuretic, vermifuge and tonic. Preliminary phytochemical screening of this plant revealed the presence of tannins, flavonoids, alkaloids, quinones and traces of saponins and amino acids.

Collection of plant material:

The *Tecoma stans* plant was collected in the month of November 2021 from B. Raghunath College Surrounding area, Dist- Parbhani. Authentication of the plant material was made by Dr. Sunil Modak, Professor in Dept. of Botany, B.Raghunath College, Dist- Parbhani. The botanical classification is

Kingdom: Plantae

Unranked: Angiosperms

Unranked: Eudicots

Unranked: Asterids

Order: Lamiales

Family: Biognoniaceae

Genus: *Tecoma*

Species *stanus*

Figure 1: Sample of *Tecoma stans*

Preparation of extract:

The leaf and stem where detached and dried in shade. About 100 grams of dried leaf and flowers were grinded sieved to powder. A known quantity of powdered leaf and stem were subjected to extraction with two different solvents methanol and ethanol. Later the extracts were filtered with Whatsmann filter paper No. 1 and subjected to various phytochemical analyses [19, 20] as per standard.

Figure 2: Preparation of extract *Tecoma Stan* stem and leaves

Results and Discussion:

The result obtained in the phytochemical screening of the *Tecoma stans* plant (Table 1 and2) alcoholic extracts of the leaf and stem showed the presence of most of the secondary metabolites studied flavonoids, tannins, saponins, phenolic compounds, alkaloids, terpenoids, steroids and glycosides which would be the active principle of the plant. Plant phenolic compounds flavonoids, alkaloids, tannins, saponinsfrom plant extract and plant-based steroids have been found to possess potent antioxidantand antimicrobialand antidiabetics' properties.

Table 1: Phytochemical analysis of the ethanolic extract of *Tecoma stans*

Sr. No.	Phytochemicals	ethanolic extract of *Tecoma stans* leaf	ethanolic extract of *Tecoma stans* stem
1	**Flavonoids**	+	+
2	**Tannins**	-	-
3	**Saponins**	+	+
4	**Phenolic gr.**	-	+
5	**Alkaloids**	+	+
6	**Terpenoids**	-	+
7	**Steroids**	+	+
8	**Glycosides**	+	+

Table 2: Phytochemical analysis of the methanolic extract of *Tecoma stans*

Sr. No.	Phytochemicals	Methanolic extract of leaf	Methanolic extract of stem
1	**Flavonoids**	+	+
2	**Taninins**	+	+
3	**Saponins**	+	+
4	**Phenolic gr.**	+	+
5	**Alkaloids**	+	+
6	**Terpenoids**	+	+
7	**Steroids**	+	+
8	**Glycosides**	+	+

Antimicrobial activity:

The methanol extracts of the leaves and stem bark of Bignoniaceae Linn *Tecoma stans* was studied for their antimicrobial activity using a wide range of Gram-positive and Gram-negative bacteria. It was observed that the extracts of stem bark generally showedbetter antimicrobial activity than those of the leaves and some organisms were selectively more sensitive to the extracts than others [21].The antimicrobial efficacy of was determined by agar disc diffusion method [22, 23]. To check the susceptibility pattern of against selected bacteria nutrient agar medium was inoculated with specific organisms.The different dilutions of the extracts are made and by disc method followed from standard procedure we measured the antimicrobial study of the *Tecoma Stans.* The extract of plant is found to be antimicrobial active and it is measure against *Bacillus Subtalis*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by zone of clearance in mm. It is observe that the extract shows good antimicrobial activity.

Table 3: Antimicrobial study

Sr. No.	Micrrganism	Leaf Extract	Stem Extract
1.	***Escherichia coli***	2 mm	2 mm
2.	***Staphylococcus aureus***	1 mm	1.5 mm
3.	***Bacillus subtilis***	3 mm	3.5 mm
4.	***Pseudomonas aeruginosa***	2 mm	2.5 mm

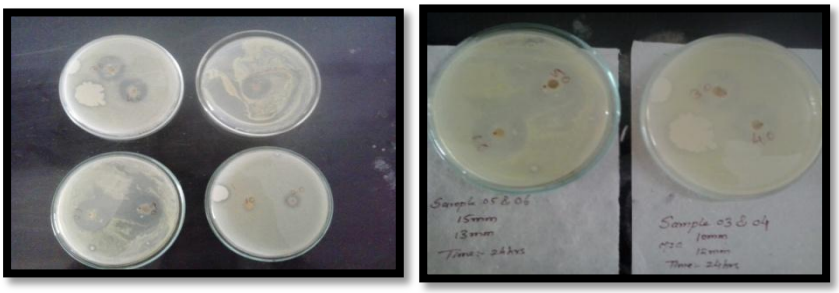

Figure 3: Zone of Clearance for measurement of Antimicrobial activity

Conclusion:

The present study was carried the phytoconstituents followed by the antimicrobial activity of extract of *Tecoma stans*. The study leads to a broader perspective for industries in usage of a new class of preservative agent for good pharmacological and therapeutic agents.

Further studies are crucial towards isolation, identification, purification, characterization and biological activity of specific bioactive compounds of this plant for industrial drug formulation, identification, activity of specific bioactivecompounds of this plant for industrial drug formulation.

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ECONOMIC IMPORTANCE OF PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

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Plant growth promoting rhizobacteria (PGPR) are a collection of bacteria that strongly colonize plant roots and enhance plant growth and yield (Wu *et al*., 2005). The mechanisms by which PGPR promote plant growth are not completely understood, but are considered to consist of the capacity to produce phytohormones against phytopathogenic microorganisms through the production of siderophores (Sharoona *et al*., 2006; Egambardiyeva, 2007) and the synthesis of antibiotics, enzymes and/ or fungicidal compounds (Ahmad *et al*., 2006). Considerable increase in growth and yield of agronomical important crops in response to inoculation with PGPR has been reported (Biswas *et al*., 2000; Asghar *et al*., 2002). *Pseudomonas* strains could affect seed germination and seedling growth (Shaukat *et al*., 2006). Strains of *Pseudomonas putida* and *Pseudomonas fluorescens* were reported to increase root and shoot elongation in canola (Glick *et al*., 1997). Therefore, it has been shown that *Pseudomonas* has the potential for agriculture utilization and could be used as biofertilizers (Cakmakc *et al*., 2006). Fluorescent *Pseudomonas*, capable of multiple mechanisms for biocontrol of phytopathogens and plant growth promotion, are being used widely (Pierson and Weller, 1994; Banasco *et al*., 1998; Dileep *et al*., 1998) as they produce a wide variety of antibiotics, chitinolytic enzymes, growth promoting hormones, siderophores, HCN and catalase. The use of PGPR has become a common employ in many regions of the world. Larger use of PGPR is possible in agriculture for biocontrol of plant pathogens and biofertilization (Siddiqui, 2006; Lugtenberg and Kamilova, 2009; Das *et al*., 2010; Saharan and Nehra, 2011). The bacterial strains isolated from *Lolium perenne* rhizosphere are capable of acting as plant growth promoting bacteria and show various plant growth promoting activities (Shoebitz *et al*., 2007)

Pseudomonads are Gram-negative bacteria comprising chemoheterotrophs predominantly inhabiting the soil and having versatile functions. They are potential colonizers of rhizosphere of many crops like cereals, pulses, oilseeds and vegetables (Johri *et al.*, 1997). Both fluorescent and

non-fluorescent species constitute the genus *Pseudomonas.* The fluorescent species produce diffusible yellow-green pigment and fluoresce under low-wavelength UV radiation.

The Marathwada region is well known for its hot summer and general dryness throughout the year except during the monsoon season. The Marathwada region comprises eight districts i.e. Aurangabad, Beed, Hingoli, Jalna, Latur, Nanded, Osmanabad and Parbhani. It is situated between 17.5° to 20.5° latitude and 75.5° to 78.5° longitude and at an average height of about 300 m above mean sea level (MSL). It is one of the four divisions of Maharashtra State. The main river Godavari of the region flows from west to east collect waters from several large and small tributaries. In spite of these water resources, the region suffers from heavy drainage due to west-east slope ultimately affecting the agriculture to a considerable extent. Some of the important cereal crops of the region are Bajra - pearl-millet (*Pennisetum typhoides* Burm.), Jowar (*Sorghum vulgare* pers.), Maize - corn (*Zea mays* L.,) and Wheat (*Triticum aestivum* L.)

Wheat is widespread and significant staple food crops for the people of the India it grown on about 26 million hectors which is about 20 per cent area under cereal sand the annual production is about 69 million tonnes of grain (Rangaswami and Mahadevan, 1999).

Safflower is one of the oilseeds of India, china, Egypt and other Far Eastern countries. In India it is grown in Maharashtra, Madhya Pradesh, Andhra Pradesh, Karnataka, Tamil nadu and other states and total area under this crop is about 2.3 lakh hectors (Rangaswami and Mahadevan, 1999).

In the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the preservation of soil health and quality, soil micro-organisms are very important (Jeffries *et al.,* 2003). The use of some PGPR as growth promoter and natural bio-control has been developed and commercialized (Dey *et al.,* 2004; Herman *et al.,* 2008; Minorsky, 2008). Biological control of plant diseases is gaining attention due to increased pollution concerns because of pesticides use for crop protection and the consequent development of pathogen resistance to such chemicals.

Soil borne pathogens have disturbing effect on plant health and yield. For successful disease Management it is important to found the most helpful and economical ways to protect the plant from various pests or diseases (Zahir *et al* 2004). In recent years, the use of PGPR as an inducer of systemic resistance in crop plant against different pathogen has been verified under field condition (Wei *et al.,* 1991 and 1996; Vidhayasekharan and Muthamilan, 1999; Viswanathan and Samiyappan, 1999).

Genetic analysis has shown a large diversity of soil bacteria, while the individuals have the low culturability. Bacterial species identification is traditionally based on functions rather than genetics, and thus agricultural and natural soil bacterial communities are to a large extent composed of unknown species. These are a reservoir of undiscovered genetics resources for mankind and merits protection in them. Molecular and biochemical techniques of estimating abundance and number of each species are mean of approaching an understanding of the community composition and diversity of soil bacterial communities at the genetic level.

The indirect effect of PGPR on plant growth is exerted by preventing harmful effects of plant pathogens by the production of secondary metabolites including HCN (Owen and Zlor, 2001), ammonia, antibiotics, and volatile metabolites. Plant growth promoting rhizobacteria actively colonize in plants rhizospHere and avoid the deleterious effects of phytopathogens (Rangajaran *et al*., 2003; Saikia *et al*., 2005). Biological control of a large array of pHytopathogens by the induction of systemic resistance has received much importance in last few decades (Jetiyanon *et al*., 2003). The ability of the antagonistic rhizobacteria is highly influenced by their morphological characteristics to inhibit the pathogens. High morphological activities results in production of more

Secondary metabolites to suppress the pathogens. The diversity of rhizobacteria in the suppressive soils is high as compared to conductive soil. In this respect the suppressive soil provide more chances to screen the antagonistic microbe that can be a potent biocontrol agent (Garbeva *et al*., 2004) Fluorescent *Pseudomonads* have emerged as most potential group implicated for promoting plant growth and biological control of plants diseases. *Pseudomonas fluorescens* are reported antagonist to various plant pathogens and also recommended for seed dressing.

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A COMPREHENSIVE OVERVIEW ON DIETARY SUPPLEMENTS

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Introduction:

The diet is believed to be much richer than before. However, the abundance of the food industry and the ignorance of the basic principles of nutrition have led to a large population leading to an unbalanced diet that is high in calories, high in fat and low in protein, vitamins and minerals. This long-term situation leads to the development of various degenerative diseases. This is where dietary supplements come in to solve the problem. Dietary supplements are Termed as those products which contain one or more concentrated nutrients. Its purpose is to supplement an individual's daily diet when the individual's diet is unbalanced and does not belong to the general food category rather than medicine. A dietary supplement is a medicinal product or specialty food product and is not intended for a specific group of people. Dietary supplements provide the body with the ingredients it needs to keep it in good physical and mental condition. In this way, the human body is not exhausted and at the same time avoids injury and fatigue.Dietary supplements have grown in number over the last few decadeswhich are formulated as tablets or powders. Side effects of dietary supplements are listed which increases with their consumption or overdose.

People who systematically consume dietary supplements may be consuming more vitamins and other nutrients than the organism can tolerate. You need to know that there is. This poses a health risk from overdose of dietary supplements. The problem is exacerbated as people can get these supplements themselves without a prescription or medical supervision. The purpose of this summary report is to show the relationship between dietary supplements and the human body. It helps protect and promote health. Medical and nursing journals and books were searched through the electronic databases MEDLINE, IATROTEK, and their respective libraries.

Keywords: Nutrition, dietary supplements, and health.

Review literature:

Maughan *et al.* revealed disclosed supplements enable more consistent and focused training by promoting recovery between training sessions, reducing training interruptions due to illness or injury, and improving competitive performance often regarded as facilitating coordination. Studies have shown that the use of supplements is widespread among athletes, but the use of some of these products is backed by solid research, and some are even harmful to athletes. There is a possibility. Certain sports foods, such as energy bars and sports drinks, play a real role, and some protein supplements and meal replacements may also help. Lin *et al.* revealed the use of herbs and dietary supplements (HDS) alone or with medications may increase the risk of side effects for the patient. This review aims to assess documented HDS drug interactions and contraindications contraindications.

Sobal *et al.* revealed about Vitamin/mineral supplements are often used by athletes as ergogenic aids to improve performance. This paper reviews studies of the prevalence, patterns, and explanations for vitamin/mineral supplement use among athletes. Fifty-one studies provided quantitative prevalence data on 10,274 male and female athletes at several levels of athletic participation in over 15 sports. The overall mean prevalence of athletes' supplement use was 46%. Most studies reported that over half of the athletes used supplements (range 6% to 100%), and the larger investigations found lower prevalence levels. Elite athletes used supplements more than college or high school athletes.

Blendon et al. revealed about about vitamin / mineral supplements is often used by athletes as a performance-enhancing aid to improve performance. This article reviews prevalence studies, patterns, and explanations for the use of vitamin / mineral supplements in athletes. Fifty-one studies provided quantitative prevalence data from 10,274 male and female athletes at various levels of participation in more than 15 sports. The average overall prevalence of dietary supplement use was 46%. Most studies reported that more than half of athletes consumed supplements (range, 6% to 100%), and larger studies found lower prevalence values.

Louise *et al.* revealed about Dietary supplements disclosed for health purposes. Based on the results of 4,444 different national polls, including the opinions of dietary supplement users and non-users, a significant proportion of Americans surveyed regularly take dietary supplements as part of their daily health care. Dwyer *et al.* told about many of the scientific and regulatory challenges that exist in dietary supplement safety, quality, and efficacy research are the same in all countries as the market becomes more and more global.

Emily *et al.* revealed these studies have not previously summarized the number of studies on the prevalence of dietary supplement (DS) use among military personnel. This article provides a systematic literature review on this subject. Berman *et al.* revealed *Citrus aurantium*

extract is an herbal weight loss product sold as a safe alternative to Ephedra, but *C. aurantium* can also have a negative effect on health. *C. aurantium* contains synephrine (oxedrin), which is structurally similar to adrenaline. Although there are no adverse events due to the use of *C. Synephrine*, an aurantlum product, can increase blood pressure in humans and other animal species and exacerbate cardiovascular events.

Scope of dietary supplement:

Dietary Supplements: The FDA regulates both finished dietary supplements and nutritional ingredients. The FDA regulates dietary supplements differently than "traditional" foods and medicines. According to the Food Supplement Health Education Act (DSHEA) of 1994:

- Food supplements and food supplement manufacturers and distributors are prohibited from selling inferior or mislabeled products. This means that these companies are responsible for assessing product safety and labeling prior to marketing to ensure that
- meets all the requirements of DSHEA and FDA regulations. ..
- The FDA is responsible for taking action against post-launch mixed or mislabeled dietary supplements.
- Example:
- Yogurt probiotics for intestinal health.
- Foods / cereals / snacks fortified with water-soluble fiber, vitamins and minerals.
- Omega 3 Milk to Prevent Heart Disease
- Canola Oil with Low Triglycerides to Lower Cholesterol Levels
- Autowheat, Bran, Obaco, Lignin for Heart Disease and Colon Cancer • Pre Controls Testicle Flora Biotics Oligofluctose
- Reducing Stanol (Benecol) Cholesterol

Classification of dietary supplement:

In accordance with the law, labeling of these substances must include:

• Declaration of a dietary supplement after the product name

• Recommended dose of product for daily consumption

• A statement that dietary supplements are not a substitute for a balanced and diverse diet. • Declaration on how to keep products out of the reach of children

According to the National Institute of Health Sciences, dietary supplements fall into two categories according to their intended use.

1. Dietary supplements as food. They complement a normal diet. 2.2. Food for special nutritional purposes as a drink. Due to its special composition, it is aimed at the special nutrition of a particular population group. For example, it targets healthy babies, children aged 2 to 5 years, and

people in a special category with disabilities. For groups of people who are in a metabolic or specific physiological state. Supplements can also be distinguished according to their origin: supplements of natural or synthetic origin. They are categorized as follows and are comparable in the nature or form they are available to.

• Whether vitamin and mineral supplements are combined in the form of multivitamins or multiminerals. • Liquid or tablet protein supplements with or without carbohydrates, fats, vitamins and minerals. • Amino acids of all shapes and compositions. • Dietary supplements that help you gain weight

• Meal exchange in the form of powder, waffles, or biscuits. • Carbohydrate supplements with or without electrolytes and vitamins. • Natural anabolic dietary supplements that are not "prohibited substances" • "Activator" supplements for growth hormone and other hormones • Supplements for basic fatty acids. • Foods or food ingredients such as yeast, garlic, seaweed and royal jelly. • Herbs Protein Food Supplements

Protein and Amino Acid Supplements are widely marketed to athletes and habitually active consumers as muscle growth and performance-enhancing products, and high-protein, low-carbohydrate diets are traditionally used for weight loss has been done. However, knowledge of nutritional importance and the effects of dietary supplements and sports supplements vary significantly between athletes and lifestyle users, especially with respect to individual levels of physical activity and overall nutritional and metabolic status. Because proteins help ensure growth in infancy, support muscle and bone metabolism, ensure normal nervous system maintenance and development, and maintain muscle mass and physical fitness in old age, humans It is a lifelong essential nutritional ingredient in the diet. Athletes have proper protein synthesis and energy production, as well as proper immune function and good bowel integrity under multi-stress conditions of targeted, more frequent, more intense, and / or longer training routines. Physiological protein requirements may increase to maintain. Protein requirements increase with increasing intensity and duration of athletic performance. Therefore, protein should be consumed with meals before and after actual performance and regularly during the day to ensure an efficient supply of essential or essential amino acids. For example, branched-chain amino acid (BCAA; valine, leucine, isoleucine) supplements are widely used by athletes and have been suggested to reduce muscle soreness and improve exercise performance after strenuous exercise. BCAA supplementation can play a role in regulating the production of neurotransmitters in the brain and causing fatigue during exercise. Due to its rapid digestion and absorption, whey protein sup.

Formulation available in market of protein dietary supplements and their composition (ingredients):

Protein content per 100g of product was 80-90g in 11.7% composition:

Sr. No.	Ingredients	Concentration
1	Protein Concentrate	58.33%
2	Protein Isolate	50%
3	Milk Protein Concentrate	23.33%
4	Milk Protein Isolate	21.66%
5	Micellar Casein	20%

The purpose of this study was to investigate the composition of protein supplements in terms of protein content, source, and cost. All dietary supplement stores in Vadodara have been identified and all protein supplements from their website are listed. Details of these products were collected from each brand's official website. Products that did not provide nutrition labeling were excluded. 59 products from 15 brands were surveyed in the form of powders (83.05%), bars (13.56%) and beverages (3.39%). For powders that needed to be reconstituted by adding to the beverage in addition to milk or water, the serving size varied from 24g to 72g. The potion size of the bar is 5080g and the drink size is 414429ml.

Future aspects:

The popularity and little unregulated sales of herbal preparations in the United States over the past few decades have raised concerns about their potential health consequences. Scientific research on the physiological and pathological effects of herbs is relatively sparse. The recent

Ephedra sinica ban (l) by the Food and Drug Administration, long after its clinical association with stroke, heart attack, hypertension, and psychological problems, indicates the need for a reassessment of the regulatory environment for Chinese herbs increase. There is also a need for more basic and clinical research in this area. *C. aurantium* (also known as Seville orange or sour orange) is a small citrus tree about 5 meters high with fragrant white flowers. *C. aurantium* is too acidic to eat, but ripe fruits can be eaten in Iran, and in Mexico fresh fruits can be eaten with salt and pepper paste. Unripe fruits are sometimes pickled and used as spices. *C. aurantium* rind is often used for jams, and dried pods are used to flavor Belgian beer called Bouquet Garni and Orange Muscat. Essential oils from the dry skin of immature fruit flavors Curacao, Cointreau and Triple Sec. Flowers are used in tea, and the essential oil of flowers called neroli is used in the water of orange blossoms, which is used to flavor perfumes, liqueurs and sweets. The most common use of *C. aurantium* is medicinal rather than cooking. Dried immature fruits are mainly used in Asian herbal medicine to treat digestive problems. It is called Zhishi in Chinese, Kijitsu in Japanese, and Chisil in Korean. Dried skins of immature or ripe fruits are also used in Western herbal medicines to stimulate appetite and gastric secretions (as opposed to recent sales of Daidai-containing products as a weight loss aid). It is a common ingredient in Swedish bitters and other gastrointestinal remedies. Flowers are sometimes used as a mild sedative in folk remedies. Therefore, there is a history of benign human consumption

Although it is a fruit of *C. aurantium*, the culinary or medicinal use of herbs is restricted and daily intake will not increase significantly.

Active Ingredients and Pharmacology:

The most effective active ingredients in the fruits of Daidai are synephrine (also known as synephrine or oxedrin) and octopamine. *C. Daidai* also contains flavonoids such as limonene, hesperidin, neohesperidin, naringin and tangaletin. Furanocoumarin is also available. Structurally, C. The active ingredient of aurantium is closely associated with the endogenous neurotransmitter and ephedrine (Figure 1). Synephrine is structurally similar to epinephrine, and octopamine is similar to noradrenaline (only the number of hydroxyl groups in the aromatic ring is different; see 6). Ilsynephrine (Phenylephrine, Neosynephrine) is closely related to synephrine. Phenylephrine is an alpha adrenergic receptor agonist used in conventional medicine as a decongestant nasal spray and mydriatic. It doesn't exist in C, so I won't explain it here. Daidai Synephrine and octopamine are both endogenous trace bioamines and are widely distributed in vertebrates, including plants, bacteria, invertebrates, and humans. Octopamine is found in the sympathetic nerves in the same area as norepinephrine, but synephrine and m-synephrine are found only in the adrenal glands. A recent study of healthy men and women found that plasma in all 16 subjects contained octopamine, 15 subjects contained synephrine, and 6 subjects contained octopamine and the precursor of synephrine, tyramine understand. Octopamine and synephrine

were detected in the platelets of most subjects, but tyramine was not. In rats, poctopamine was found in the adrenal glands, heart, spleen, vas deferens, brain, liver, kidneys, colon, bladder and lungs. Dopamine beta-hydroxylase converts tyramine to octopamine. This biosynthesis is enhanced by inhibition of monoamine oxidase. Phenylethanolamine methyltransferase breaks down octopamine into synephrines. Synephrine has an alpha-adrenergic effect and activates about 3 (although) ~ 1 or ~ 2) Adrenaline receptor. Octopamine appears to be a selective ~ 3-adrenergic receptor agonist (10). Both synephrine and octopamine appear to inhibit cAMP production. The function of endogenous synephrines and octopamines is not well explained. Synephrine, octopamine, and tyramine, formerly known as "fake neurotransmitters," can actually be true neurotransmitters. These amines affect platelet-mediated signaling events and may contribute to the pathophysiology of migraine and other types of headache.

Compound d, Imoctopamine, exhibits an antifat-producing effect on angiotensin II-induced fluid intake in rats. This effect is clearly mediated by et2adrenoreceptors because it is blocked by yohimbine. Synephrine had antidepressant effects in a mouse model using immobility trials, but the effects were not dose-dependent. No effect was seen at the lowest (0.3 mg / kg) or highest dose (30 mg / kg), and the effects at 3 mg / kg and 10 mg / kg were similar. The effect of synephrine was reversed by administration of the ol antagonist prazosin, so it seems that alpha adrenergic receptors are involved. In a later study by the same group, S (+) p-synephrine was more effective than R () pseudosynephrine in reducing immobility in the tail hang test. Effects on Weight Loss and Lipolysis There is little evidence to support the use of

C. Daidai is included in commercial weight loss products, but is used for weight loss. The combination product was tested for weight loss in the only clinical trial using Daidai. A double-blind, randomized, placebo-controlled, three-group study of 23 subjects with a body weight index of> 25 kg / m2 was conducted under the guidance of an exercise physiologist and a 1800 kcal American Heart Association Step I diet. Strength circuit training program 3 days a week. The combination tested contained 975 mg of *C. Daidai* extract (6% synephrine alkaloid), 528 mg caffeine, 900 mg St. John's wort (3% Hypericum erectum), taken daily for 6 weeks. Measurements of the results included weight, fat loss, and mood. Twenty subjects completed the study. This study reports that treated subjects lost significant body weight (1.4 kg) compared to the placebo group (lost 0.9 kg) and the control group (lost 0.04 kg). However, the publication table seems to suggest that the differences are important only when compared to the baseline, and not when compared.

Effects on weight loss and lipolysis:

Little proof helps the usage of *C. aurantium* for weight reduction, in spite of its inclusion in over-the- counter weight-loss products. The best scientific trial of *C. aurantium* for weight

reduction examined a aggregate product. A double-blind, randomized, placebo-controlled, three-armed take a look at of 23 topics with frame mass index >25 kg/m2 as compared remedy, placebo, and no remedy as an accessory to a 1800 kcal American Heart Association Step I food regimen and a weight circuit schooling exercising application three days every week beneathneath the course of an exercising physiologist . The examined aggregate contained 975 mg *C. aurantium* extract (6% synephrine alkaloids), 528 mg caffeine, and 900 mg St. John`s wort (three% hypericum [sic]), taken each day for six weeks. Outcome measures covered weight, fats loss, and mood. Twenty topics finished the take a look at. The take a look at reviews that dealt with topics 'misplaced a extensive quantity of weight (1.four kg) as compared with the placebo organization (which misplaced 0.nine kg) and the manipulate organization (which misplaced 0.04 kg). However, the desk withinside the guide seems to signify that the variations are extensive best as compared with baseline however now no longer in contrast with the opposite corporations on the quit of the trial.

The remedy institution misplaced 2.9% of fat, while there has been no full-size alternate within side the placebo or manipulates groups. No full-size modifications have been visible in any institution in a Profile of Mood States Questionnaire, blood lipids, blood pressure, coronary heart rate, electrocardiogram, serum chemistries, or urinalyses. The handled institution skilled a full-size growth in basal metabolic rate, while the placebo institution skilled a full-size lower in basal metabolic rate. There became no alternate within side the untreated manipulate institution. No facet outcomes have been reported.

Assuming that the 3% hypericum clearly approach 0.3% hypericin, the dose of St. John`s wort (Hypericum perforatum) on this specific product might be a healing antidepressant dose. There isn't anyt any scientific proof that St. John's wort facilitates weight loss, however despair can simply predispose to overeating.

This product carries a beneficiant quantity of caffeine, the equal of approximately four cups of espresso or 10 cups of tea. Caffeine has a thermogenic impact, and this impact is synergistic with different sympathomimetic agents. Even one hundred mg caffeine has a thermogenic impact lasting 1-2 hrs, and dosages >six hundred mg/day boom 24-hr electricity expenditure below respiration chamber conditions.

Beta-three adrenoreceptor agonists do have lipolytic consequences withinside the fats cells of rats, hamsters, and dogs, however they're a great deal much less energetic in human fats cells. Octopamine became stronger than synephrine (however a ways much less mighty than norepinephrine) for exciting lipolysis in adipocytes from rats, hamsters, or dogs; however, the impact became now no longer sizable in fats cells from guinea pigs or humans (10). Octopamine became completely lipolytic in adipocytes from the lawn dormouse and Siberian hamster.

In summary, the most effective posted trial of a C. aurantiumcontaining weight loss product discovered that the product became now no longer advanced to placebo for weight loss. There isn't anyt any proof that synephrine and octopamine in degrees that could be discovered in weight loss merchandise might have any lipolytic impact on human adipocytes.

Adverse Effects and Drug Interactions

C. aurantium might be predicted to have sympathomimetic consequences, however *C. aurantium* extracts have now no longer been related to unfavourable consequences to date. A thinly defined case document related a big myocardial infarction in a 28-yearold male to the abuse of synephrine tablets .

C. aurantium, grapefruit (*C. paradisi*), and pomelo (*C. maximi*) comprise numerous flavonoids that have an effect on drug metab- olism, together with 6',7'-dihydroxybergamottin, that's used to selectively block intestinal cytochrome P450 isoenzyme CYP3A4 in bioavailability research. C. aurantium, however now no longer grapefruit, additionally carries a furocoumarin, bergapten, that still inhibits CYP3A4 in cultured intestinal epithelial cells, however the impact is weaker than that of 6',7'- dihydroxybergamottin .

CYP3A4 metabolizes extra than 1 / 4 of pharmaceuticals, and grapefruit juice will increase blood degrees of many drugs. C. aurantium, predictably, additionally will increase drug degrees, and as it carries bergapten in addition to 6',7'-dihydroxybergamottin, might also additionally have a fair more potent impact than grapefruit juice.

A current scientific pharmacokinetics have a look at discovered that *C. aurantium* juice, however now no longer grapefruit juice, considerably elevated plasma degrees of simultaneously administered indinavir. Another scientific have a look at discovered that *C. aurantium* juice affected felodipine pharmacokinetics in addition to grapefruit juice, growing most awareness and AUC (place below the awareness-time curve) with out affecting' terminal removal half-lifestyles . And a 3rd pharmacokinetics have a look at discovered that each *C. aurantium* juice and grapefruit juice elevated the bioavailability of dextromethorphan

Background: Multiple sclerosis (MS) constitutes a persistent modern demyelinating sickness which negatively influences the significant frightened system. MS signs and symptoms detrimentally have an effect on the nice of lifestyles, in addition to the lifestyles expectancy of MS sufferers. In this aspect, the existing have a look at objectives to seriously summarize and examine the presently to be had scientific research focusing at the capability useful consequences of nutritional dietary supplements on controlling MS symptomatology and relapse.

Methods: PubMed database became comprehensively searched, the usage of relative key phrases to pick out scientific trials that investigated the useful consequences of nutritional

supplementation towards MS symptomatology and progression. forty scientific trials have been discovered, which have been divided into categories.

Results: Nutritional fame of MS sufferers, in addition to supplementation were recommended as capability elements affecting progression. Several giant research have documented a systematically excessive occurrence of diet A, B12 and D3 deficiency among MS sufferers. At present, scientific facts have recommended that maximum of the nutritional dietary supplements below have a look at might also additionally exert antioxidant and anti inflammatory properties, enhancing despair symptomatology and nice of lifestyles overall. However, malnutrition chance in MS sufferers has now no longer been safely explored so as for extra unique conclusions to be drawn. The dietary supplements which can have a nice impact on MS are vitamins, fatty acids, antioxidants.

Adverse effects and drug interactions:

C. aurantium would be expected to have sympathomimetic effects, but *C. aurantium* extracts have not been associated with adverse effects to date. A thinly described case report linked alarge myocardial infarction in a 28-yearold male to the abuse of synephrine tablets.

C. aurantium, grapefruit *(C. paradisi),* and pomelo *(C. maximi*) contain several flavonoids that affect drug metab- olism, including 6',7'-dihydroxybergamottin, which is used to selectively block intestinal cytochrome P450 isoenzyme CYP3A4 in bioavailability studies. C. aurantium,but not grapefruit, also contains a furocoumarin, bergapten, that also inhibits CYP3A4 in cultured intestinal epithelial cells, but the effect is weaker than that of 6',7'- dihydroxybergamottin .

CYP3A4 metabolizes more than a quarter of pharmaceuticals, and grapefruit juice increases blood levels of many drugs. C. aurantium, predictably, also increases drug levels, and because it contains bergapten as well as 6' ,7'-dihydroxybergamottin, may have an even stronger effect thangrapefruit juice.

A recent clinical pharmacokinetics study found that *C. aurantium* juice, but not grapefruit juice, significantly increased plasma levels of concurrently administered indinavir . Another clinical study found that *C. aurantium* juice affected felodipine pharmacokinetics similarly to grapefruit juice, increasing maximum concentration and AUC (area under the concentration-time curve) without affecting' terminal elimination half-life . And a third pharmacokinetics study found that both *C. aurantium* juice and grapefruit juice increased the bioavailability of dextromethorphan

Background: Multiple sclerosis (MS) constitutes a chronic progressive demyelinating disease which negatively affects the central nervous system. MS symptoms detrimentally affect the quality of life, as well as the life expectancy of MS patients. In this aspect, the present study aims to critically summarize and evaluate the currently available clinical studies focusing on the

potential beneficial effects of dietary supplements on controlling MS symptomatology and relapse.

Methods: PubMed database was comprehensively searched, using relative keywords to identify clinical trials that investigated the beneficial effects of dietary supplementation against MS symptomatology and progression. 40 clinical trials were found, which were divided into categories.

Results: Nutritional status of MS patients, as well as supplementation has been suggested as potential factors affecting progression. Several substantial studies have documented a systematically high prevalence of vitamin A, B12 and D3 deficiency amongst MS patients. At present, clinical data have suggested that most of the dietary supplements under study may exert antioxidant and anti-inflammatory properties, improving depression symptomatology and quality of life overall. However, malnutrition risk in MS patients has not been adequately explored in order for more precise conclusions to be drawn. The supplements that may have a positive effect on MS are vitamins, fatty acids, antioxidants, phytochemicals and melatonin.

Conclusion:

Individuals who systematically take nutritional dietary supplements have to be knowledgeable that they could take extra vitamins than their organism is capable of tolerate. Upper tolerable tiers are the ones, which if the attention of the substance is exceeded, then, aspect consequences and toxicity begin to seem to the human organism. People might not be conscious that there are dangers which can rise up from the ones formulations. So, they regularly devour immoderate amounts. The hassle turns into extra critical for, they are able to acquire those materials with the aid of using themselves, without a prescription and scientific supervision. Therefore, customers of dietary dietary supplements ought to examine cautiously the labels on dietary supplements and enriched meals and keep away from taking more than one doses that exceed the Recommended Dietary benefits (RDAs). In instances of doubt, human beings have to are looking for the recommendation of a of a consultant earlier than they pick out a nutritional supplement

Several nutritional dietary supplements can also additionally lower infection and fatigue, additionally growing additionally autoimmunity tolerance in MS patients, and as a consequence enhancing excellent of existence and existence expectancy. Currently, there's no powerful medical indication for making use of nutritional supplementation as complementary remedy in opposition.

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SEED SURFACE CHARACTERISTICS AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF SOME LAMIACEAE (LABIATAE) SEEDS

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Abstract:

The family Lamiaceae also known as Labiatae. It is also known as mint family. This family is a flowering plant family. For the study of seed morphology and preliminary phytochemical observations about 04 species were studied of Lamiaceae. In Lamiaceae, *Ocimum sanctum*, *Ocimum americanum*, *Ocimum basilicum* and *Salvia officinalis* were well studied. In the morphological observations seed surface irregular with grooves and furrows, some fibrous, cellular deposition on the surface. In the preliminary phytochemical observations with aqueous extract of seed powder treated with various chemical for detection of alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoids, terpenoides, saponins protein, tannins, amino acids, volatile oil or essential oil etc. Biuret and Xanthoproteic test were also done for detection of protein. Phytochemical analysis were done for correct identity and evaluation. This study is also useful to isolate the pharmacologically active principles present in the drug. Scanning electron microscopy (SEM) study is useful for detection of variation on seed surface. The micromorphological observation helps to identify the seed also. The family shows fragrance also.

Keywords: Phytochemical, preliminary screening, seed morphology, SEM, Lamiaceae (Labiatae)

Introduction:

Seed coat morphology is an important study for seed identification because seed identification is important in farming. Seed is a mature ovule. Outer coat of seed is known as seed coat, a small embryonic plant enclosed in a covering and usually with some stored food. For study of seed coat characters all the parameters are very important like seed size, shape, colour, hilum shape, hilum size, surface features, seed weight etc. The scanning electron microscopy is a modern technique for surface identification. Seeds have also the medicinal value. Lamiaceae is

one of the most important angiospermic family and having an aromatic smell. About 16 members of this family were reported from vidharbha region (MS) India (Dhore and Joshi, 1988). Seeds of this family are small, minute. Phytochemical analysis of some members of Lamiaceae family shows various chemical constituents which is present in stem, leaves and seeds also. The Lamiaceae seed having various medicinal values which is used on wound, bronchitis, liver disease, gastric disorders etc.

Figure 1: *Ocimum sanctum* Linn.

Figure 2: *Ocimum basilicum* Linn.

Figure 3: *Ocimum americanum* Linn.

Figure 4: *Salvia officinalis* Linn.

Figure 1 to 4: Habit of plant *Ocimum sanctum* Linn., *Ocimum basilicum* Linn., *Ocimum americanum* Linn., *Salvia officinalis* Linn. respectively

Materials and methods:

Sample collection:

Some seeds of Lamiaceae family like *Ocimum sanctum* Linn., *Ocimum americanum* Linn., *Ocimum basilicum* Linn., and *Salvia officinalis* Linn. were collected from various local regions in Amravati district and BSIP institute of Lucknow.

For seed coat study all the seeds parameters were studied using dissecting and binocular microscope. Digital weighing balance was used for weighing the seeds in mg. The

morphological observations of seeds were done followed by their photography, using 1 cm. scale.

Seed coat morphology (SEM):

To study the seed coat morphology scanning electron microscopy is most important. For this purpose, the individual seeds were dipped in alcohol for 5-10 min., to remove the dust from them. The seed mounted on pin type stubs using double sided adhesive tape or conductive silver paint to prevent charging of the surface during scanning and then coated with a very thin layer of gold in a polaron sputter coating unit. For spermoderm study of seed photomicrograph were taken in the scanning electron microscope (SEM) (LEO 430) at Birbal Sahani Institute of paleobotany, Lucknow.

Preliminary phytochemical tests:

The preliminary phytochemical analysis is most important for detection of various chemical constituents. Trease and Evans (1989) test were done. Qualitative phytochemical analysis of the crude powder of the 04 plants for the identification of phytochemicals like alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, saponine, protein, tannins, amino acids, volatile oil or essential oil. Preliminary photochemical test were done using aqueous extract.

Qualitative test:

Test for protein: About 04 seed samples were used for detection of protein .For this process Biuret and Xanthoproteic test were done.

In Biuret test: 1ml seed pulp/powder in a test tube treated with 2 ml solution of 20% NaOH and a drop of 1% $CuSO_4$.Then the test tube was shaken for few minutes. The pulp turns violet which indicates the presence of protein.

In Xanthoproteic test: 1 ml seed pulp in a test tube treated with 2 to 3ml of Conc. nitric acid. The pulp turns yellow colouration which clearly indicated the presence of protein. The concentration of protein in seeds was given in symbols (+,++,+++) are as follows.

Observations:

Scanning Electron Microscopy (SEM) study:

In Scanning electron microscopy (SEM) investigation shows that in *Ocimum sanctum* Linn. seed oblong shape with small, granular bodies deposited all over the surface. These granulated deposition present all over the surface. Hilar region slightly tapered.

Ocimum americanum seed shows irregular surface. Seed surface contain cellular thread like fibrous network all over the surface. Surface irregular, granulated. Hilar region circular, granulated. *Ocimum basilicum* seed shows irregular, cellular network, granulated deposition.

Salvia officinalis seeds also shows deep, irregular, thick cellular network. Surface shows no. of thick ridges and deep furrows are present. Some granulated small bodies present on the surface. Surface more irregular than other *Ocimum* species.

Figure 5: *Ocimum sanctum* Linn.

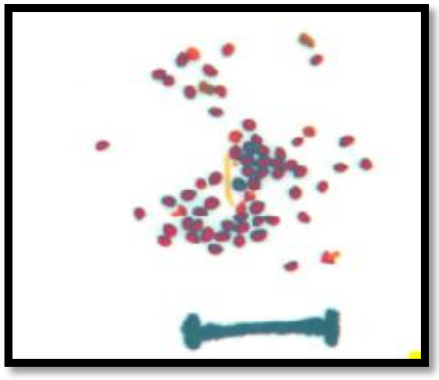


Figure 6: *Ocimum americanum* Linn.

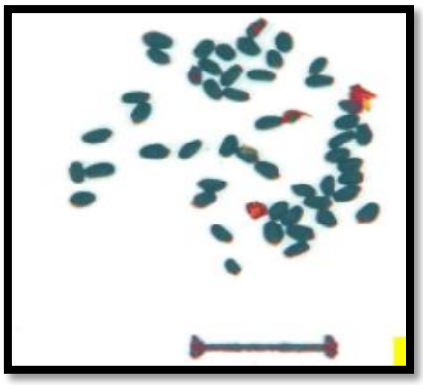


Figure 7: *Ocimum basilicum* Linn.

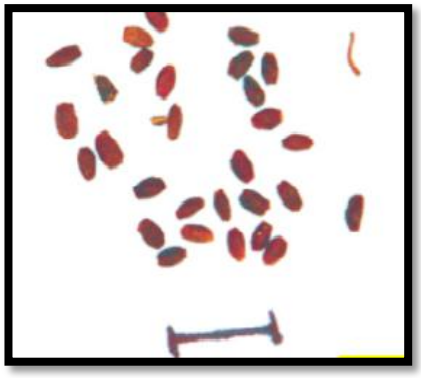


Figure 8: *Salvia officinalis* Linn.

Figure 5 to 8: *Ocimum sanctum* Linn., minute seeds, *Ocimum americanum* Linn .minute, oblong seeds *Ocimum basilicum* Linn .seeds oblong ovate .*Salvia officinalis* Linn .obovoid, brown seeds respectively

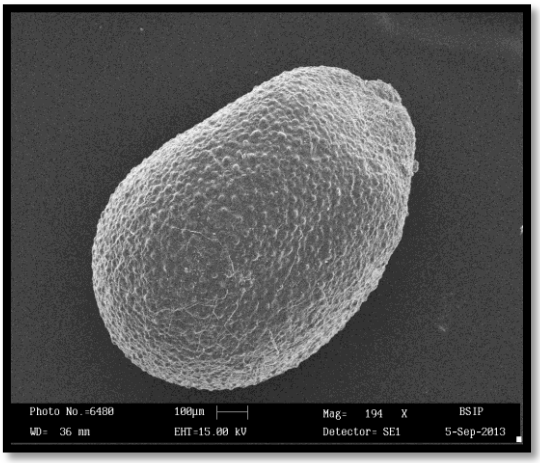


Figure 9: *Ocimum sanctum* Linn. 194X

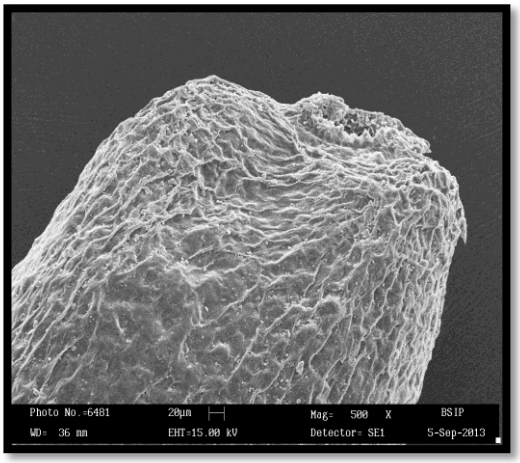
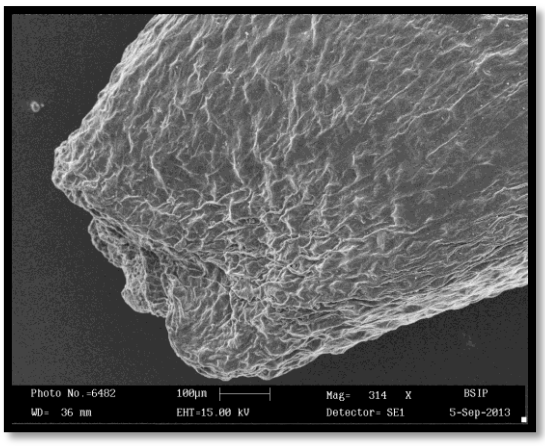


Figure 10: *Ocimum americanum* Linn. 500X



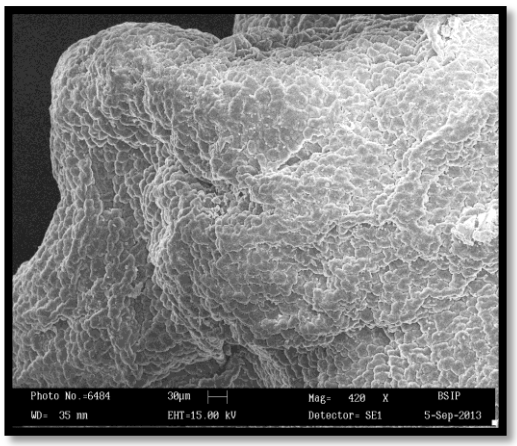


Figure 11: ***Ocimum basilicum*** **Linn.314X** **Figure 12:** ***Salvia officinalis*** **Linn.420X**

Figure 9 – 12: ***Ocimum sanctum*** **Linn. 194X, oblong seeds with granular deposition,** ***Ocimum americanum*** **Linn. 500X. irregular rough surface,** ***Ocimum basilicum*** **Linn314X. irregular deep ridges and furrows** ***Salvia officinalis*** **Linn.420X .irregular cellular ridges and furrows respectively through scanning electron microscope study**

Table 1: Protein test

Sr.No.	Botanical name	Family	Biuret test	Xanthoproteic test
1	*Ocimum sanctum* Linn.	Lamiaceae (Labiatae)	+	+
2	*Ocimum americanum* Linn.	Lamiaceae (Labiatae)	+	+++
3	*Ocimum basilicum* Linn.	Lamiaceae (Labiatae)	+++	++
4	*Salvia officinalis* Linn.	Lamiaceae (Labiatae)	++	++

Present **(+)**, Absent **(-)**

From the above observation we detected protein concentration in seed samples of *Ocimum sanctum* Linn., *Ocimum americanum* Linn., *Ocimum basilicum* Linn. and *Salvia officinalis* Linn.etc. The Biuret test and Xanthoproteic test shows *Ocimum basilicum* Linn. have more quantity as compare to others *Ocimum sanctum* Linn. have less quantity.

In phytochemical observation the qualitative chemical tests revealed the presence of sugars,steroids,glycosoides,flavonoides,terpenoides,saponine,protein,tannin,amino acids, volatile oil or essential oil using the seed extract treated with various chemicals for detection of phytoconstituents. Using the preliminary phytochemical analysis shows presence or absence of various chemical constituents in them.

Table 2: Phytochemical study of following plant (seed) extracts

Sr No.	Phytochemicals	*Ocimum sanctum*	*Ocimum americanum*	*Ocimum basilicum*	*Salvia officinalis*
01	Alkaloids	+	+	+	+
02	Carbohydrates	+	+	+	+
03	Reducing sugars	-	-	-	+
04	Steroids	+	+	+	+
05	Glysoides	+	+	+	+
06	Flavonoides	+	+	+	+
07	Terpenoids	+	+	+	+
08	Saponine	+	+	+	+
09	Protein	+	+	+	+
10	Tannins	+	+	+	+
11	Amino acids	+	+	+	+
12	Volatile oil or essential oil	_	_	+	+

Present **(+)**, Absent **(-)**

Table 3: Medicinal uses of seeds

Sr. No.	Name of seed sample	Local name	Medicinal uses
1	*Ocimum sanctum* Linn.	Krishna tulas	Seeds yield an essential oil containing eugenal, carvacrol, methyl eugenal and caryophyllene. Antibacterial and insecticidal properties. Seeds are mucilaginous, demulcent, useful in complaint of the urinary systems.(Sharma 2003)
2	*Ocimum basillicum* Linn.	Sabja	Seeds mucilaginous , Dimulcent, aphrodiasiac and diuretic properties. Useful in catarrh, chronic diarrhoea, dysentery, gonorrhea, naphritis, cystitis and internal piles (NIIR Board of consultant and engineers)
3	*Salvia officinalis* Linn.	Kamar kas	Anthilmintic, diuretic
4	*Ocimum americanum* Linn.	Tulsi	Aromatic ,antibacterial

In *Ocimum sanctum*, *Ocimum americanum* and *Ocimum basilicum* shows presence of alkaloids, carbohydrates, steroids, glycosides, flavonoides, terpenoids, saponine, protein, tannins, amino acids etc. In *Salvia officinalis* shows presence of carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoides, tannins, volatile or essential oil. Reducing sugars absent in *Ocimum sanctum, Ocimum americanum, Ocimum basilicum* etc . Volatile oil or essential oil absent in *Ocimum sanctum, Ocimum americanum* etc. Above study help to determine various chemical constituents present in it.

Discussion:

From the above observations we studied the seed morphological and preliminary phytochemical analysis of some seeds of Lamiaceae. In SEM observations of *Ocimum sanctum* shows more granulated deposition than *Ocimum americanum* and *Ocimum basilicum*. *Ocimum americanum* shows more fibrous network. *Ocimum basilicum* shows compact cellular network with fibrous deposition on the surface. *Salvia officinalis* shows presence of deep ridges and furrows on the surface, more irregular than other species. On the above observation the important micromorphological characters were well studied. According to Chaung and Heckard (1972) the seed coat pattern is diversified among species and furnishes an important feature for classification.

The preliminary phytochemical studies of 4 seed samples were studied. The qualitative analysis of seed samples were used for detection of alkaloids, carbohydrates, reducing sugars, steroids, glycosides, flavonoides, terpenoids, saponine, protein, tannins, aminoacids, volatile oil or essential oil etc.

In Biuret and Xanthoproteic test in which *Ocimum americanum* and *Ocimum basilicum* shows more protein content than others. So seed identification is an important feature for detection of micromorphological characters and detection of various chemical constituents. Seed identification both externally and internally provide various information and reliable criteria about unknown seeds. Seed shows high medicinal value, used in preparation of various drugs. Various chemical compositions present inside the seed are effective for drug preparations. All the above study helps for seed identification, drugs preparation on various types of diseases.

So identification, detection and analysis are most important for basic research for various purposes and economic use also.

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BENEFITS AND PHARMACOLOGICAL PROPERTIES OF CUMIN - AN UPDATED REVIEW

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Abstract:

Cumin is an ancient spice which has been used in Indian cooking as far back as 5000 years ago, use in Egypt, Greeks and Europe. Cumin is the best source of natural iron and commonly known as Jeera. It is usually used both as whole seed or ground in any curry dish which makes it one of the most important of all Indian spices and as flavouring agent. Despite of its flavouring property, cumin has been found to possess various pharmacological activities such as antiseptic, astringent, diuretics, stimulants, anticancer, antimicrobial, antidiabetic, antifertility, antileptics, antioxidants and Immunomodulatory also helps to improve amnesia and mental function. Its various pharmacological activities may be due to the presence of different chemical constituents such as volatile oil, fixed oil, proteins, amino acids, a variety of flavonoid glycosides including derivatives of apigenin and luteolin. Hence in this review we are highlightening the important Pharmacological and medicinal properties of cumin seeds.

Keywords: Cumin, Pharmacology, Phytochemicals, Volatile oil

Introduction:

Cumin (*Cuminum cyminum*) is a small annual herbaceous plant native to the Mediterranean region, where it is cultivated extensively. Cumin is a member of the aromatic plant family (Umbelliferae). The seeds of the plant are used in cooking to add flavour to spicy dishes. The dried seeds resemble those of caraway, but are straighter in form and have a coarser taste and odour [1]. Major cumin seed producers include Egypt, Iran, India, and Morocco [2].The unites States is one of the largest producers of cumin oil. This spice should not be confused with sweet cumin, which is a common name for anise (*Pimpinella anisum)* [3]. Black cumin *(Bunium persicum*) has smaller and sweeter seeds than C. cyminum, but is not commercially important. They are also used as an appetite stimulant and to ease several stomach disorders [4]. Its

micronutrients include about 1.4 mg iron, 38 mg potassium and 8 mg magnesium. Magnesium serves a host of functions, including promoting heart health, controlling blood pressure and aiding the absorption of calcium [5]. A cumin seed has been found to possess essential oils such as cuminaldehyde (4-isopropylbenzaldehyde), pyrazines, 2-methoxy-3-sec-butylpyrazine, 2 ethoxy-3-isopropylpyrazine, and 2-methoxy-3-methylpyrazine [6]. Moreover, Cumin seeds contain possess numerous phyto-chemicals that are known to have antioxidant, carminative and anti-flatulent properties. The active principles in the cumin may increase the motility of the gastro-intestinal tract as well as increase the digestion power by increasing gastro-intestinal enzyme secretions. This spice is an excellent source of minerals like Iron, calcium, copper, potassium, manganese, selenium, zinc and magnesium. It also contains very good amounts of B-complex vitamins such as thiamin, vitamin B-6, niacin, riboflavin, and other vital anti-oxidant vitamins like vitamin E, vitamin A and vitamin C.

Geographical distribution:

World scenario:

Cuminum cyminum is a yearly plant. The estimated world production is around 300,000 tons. Today cumin production is mainly concentrated in Central and South Asia. Nowadays, India is the largest producer (70% of world production), exporter and consumer of cumin seed in the world. India scenario: India holds a major position in the world production of Cumin. Rajasthan produces 56% while as production from Gujarat accounts for 44% [7].

Scientific classification, local and vernacular name:

Botanically cumin is *Cuminum cyminum* and a member of Apiaceae family (Parsley family)

Scientific classification:

Kingdom: Plantae

Subkingdom: Viridiplantae

Division: Tracheophyta

Sub division: Spermatophytina

Class: Magnoliopsida

Order: Apiales

Family: Apiaceae (Umbeliferar)

Genus: Cuminum – cumin

Species: *cuminum cymimum*-cumin

Vernacular names:

English: cumin, white cumin; Persian: Zirah-sabz or cravich; Spanish: Comino; French: cumin; Italian: cumino; German: Kreuzkummel; Romische kummel

Local names:

Hindi: Jeera, Jira, SafedJeera; Marathi: Pandharejire; Sanskrit: Ajaji, jiraka, Ajajika; Gujarati: Jirautmi, Jirn, Jiraugi, Jeeru; Malayalam: Jeerakam; Oriya: Dalajira, jira

Morphology of *Cuminum cyminum*:

Cumin is the dried seed of the herb *Cuminum cyminum,* a member of the Parseley family. The cumin plant grows to 30-50cm tall andis harvested by hand. It is an annual herbaceous plant with a slender, glabrous, branched stem that is 20-30 cm tall and has a diameter of 3-5cm. Each branch has 2 to 3 sub-branches. All the branches attain the same height so the plant has a uniform canopy [8].

Figure 1: Image of Cumin Plant and Seed

The stem is colored grey or dark green. The leaves are 5-10 cm long, pinnate or bipinnate with thread like leaflets. The flowers are small, white or pink and borne in umbels, each umbel has 5 to 7 umbellts [8]. The fruit is a lateral fusiform or ovoid achene 4-5 mm long containing two mericarps with a single seed. Cumin seeds have 8 ridges with oil canals.

Traditional uses:

Cumin traditionally used to reduce inflammation, decrease urination, prevent gas and suppress muscle spasms. It has also used as an aid for Indigestion, Jaundice, diarrhea and flatulence. Cumin powder has been used as a poultice and suppository and has been smoked in a pipe and taken orally [9-14]. Inaddition, cumin has been used historically for the treatment of toothaches and Epilepsy in Iran [15]. Cumin is a major component of curry and chilli powders and has been used to flavor a variety of food products [16]. The oil which is derived by steam distillation [17]. It is used to flavor a variety of food products like alcoholic beverages, desserts and condiments. It is also used as afragrant component of creams, lotions and perfumes [16].

Phytochemical:

Cumin seeds contain up to 5% of a volatile oil composed primarily of aldehydes (up to 60%). In addition, the seeds yield about 22% fats, numerous free amino acids, and a variety of flavonoid glycosides, including derivatives of apigenin and luteolin [10-18]. The cuminaldehyde content varies considerably, depending on the source of the oil (fresh vs ground seeds). Fine grinding of the seed can result in the loss of up to 50% of the volatile oil with the greatest loss occurring within 1 hour of milling [16]. Monoterpene hydrocarbons are another major component of the oil, sesquiterpenes are minor constituents [11, 17].

The chief components of the characteristics aroma of untreated whole seeds are 3p-menthen-7al and cuminaldehyde in combination with other related aldehydes. Cumin also contains safrole, a mutagen, which is degraded by cooking [19].Cumin seeds contain2 to4% essential volatile oils, also called cumin oil.

The main phytochemical present in the cumin oil are as follows;
Cuminaldehyde, Cinnamaldehyde, 8-Cineole,Cuminalcohol,Limonene,Linalool,Perillaldehyde, Terpinen-4-ol, Terpenoid aldehydes – Cuminic aldehyde and somericmenthadiencarboxaldehydes..Monoterpenes –Beta-pinene, p-cymene and Gamma-Terpinene.

Pharmacological properties:

Antioxidant effects:

Cumin oils as well as their aqueous and solvent derived extracts have significant antioxidant activity in several test methods. These effects are documented as their ability to prominently quench hydroxyl radicals, 1.1-diphenyl-2-picrylhydrazyl (DPPH) radicals and liquid peroxides. The other assays employed were ferric thiocyanate method in linoleic acid system, Fe2+ ascorbate-induced rat liver microsomal liquid peroxidation (LPO), soybean lipoxygenase dependent lipid peroxidation and ferric reducing ability [20]. The cumin oil exhibited high antioxidant activity which has been attributed largely to thresence of monoterpene alcohols, flavonoids and other polyphenolic compounds [21].

Antidiabetic effects:

The antidiabetic effects of cumin products are amply documented [1]. In a glucose tolerance test conducted in rabbits, cumin significantly increased the area under the glucose tolerance curve and hyperglycemic peak [2]. A methanolic extract of cumin seeds reduced the bloodglucose and inhibited glycosylated hemoglobin, creatinine, blood urea nitrogen and improved serum insulin and glycogen (liver and skeletal muscle) content in alloxan and

streptozotocin (STZ) diabetic rats [3, 4]. The collateral benefits included decreased creatinine, urea nitrogen and improved insulin and glycogen in tissue and skeletal muscles, accompanied by a reduction in rat tail tendon collagen-linked fluorescence and pepsin digestion which are implicated in the pathogenesis of diabetic microvascular complications [4]. In another study, an aqueous extract of cumin prevented *in vitro* glycation of total soluble protein, α-crystallin, and delayed the progression and maturation of STZ-induced cataract in rats. Cumin prevented loss of chaperone activity in diabetic rats and also attenuated the structural changes of α-crystallin in lens, which is a long-lived protein and is susceptible to several post-translational modifications in certain diabetic conditions [5]. Eight-week sub-acute administration of cumin to STZ-diabetic rats reduced hyperglycemia and glucosuria accompanied by an improvement in body weight, blood urea and reduced excretion of urea and creatinine [6]. Oral administration of cumin also showed hypoglycemic effect in normal rabbit, resulting in significant decrease in the area under the glucose tolerance curve [2] .The biologically active constituent of cumin seed oil was characterized as cuminaldehyde which inhibited aldose reductase and alpha-glucosidase isolated from rat [7]. In hyperglycemia associated with diabetes, the use of aldose reductase inhibitors has shown efficacy in attenuating diabetic complications.

Hyperlipidemia is an associated complication of diabetes mellitus. Oral administration of cumin to alloxan diabetic rats reduced body weight, plasma and tissue cholesterol, phospholipids, free fatty acids and triglycerides. Histological observations demonstrated significant decrease in fatty changes and inflammatory cell infiltrates in diabetic rat pancreas [3]. Cumin suppressed alcohol and thermally oxidized oil induced hyperlipidemia. It decreased aspartate transaminase (AST), alkaline phosphatase (ALP) and γ-glutamyltransferase (GGT) activities and decreased the tissue (liver and kidney) levels of cholesterol, triglycerides and phospholipids and prevented the changes in the composition of fatty acids in the plasma of rats administered with alcohol and/or thermally oxidized oil. The activity of phospholipase A and C decreased significantly [9, 10]. Hypocholesterolemic effect of methanolic extract of cumin is also documented in ovariectomized rat in relation to its anti-osteoporotic effect [11]. Cumin added to hypercholesterolemic diet decreased serum and liver cholesterol in rats [12].

Antimicrobial effects:

The antimicrobial action of cumin both oil and aqueous has assessed against a wide range of valuable and pathogenic gram-positive and gram-negative microbial strains. Cumin seed oil and alcoholic extract inhibited the growth of *Klebsiellapneumoniae* and its clinical isolates and caused improvement in cell morphology, capsule expression and decreased urease activity. Cumin has also found the biofilm-formation preventive properties against Streptococcus mutans

and *Streptococcus pyogenes* [13, 14]. Cumin has shown the anti-fungal activity against food, soil, animal and human pathogens, yeasts, aflatoxins and mycotoxin producers [15-17].

Anticarcinogenic/antimutagenic:

The dietary supplements of cumin have prevented the occurrence of rat colon cancer induced by a colon-specific carcinogen and also decrease the activity of β-glucuronidase and mucinase enzymes. In cumin-colon treated rats, the levels of cholesterol, cholesterol/phospholipids ratio and 3-methylglutaryl COA reductase activity were reduced [18, 19]. The other inhibition activities of dietary cumin in mice are benzopyrene-induced for stomach tumorigenesis, 3-methylcholanthrene induced uterine cervix tumorigenesis, and 3-methyl-4-dimethyaminoazobenzene induced hepatomas.

Immunomodulatory:

In a recent study, oral treatment with cumin showed immunomodulatory properties in normal and immune-suppressed animals via modulation of T lymphocytes' expression in a dose-dependent manner. It stimulated the T cells' (CD4 and CD8) and Th1 cytokines' expression in normal and cyclosporine-A induced immune-suppressed mice. In restraint stress-induced immune-suppressed animals, the active compound of cumin countered the depleted T lymphocytes, decreased the elevated corticosterone levels and size of adrenal glands and increased the weight of thymus and spleen [19].

Gastrointestinal disorders:

Cumin is extremely good for digestion and related problems. The very smell (aroma) of it, which comes from an aromatic organic compound called Cuminaldehyde, the main component of its essential oil, activates our salivary glands in our mouth (the mouth watering flavor), facilitating the primary digestion of the food [20]. Next is Thymol, a compound present in cumin, which does same to the glands which secrete acids, bile and enzymes responsible for complete digestion of the food in the stomach and the intestines, due to its Stimulating properties. Cumin is also Carminative i.e. relieves from you from gastroubles and thereby improves digestion and appetite. Due to its essential oils, magnesium and sodiumcontent, it promotes digestion and also gives relief in stomach-ache when taken with hot water (like aqua ptycotis and mint) [21]. Perfusion of an aqueous extract of cumin via the stomach of pentobarbitone-anesthetized rats under the aspirin-induced gastric mucosal injury showed an increased acid secretion by a cholinergic mechanism [22]. Aqueous and solvent derived extracts of cumin increased amylase, protease, lipase and phytase activities [23].

Anti-osteoporotic:

Cumin seeds are reported to be estrogenic. The presence of phytoestrogens in cumin has been shown and also related to its anti-osteoporotic effects. In the animals receiving a methanolic extract of cumin, a significant reduction in urinary calcium excretion and augmentation of calcium content and mechanical strength of bones was found. Animals showed greater bone and ash densities and improved microarchitecture, with no adverse effects like body weight gain and weight of atrophic uterus [24].

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ANTIBACTERIAL ACTIVITY OF BIOPESTICIDES AGAINST SEED BORNE FUNGI OF JOWAR

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Abstract:

The effect of biocontrol agents, antagonists *Pseudomonas fluorescence* and *Bacillus subtilis* and effect of were observed on dominant fungi like *Aspergillus flavus* (Link), *Fusarium moniliforme* (J. Schled.), and *Alternaria Alternata* (Fr. Keissl). The present investigation and seeds can be stored for a longer time and disease can be stored for a longer time and disease can be controlled. Hence this investigation will be useful in increasing the productivity of Jowar seeds.

Keywords: Antagonists, *Pseudomonas fluorescence* and *Bacillus subtilis* and Seed borne Pathogens.

Introduction:

Biocontrol offers a solution to many of the persistent problems in agriculture including problems of health hazards, environmental pollution, residual effects, overreliance on pesticides/fungicides, resource limitations, etc. Biocontrol mechanism to suppress fungal pathogens by *Trichoderma* spp., *Pseudomonas* and *Bacillus spp*. generally involves the production of antibiotics, siderophores, volatile compounds, hydrocyanic acid (HCN), enzymes phytohormones and subtilin, phosphatidylethanolamine, and lysylphosphatidylglycerol. The effect of biocontrol agents i.e., bacterial species including *Pseudomonas fluorescens* and *Bacillus subtilis* were tested by dual culture technique. The Petri plates were incubated with pathogenic fungi and biocontrol agents. The zone of inhibition was measured concerning its growth and results were recorded. Biological control of seed-borne pathogens continues to inspire research and development in many fields. However, the interrelationships of many environmental variables can result in multiple interactions among organisms and their environment, several of which might contribute to effective biological control as a biological control agent, *P. fluorescens* has been shown to have a beneficial effect on plant growth and health (Anuratha and Gnanamanickam, 1990; Shanmugam *et al.*, 2002; Chin-A-Woeng *et al.*, 2003). Furthermore,

natural products and chemical compounds were discovered as a result of basic research into the molecular mechanisms of pathogenesis and we use the term biological control in the broader sense as we describe the current status of research, commercial development, and application of biocontrol strategies targeted at plant pathogens (De Boer, 1999; Reddy *et al.*, 2007, 2009). Cook and Baker (1983), Georgakopoulos *et al.* (2002), Crowley (2006), Girija and Manojkumar (2005) and Roy Manidipa1 *et al.* (2013) studied the biological control of Plant Pathogens. The mechanisms of biological control of plant pathogens by antagonistic bacteria and fungi have been the subjects of many studies in the past two decades (Janisiewicz *et al.*, 2000; Fravel, 2005; Megha *et al.*, 2007). Mechanisms of biocontrol of root and soil-borne pathogens are as a result of the direct action of antagonists on plant pathogens, through antibiosis, predation or parasitism, induced resistance of the host plant, and direct competition for space and limited resources (Janisiewicz *et al.*, 2000; Gupta *et al.*, 2001; Nagarajkumar *et al.*, 2004; Romero, 2004; Lwin and Ranamukhaarachchi, 2006).

Materials and Methods:

Dual culture technique:

Antagonistic properties of bacterial strains (*Pseudomonas fluorescens* and *Bacillus subtilis*) were tested against *Aspergillus flavus* (Link), *Fusarium moniliforme* (J. Schled.), and *Alternaria alternata* (Fr. Keissl) on PDA plates using a dual culture technique (Skidmore and Dickinsom, 1976). Agar blocks (5 days old, 5mm diameter) containing 5 days old mycelium were placed in the center of the PDA plate. A loop full 24 hours old culture of bacterial strain was inoculated at 2cm juxtaposed to the pathogen on each plate. The fungal pathogen was inoculated centrally on the PDA plate but uninoculated by bacteria strain served as control. The plates were incubated at 28± 10C for 5 days and colony growth inhibition (%) was calculated. Observe the development of the inhibition zone. The diameter of the mycelial growth was measured when controlled plates showed the maximum growth, after 8 days of incubation. The percentage inhibition of mycelial growth was calculated as per the formula given by Vincent (1947).

Experimental Results:

Table 1 shows that the growth of *Aspergillus flavus* was 4.0 cm in presence of *Pseudomonas fluorescens,* while 4.5 cm in the presence of *Bacillus subtilis.* The percent growth of inhibition of *Pseudomonas fluorescens* and *Bacillus subtilis* were 46.66% and 40.00%

respectively. The growth of *Aspergillus flavus* on the control plate was 7.5 cm. *Pseudomonas fluorescens* inhibit the maximum growth of *Aspergillus flavus* as compared to *Bacillus subtilis.*

The growth of *Fusarium Fusarium moniliforme* (J. Schled) in presence of *Pseudomonas fluorescens* was 2.5 cm and the percentage growth of inhibition was 58.33. In presence of *Bacillus subtilis* growth of the fungus was 3.5 cm and the percentage growth of inhibition was 41.66. The growth of *Fusarium moniliforme* on the control plate was 6.0 cm from Table 2 and Fig 1. So, it is clear that *Pseudomonas fluorescens* inhibits the maximum growth of *Fusarium moniliforme* as compared to *Bacillus subtilis.*

Table 3 reveal that the growth of *Alternaria Alternata* in presence of *Pseudomonas fluorescens* was 2.0 cm and the percentage growth inhibition was 50.00 while in the presence of *Bacillus subtilis* growth was 3.0 cm and the percentage of growth inhibition was 25.00. The growth of *Alternaria Alternata* on the control plate was 4.0 cm. The percentage of growth inhibition was maximum by *Pseudomonas fluorescens.*

Table 1: Effect of Bacterial species on the growth of *Aspergillus flavus* Link. in Dual culture

Sr. No.	Bacterial antagonists	Growth of *Aspergillus flavus* against bacterial species in cm	% of growth inhibition
1	***Pseudomonas fluorescens***	4.0	46.66
2	***Bacillus subtilis***	4.5	40.00
3	Control	7.5	-
	S.E. ±	0.89	2.35
	C.D. at p=0.01	8.83	149.60
	C.D. at p=0.05	3.85	29.86

Table 2: Effect of Bacterial species on the growth of *Alternaria Alternata* (Fr. Keissl) in Dual culture

Sr. No.	Bacterial antagonists	Growth of *Alternaria alternata* against bacterial species in cm	% of growth inhibition
1	***Pseudomonas fluorescens***	2.0	50.00
2	***Bacillus subtilis***	3.0	25.00
3	Control	4.0	-
	S.E. ±	0.46	8.86
	C.D. at p=0.01	4.56	564.02
	C.D. at p=0.05	19.17	112.61

Discussion:

Vincent (1947) studied the Distortion of fungal hyphae in the presence of certain inhibitors. Agrawal (1976) observed a Technique for the detection of seed-borne fungi. Skidmore and Dickinsom (1976) observed colony interaction of hypal interference between *Septoria nodorium* and Phylloplane fungi. Loper (1988): studied the Role of fluorescent siderophore production in biological control of *Pythium ultimum* by a *Pseudomonas fluorescens* strain. Anuratha and Gnanamanickam (1990) studied the biological control of bacterial wilt caused by Pseudomonas solanacearum. Smith Handelsman and Goodman (1997) studied Modeling dose-response relationships in biological control: partitioning host responses to the pathogen and biocontrol agent. Vidhyasekaran and Muthamilan (1999) evaluated a powder formulation of *Pseudomonas fluorescens*. Janisiewicz *et al.* (2000) studied the mechanism of biological control of postharvest diseases on fruits Whipps (2001) studied Microbial interactions and biocontrol in the rhizosphere. Gupta *et al.* (2001) observed Antibiosis mediated necrotrophic effect of *Pseudomonas* against two fungal pathogens. Georgakopoulos *et al.* (2002) evaluated the Biological control of cucumber and sugar beet damping-off caused by Pythium ultimun with bacterial and fungal antagonists. Shanmugam *et al.* (2002) observed the Interaction of *Pseudomonas fluorescens* with Rhizobium for their effect on the management of peanut root rot. Chin-A-Woeng *et al.* (2003) studied Phenazines and their role in biocontrol by Pseudomonas bacteria. Suryakala *et al.* (2004) studied Chemical characterization and in vitro antibiosis of siderophores of rhizosphere fluorescent pseudomonads. Wandersman and Delepelaire (2004) observed Bacterial iron sources: From siderophores to hemophores. Nagarajkumar *et al.* (2004) studied the involvement of secondary metabolites and extracellular lytic enzymes produced by *Pseudomonas fluorescens* in inhibition of *Rhizoctonia solani*, the rice sheath blight pathogen. Romero *et al.* (2004). Isolated and evaluation of antagonistic bacteria towards the cucurbit powdery mildew fungus *Podosphaera fusca*. Manwar *et al.* (2004). Observed Siderophore production by a marine *Pseudomonas aeruginosa* and its antagonistic action against phytopathogenic fungi. Fravel (2005) studied the Commercialization and implementation of biocontrol. Girija Ganeshan and Manojkumar (2005) screened *Pseudomonas fluorescens*, a potential bacterial antagonist to control plant diseases. Crowley (2006) studied Microbial siderophores in the plant rhizospheric. Lwin and Ranamukhaarachchi (2006) studied the Development of biological control of *Ralstonia solanacearum* through antagonistic microbial populations. de Bruijn *et al.* (2007) Genome-based discovery, structure prediction, and functional analysis of cyclic lipopeptide antibiotics in *Pseudomonas* species. Megha, *et al.*

(2007) observed Multiple Beneficial Functions of Fluorescent Pseudomonads of Western Ghats of Uttar Kannada Reddy *et al.* (2007) screened Antifungal metabolites of *Pseudomonas fluorescens* isolated from the rhizosphere of rice crop. Reddy and Rao (2009) observed Biochemical and PCR-RAPD characterization of *Pseudomonas fluorescens* produced antifungal compounds inhibit the rice fungal pathogens in vitro. Roy Manidipal *et al.* (2013) studied Pseudomonads: Potential Biocontrol agents of Rice Diseases.

Conclusion:

Seed act as a carrier for the transport of seed-borne pathogens harmful to the plants. The abundance of seed viability of seed-borne fungi is responsible for loss in seed viability and poor growth of the crop, which brings about varied pathogenic effects on plants. Biological control is an ecology-conscious, cost-effective, and sustainable alternative method in disease management. As reported by many researchers that *Pseudomonas* and *Bacillus* produce different antibiotics which suppress the disease-causing pathogens. The ability of bacterial siderophores and antibiotics to suppress phytopathogens and seed-borne pathogens could be of significant agronomic importance. We conclude by describing prospects for using biological control to limit the damage of seed-borne pathogens in both conventional and organic agriculture.

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PHYTOCHEMISTRY, ETHNOMEDICINAL USE AND PHARMACOLOGICAL PROFILE OF *ADHATODA VASICA* NEES - AN ETHNOMEDICINAL PLANT

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Abstract:

Medicinal plants are important for pharmacological research and drug development. It provides raw materials for the herbal medicine trade and the pharmaceutical industry. Herbal medicines are derived from the leaves, roots or other parts of the medicinal plants. A large number of modern pharmaceutical drugs are derived from plant secondary metabolites. *Adhatoda vasica* Nees, is a well-known medicinal plant belonging to family Acanthaceae, used for the treatment of various diseases, primarily for the respiratory tract ailments. A significant amount of research has been carried out to explore the chemistry and pharmacological effect of *Adhatoda vasica*. The plant is known to contain pyrroloquinazoline alkaloids and its derivatives, such as vasicine and vasicinone. It shows various pharmacological activities like antimicrobial, antiulsar, anti inflammatory, anti cancer, anti antitussive, andanti-oxidant activities etc. This review describes the phytochemistry, ethnomedicinal uses, and pharmacological activities of *Adhatoda vasica*. Shortly it also describes the genetic diversity studies, biotechnological applications such as micropropation and elicitation studies for the propagation and enhanced metabolites production of this plant.

Keyword: *Adhatoda vasica*, Phytochemistry, pharmacological activity, tissue culture, molecular studies.

Introduction:

Medicinal plants have been used as a source of drugs by mankind for several thousand years to cure different illnesses. India has a rich heritage of traditional medicine and health care system. The Indian system of medicine heavily relies on medicinal plants and Ayurveda have

their roots in folk medicine (Ravishankar and Shukla, 2007). Among the variety of modern medicines too, many of them are produced directly or indirectly from medicinal plants. Studying medicinal plants helps to understand plant diversity, phytochemistry and their pharmacological properties. The medicinal effects of plants depend on the presence of secondary metabolites that are affected by environmental stress.

***Adhatoda vasica* Nees**

Adhatoda vasica Nees belongs to the family Acanthaceae, is a small evergreen shrub with many long opposite branches having large lance-shaped, opposite and exstipulate leaves (Figure 1 and 2). The leaves, flowers, fruits and roots are used as medicinal parts .The leaves of *Adhatoda vasica* contains phytochemicals such as alkaloids, tannins, saponins, phenolics and flavonoids etc. The leaves carry an unpleasant smell and have a bitter taste. The flowers are spikes or panicles, small irregular zygomorphic, bisexual, hypogynous and either white or purple in colour. It has capsular four seeded fruits. The plant is used for the treatment of various diseases and disorders, primarily for the respiratory tract ailments.

Figure 1: *Adhatoda vasica* plant

Figure 2: *Adhatoda vasica* leaf and inflorescence

Origin and distribution

Adhatoda vasica Nees is native to India. It's distributed throughout India up to an altitude of 1,300 m and mainly found in lower Himalayas. It is also found in few parts of Sri-Lanka, Bhutan, Pakistan, Afghanistan, and is additionally introduced to other countries like China, Hong kong, Taiwan, Ethiopia etc. This plant is also found in tropical regions of Southeast Asia and a couple of part in Germany and Sweden**.**

Phytochemical analysis

The phytochemical analysis of ethanolic leaf extract of *Adhatoda vasica* revealed the presence of alkaloids, flavonoids, terpenoids, saponins, phenols and steroids (Bajpai *et al.*, 2015, Sudevan *et al.*, 2019). Six different quinazoline alkaloids (vasicoline, vasicolinone, vasicinone,

vasicine, adhatodine and anisotine) have been detected in the leaf of *Adhatoda vasica* (Jha *et al.,* 2012). Quantification of Vasicine and Vasicine acetate by HPLC-DAD analysis showed their contents to be 0.2293% and 0.0156%, respectively, on dry weight basis of the leaves (Duraipandiyan *et al.,* 2015). Eleven compounds such as, 1,2,3, trimethyl benzene (1.51 %), borneol (58.60%), ethanonaphthalene (2.82%), 1,1,4a trimethyl-5,6-dimethylenedecahydro naphthalene (5.28 %), 2,tert-butyl-1,4- dimethoxy benzene (6.50%), bicyclo[jundec-4-ene,4,11-trimethyl-8-methylene (14.56%), hexa- methyl dewar benzene (0.87%), alphacaryophyllene (1.95%), cycloproplejazulene (1.48 %), caryophyllene oxide (2.35%) and 2-naphthalenemethanol (1.46%) has also been identified from the essential oil obtained by hydrodistillation of *Adhatoda vasica* leaves (Sarkera *et al.,* 2011). A rapid, efficient, and sensitive method has been developed for the estimation of vasicine (Madhukar *et al.,* 2014). A wide range of fatty acids and the heterocyclic compound has also been identified from leaves, shoot and flowers of *Adhatoda vasica* (Khan and Bhadauria, 2017, Jayapriya and Shoba, 2015)**.**

Ethnomedicinal use of *Adhatoda vasica*

Plants have been used since ancient times for the treatment of various diseases. Traditionally, *A. vasica* is used for the treatment of various ailments, which is described in table 1.

Pharmacological activity

During the last 2-3 decade, several scientific studies on the phytochemistry, pharmacological activities and molecular biology of *Adhatoda vasica* have been carried out. A wide range of phytochemical constituents have been isolated from this plant which show pharmacological activities like antimicrobial, antiulcer, anti inflammatory, anti cancer, anti antitussive**,** anti-oxidant activities etc.

Anti-ulcer activity

The anti-ulcer activity of *Adhatoda vasica* leaves using two ulcer models: Ethanol-induced and Pylorus ligation plus aspirin-induced models have been studied. *Adhatoda vasica* leaf powder showed a considerable degree of anti-ulcer activity in experimental rats when compared with a control. The highest degree of activity (80%) was observed in the ethanol-induced ulceration model. Results of the study suggest that in addition to its classically established pharmacological activities, the plant also has immense potential as an anti-ulcer agent of great therapeutic relevance (Shrivastava *et al.,* 2006).

Table 1: Ethnomedicinal use of *Adhatoda vasica*

Ethnomedicinal use	Parts used	Mode of administration
Bronchitis, cough and cold	Leaf	5–6 leaves mixed with ginger is boiled half to its original amount and taken with honey for curing cough (Raj *et al.,* 2018). Leaf juice taken for several days as expectorant to treat chronic bronchitis, cough and cold (Dutta *et al.*, 2014). Flower powder used for cough and cold (Singh *et al.,* 2017).
Diabetes	Leaf Root	Juice of leaves is used with an empty stomach. A decoction of roots (~ 50 g) with cow milk (125 mL) is taken daily in the morning (Kumar *et al.,* 2019).
Stomachache and fever.	Leaf	Leaf buds (5–10) decoction 100 ml is taken thrice a day for treatment of stomachache and fever (Singh *et al.*, 2017). Decoction prepared from leaves of *Adhatoda vasica* is used orally for treatment of fever (Venkatachalapathi *et al.,* 2018).
Tuberculosis.	Leaf	Juice obtained from crushed leaves is orally taken (Kabir *et al.,* 2014).
Nose bleeding, dysentery and blood vomiting	Flower Leaf	Fresh flowers and leaves are boiled in water and decoction is prepared which is consumed once in a day (Sajem and Gosai, 2006)
Itching and Skin infections.	Leaf	Leaves are boiled and the solution is used for taking bath for curing itching (Raj *et al.,* 2018). Young leaves are made into a paste and applied over infected area in skin (Kabir *et al.*, 2014)**.**
Cut and wound	Leaf	Leaf extract or juice applied for treatment of cut and wounds (Singh *et al.,* 2017).
Snakebite	Leaf	Paste is applied externally (Sulochana *et al.,* 2015)**.**
Teeth cleaning	Stem	Stem used for cleaning teeth (Singh *et al.,* 2017).

Antibacterial Activity

Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* showed antibacterial activity against *M. Luteus*, *E. Aerogenes*, *S. epidermidis*, and *P. aeruginosa*. Vasicine acetate exhibited good zone of inhibition against bacteria: 10 mm

against *E. aerogenes*, 10 mm against *S. epidermidis*, and 10 mm against *P. aeruginosa*. Vasicine acetate showed minimum inhibitory concentration values against bacteria: *M. luteus* (125 μg/mL), *E. aerogenes* (125 μg/mL), *S. epidermidis* (125 μg/mL), and *P. aeruginosa* (125 μg/mL) (Duraipandiyan *et al.,* 2015). Sudevan *et al.,* also reported antibacterial activity against *Salmonella* and *Escherichia coli* (Sudevan *et al.,* 2019). Vasicine showed moderate activity against *M. luteus*, and *S. Typhimurium* (Shahwar *et al.,* 2012). Antimicrobial activity of isolated compounds was assessed by using the micro dilution method. The strong antibacterial activity was exhibited by vasicine at 20 µg/ml dose against *E. coli* (Singh and Sharma 2013). Phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica* showed antibacterial activity against *Bacillus licheniformis*, a pathogenic bacteria which cause high mortalities and economic losses among the ornamental fish farms of India (Saha and Bandyopadhyay 2020). Copper Oxide/Carbon nanocomposites developed using the leaf extract of *Adhatoda vasica* showed significant antibacterial activity against the pathogenic bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus (*Bhavyasree and Xavier 2020). Nithya and Sundrarajan, prepared noble metal such as Ag and Au doped CeO_2 nanoparticles by ionic liquid assisted hydrothermal method in the presence of *Adhatoda vasica* leaves extract and used as an antibacterial and anticancer agent. The AgAu loaded CeO_2 nanoparticles exhibited the highest zone of inhibition against *E. coli* and *S. aureus* strains when compared with pristine CeO_2, Ag loaded CeO_2, and Au loaded CeO_2 nanoparticles (Nithya and Sundrarajan 2020).

Antifungal activity

The nanocomposites prepared by mixing the copper sulphate penta hydrate solution with the plant extractposses antifungal activity against the fungi *Aspergillus niger* and *Candida albicans (*Bhavyasree and Xavier 2020). In another experiment, vasicine showed antifungal activity against *C. albicans* (Singh and Sharma 2013).

Antioxidant activity

Adhatoda vasica is known to posses significant antioxidant and free radical scavenging activities In an experiment ethanolic leaf extract of *Adhatoda vasica* showed antioxidant capacity, with inhibition of the 1,1-diphenyl2-picrylhydrazyl (DPPH) radical, scavenging nitric oxide, superoxide and hydroxyl radicals. Moreover, it also displayed a concentration-dependent reducing power activity and potent inhibitory effects on ferric ion-induced lipid peroxidation in bovine brain extract (Bajpai *et al.,* 2015). Vasicine isolated from the ethanolic extract exhibited significant antioxidant activity. In an experiments vasicine showed significant antioxidant

activity as infered by the concentration dependent increase of the reduction of Fe^{3+} in FRAP assay and significant scavenging of free radicals in DPPH assay (Shahwar *et al.*, 2012). The radical scavenging activity of Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* was the maximum at 1000 μg/mL (66.15%) (Duraipandiyan *et al.*, 2015). Vasicinone, a quinazoline alkaloid from *Adhatoda vasica* treatment also lowered the ROS levels in A549 cells and have potential free radical scavenging (DPPH, Hydroxyl) activity and ferric reducing power in cell free systems (Dey *et al.*, 2018). These findings suggest the biological efficacy of *A. vasica* as a potential source of natural antioxidants.

Anti-inflammatory activity

The anti-inflammatory activity was tested by using carrageenan and CFA-model induced paw oedema in rats. The observed results revealed that vasicine showed most potent anti-inflammatory effects (59.51%) at the dose of 20.0 mg/kg at 6 h after carrageenan injection. Maximum inhibition rate was observed of vasicinone (63.94%) at the dose of 10.0 mg/kg at 4 days after CFA injection. All the five alkaloids viz., vasicine, vasicinone, vasicine acetate, 2-acetyl benzyl amine, vasicinolone present in the chloroform fraction demonstrated significant anti-inflammatory activities (Singh and Sharma 2013)**.** Sudevan *et al.,* also reported significant anti-inflammatory activity of the active components present in *Adhatoda* plant extract (Sudevan *et al.,* 2019).

Anticancer/Cytotoxic Activity

One of the important aims of drug discovery for cancer is to find therapeutic agents from natural products that are effective and safe for cancer treatment. Vasicine acetate obtained by acetylation of Vasicine isolated from ethanolic extract of the leaves of *A. vasica* showed prominent cytotoxic activity against A549 lung adenocarcinoma cancer cell line (Duraipandiyan *et al.*, 2015). An alkaloid, 2-acetyl-benzylamine *Adhatoda vasica*, posses significant cytotoxic properties against leukemia cells MOLM-14 and NB-4. It induced cell cycle arrest at G2/M phase in MOLM-14 cells and G0/G1 phase in NB-4 cells (Balachandran *et al.,* 2017). The anticancer activity of the *Adhatoda vasica* extract is found to be effective in human cervical cancer cell line (HeLa) has also been reported in in-vitro analysis (Sudevan *et al.,* 2019). Recently it was observed that the CeO_2, Ag/CeO_2, Au/CeO_2 and $Ag\text{-}Au/CeO_2$ nanoparticles prepared by ionic liquid assisted hydrothermal method in the presence of *Adhatoda vasica* leaves extract exhibited anticancer activity against the HeLa cells (Nithya and Sundrarajan 2020). The findings proved that these compounds could be developed as a potential therapeutic agent against cancer. Dey *et al.,* investigated the anti-proliferative effect of vasicinone and its underlying mechanism against A549 lung carcinoma cells. The A549 cells upon treatment with various

doses of vasicinone showed significant decrease in cell viability. Vasicinone treatment caused DNA fragmentation, LDH leakage, and disruption of mitochondrial potential, and lower wound healing ability in A549 cells. Moreover it was also observed that the vasicinone treatment lead to down regulation of Bcl-2, Fas death receptor and up regulation of PARP, BAD and cytochrome c, which suggest the anti-proliferative nature of vasicinone which mediated apoptosis through both Fas death receptors as well as Bcl-2 regulated signalling (Dey *et al.*, 2018).

Antimutagenic activity

Environmental and occupational exposure with cadmium affects the renal system adversely. In a study, the antioxidant and anticlastogenic efficacy of *A. vasica* against cadmium chloride (CdCl2)-induced renal oxidative stress and genotoxicity in Swiss albino mice has been evaluated. A single intra-peritoneal dose of CdCl2 resulted in significant increase in chromosomal aberration and micronuclei formation. Cadmium intoxication altered the antioxidant levels and enhanced malondialdehyde **(**MDA) formation significantly. Oral administration of *A. vasica* at two doses (50 and 100 mg/kg BW) for seven consecutive days showed significant suppression of mutagenic effects of $CdCl_2$ in plant-pretreated groups. The antimutagenic efficacy of *A. vasica* can be attributed to its restoring effects on antioxidant status and suppression of MDA level formation. Prophylactic pretreatment of *A. vasica* extract in cadmium-intoxicated mice showed marked inhibition of lipid peroxidation (LPO) and xanthine oxidase (XO) activity (Jahangir *et al.*, 2006)**.**

Antitussive activity

Antitussive drugs are amongst the most widely used medications worldwide. In traditional system of medicine *A. vasica* has been used for its antitussive activity. Leaf extract used to treat cold, cough, whooping cough, as an expectorant and to treat chronic bronchitis. It was reported that the *Adhatoda vasica* leaves extract posses good antitussive activity. The antitussive activity of *Adhatoda vasica* extract has been evaluated in anaesthetized guinea pigs and rabbits and in anaesthetized guinea pigs. Intravenously, it was as active as codeine on mechanically and electrically induced coughing in rabbits and guinea-pigs. After oral administration to the guinea-pig the antitussive activity was similar to codeine against coughing induced by irritant aerosols (Dhuley 1999). A pectic arabinogalactan has also been isolated from *Adhatoda vasica* by aqueous extraction and precipitation with ethanol. Oral administration of arabinogalactan inhibited the number of coughs induced by citric acid in guinea pigs and slightly decreased the values of specific airway resistance (Chattopadhyay *et al.*, 2011). Similarly, ethyl acetate and methanolic extract of leaves of *Adhatoda vasica* also posses

antitussive activity in Ammonium hydroxide and Sulphur dioxide induced cough models in mice. It was found that both extracts of *Adhatoda vasica* showed anti-tussive activity and obtained percentage inhibition of cough reflex is approximately comparable as standard drug Codiene phosphate (Srivastava and Choudhary 2016).

Immunomodulatory

Vinothapooshan and Sundar reported the immunomodulatory properties of different extract of leaves of *Adhatoda vasica* in experimental animals. Oral administration of extracts in adult male Wister rats significantly increased the percentage neutrophil adhesion to nylon fibers. The observed results at different doses were significant when compared to control groups (Vinothapooshan and Sundar, 2011). This indicated that the extract could be useful as chemotherapy for neutrophils. In another study, effect of *Adathoda vasica* extract in antibody production, no. of antibody producing plasma cell, release of anti-inflammatory cytokines in serum on mice was studied. The leaf extract had long term effect on humoral immune system and inhibitory potential on inflammatory response (Adhikary *et al.*, 2014).

Radio modulatory effect

Modulatory effect of ethanolic extract of *A. vasica* against radiation-induced changes in terms of histological alterations in testis, reduced glutathione (GSH), lipid peroxidation (LPO), acid and alkaline phosphatases levels, and chromosomal alterations in Swiss albino mice has been studied. *A. vasica* extract pretreated irradiated animals exhibited a significant increase in GSH content and decrease in LPO level. Similarly, a significant increase in the serum alkaline phosphatase activity and decrease in acid phosphatase activity was observed in *A. vasica* extract pretreated irradiated animals. *Adhatoda* pretreatment also significantly prevented radiation-induced chromosomal damage in bone marrow cells. Finding from these studies suggests that *Adhatoda* plant extract has significant radioprotective effects (Kumar *et al.*, 2005, Kumar *et al.*, 2007).

Anti Alzheimer

Alzheimer's disease (AD) is a neurodegenerative disease affecting older adults and is characterized by a progressive decline of memory and cognition. Various studies have been carried out to find therapeutic approaches for Alzheimer's disease. However, the proper treatment option is still not available. The herbal therapy is now anticipated to control AD progression and help to relieve the symptoms. Medicinal plants used in different systems of medicine particularly exhibit their powerful role in the management and cure of memory disorders (Akram and Nawaz 2017). *Adhatoda vasica* showed inhibitory effect on acetylcholinesterase (AChE). Additionally, itsstrong and reversible interaction against AChE, make them effective, new and promising

agents for treatment of AD in the future (Ali *et al.*, 2013, Ali *et al.*, 2016). Based on these studies researcher suggested that *Adhatoda vasica* can be used directly or indirectly for the development of efficient drug for the treatment of Alzheimer disease.

Anti-tuberculosis activity

Aqueous extract of leaves of *A. vasica*, was tested *in vitro* for its activity against two multi-drug resistant (MDR) isolates (DKU-156 and JAL-1236) and susceptible strain *M. tuberculosis* H37Rv using Lowenstein Jensen (L-J) medium and colorimetric BacT/ ALERT 3D system. Extract exhibited anti-tuberculosis activity against MDR isolate DKU-156, JAL-1236 and sensitive *M. tuberculosis* H37Rv in L-J medium. In BacT/ALERT also, extract of this plant showed significant inhibition against *M. Tuberculosis* (Gupta *et al.*, 2010). Vasicine acetate and 2-acetyl benzylamine isolated from hexane extract of *A. vasica* leaves significantly inhibited pathogenic bacteria *M. Tuberculosis* (Ignacimuthu *et al.*, 2010).

Hepatoprotective activity

Adhatoda vasica leaf extract showed significant hepatoprotective effect at doses of 50-100 mg/kg, on liver damage induced by D-galactosamine in rats (Bhattacharyya *et al.*, 2005). Hepatoprotective activity of vasicinone isolated from leaves of *Adhatoda vasica* has been reported in Carbon Tetrachloride (CCl_4)-induced acute hepatotoxicity model in mice. CCl_4 treatments lead to significant increase in serum glutamic oxaloacetate transaminase (SGOT), serum glutamic pyruvates transaminase (SGPT), alkaline phospatase (ALP) levels. Pre-treatment with vasicinone significantly decreased these enzyme levels. Furthermore, these biochemical observations were also confirmed by histopathological examinations of liver sections and were comparable with the standard hepatoprotective drug Silymarin which served as a positive control (Sarkar *et al.*, 2014). Similarly, hepatoprotective activity of aqueous extract of *Adhatoda vasica* on total protein, bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in CCl_4 intoxicated rats has also been studied. Administration of CCl_4 showed significantly increase liver marker enzymes in serum and significantly decreased total protein. *A. vasica* at 250 mg/kg and 500 mg/kg body weight showed significant increase in total protein when compared to CCl_4 treated rats. Leaf extract lowered enzymes levels, which is a designation of hepatoprotective action of the extract (Kumar *et al.*, 2015).

Antidiabetic

In an experiment it was observed that the methanolic extract from the leaves of *Adhatoda vasica* showed sucrase inhibitory activity. Compounds vasicine and vasicinol showed high sucrase inhibitory activity, and the IC50 values were 125μM and 250μM, respectively (Gao *et al.*, 2008). The effect of ethanolic extracts of leaves and roots was also studied in alloxan

induced diabetic animals. Oral administration of leaves extract to normal and experimental diabetic rats produced a significant reduction in blood glucose levels as compared to the root extract (Gulfraz *et al.,* 2011). These finding suggest use of the *A. vasica* extract as an antidiabetic agent and show a possibility that compounds present in this plant could be a useful treatment for metabolic disorders.

Antiviral activity

The preliminary report on antiviral activity of *Adhatoda vasica*, against Herpes Simplex Viruses shows its antiviral activity. The Herpes viruses (HSV) are important pathogens that can cause mild to severe viral infections in human (Koelle and Corey 2008). Methanolic and ethanoloic extract from the leaves of *Adhatoda vasica* significantly inhibits plaque formation in HSV infected tissue culture, which suggest that this herbal extract has potent anti-viral agents against herpes simplex viruses that can be exploited for development of an alternative remedy for HSV infections (Chavan *et al.,* 2013).

Genetic diversity in *Adhatoda vasica*

Systematic analysis of genetic diversity and relationship among cultivars is essential for development of appropriate conservation and breeding program. DNA-based molecular marker techniques, such as random amplification of polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) has been used to unravel the genetic variability and relationships across thirty-two wild accessions of *Adhatoda vasica*. Amplification of genomic DNA using 38 primers (18 RAPD and 20 ISSR) yielded 434 products, of which 404 products were polymorphic revealing 93.11 % polymorphism (Kumar *et al.,* 2014). Garg *et al.,* also reported genetic variation amongst the accessions of *Adhatoda vasica* collected from different sub-climatic zones of India by RAPD (Garg *et al.,* 2015). Genetic diversity study in India using Amplified Fragment Length Polymorphism (AFLP) fingerprinting also suggested a very high genetic variation in *Adhatoda vasica* (Varma and Shrivastava 2018).

In vitro* regeneration and production of secondary metabolites in *Adhatoda vasica

Conventionally, *A. vasica* is propagated through seed germination and stem cuttings. But poor seed germination and season dependent propagation via stem cuttings limit the frequency of propagation. Tissue culture technique provides a high efficiency regeneration system for successful regeneration of adventitious shoots for ex situ preservation as well as genetic improvement studies for pharmaceutical uses and future research investigations in medicinal plants. A novel protocol for indirect shoot organogenesis of *Adhatoda vasica* was also developed using petiole and leaves explants. Media with concentrations of cytokinins in combination with auxins were used to induce callus formation. Although both petiole and leaf segment explants

produced callus, higher potential for organogenic differentiation from callus derived from petiole explants was observed on MS medium supplemented with 0.25 mg l^{-1} NAA and 0.25 mg l^{-1} TDZ (Mandal and Laxminarayana 2014). Prajila and Indulekha, induced callus from axillary bud explants of *Adhatoda* and the callus showed shoot organogenesis in MS+2,4-D 0.1 mg/l and BAP 4.0 mg/l and root was induced in MS+ 2,4-D 0.1mg/l (Prajila and Indulekha 2016). Plant cell cultures, shoot cultures, root cultures and transgenic roots obtained through biotechnological means are promising potential alternative sources for the production of high-value secondary metabolites of industrial importance (Rao and Ravishankar 2002). It was observed that the *in vitro* cultured samples produced relatively higher vasicinone than that of the *in vivo* counterparts. In vivo leaves and stems produced comparatively lower quantities of vasicinone (2.41% and 1.93% of dry weight, respectively) as compared to leaves, stems and calli (6.4%, 2% and 5.22 % of dry weight, respectively) (Panigrahi *et al.*, 2017).

Madhukar *et al.*, optimized tissue culture protocol for quinazoline alkaloids that can be used at industrial level using bioreactors for large scale production and constant supply of vasicine. The addition of extra 28 mM KNO_3 and 100 mM NaCl in MS medium supplemented with 2,4-dichlorophenoxyacetic acid, benzyladenine and indole acetic acid (1ppm each) produces faster biomass and higher amount of quinazoline alkaloids (Madhukar *et al.*, 2014).

Enhanced secondary metabolites production in *Adhatoda vasica* by Elicitation

Plants and/or plant cells *in vitro*, show physiological and morphological responses to microbial, physical or chemical factors which are known as ‘elicitors’. Elicitation is a process of inducing or enhancing synthesis of secondary metabolites by the plants to ensure their survival, persistence and competitiveness (Namdeo 2007). Effect of elicitors to improve the productivity of useful metabolite in *Adhatoda vasica* cell culture has also been studied by some author. Increased vasicine production in cell suspension culture of *A. vasica* supplemented with α-naphthyl acetic acid and Mannitol at concentration as 3mg 100ml-1 and 0.32M 100ml-1 respectively has been reported (Pa and Mathew 2012). Pyrroloquinazoline alkaloids are medicinally important compounds, determined by HPLC from cell cultures of *Adhatoda vasica*. Anthranilate synthase functions as rate-limiting factor for the biosynthesis of pyrroloquinazoline alkaloids. Tryptophan and sorbitol enhanced the production of vasicinone, vasicine, 2-acetyl benzyl amine and other pyrroloquinazoline alkaloids by stimulating the anthranilate synthase activity in cell cultures of *A. vasica* (Singh *et al.*, 2017).

Conclusion:

For the last few years, there has been an increasing trend and awareness in medicinal plants research. *Adhatoda vasica* Nees, belonging to family Acanthaceae, is a well-known medicinal plant used for the treatment of various diseases and disorders, primarily for the respiratory tract ailments.It contains pyrroloquinazoline alkaloids and its derivatives, such as vasicine and vasicinone, which is responsible for its pharmacological activities. A significant amount of research has already been carried out during the past few decades in exploring the chemistry and pharmacological effect of *Adhatoda vasica*. But further extensive research and development work still needed for their better therapeutic utilization.

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PLANT PATHOLOGY: AN OVERVIEW

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Introduction:

A plant is in health reflects all its organs or parts performing their functions in natural and regular manner. It is also true that when any of the organ or parts not doing their proper work, say growth, reproduction in natural and regular manner, then plant is said to be diseased, irrespective of cause or causes of its abnormal condition. Therefore disease involves malfunctioning or malforming of various plants processes either in growing or in dormant stages. Hence the term disease is applied to any deviation from natural growth and to produce permanent visible changes on economic value. The word pathology has been derived from greek words Pathos means suffering and logos means discourse or study. Thus plant pathology is the study of the plant diseased. Also plant pathology is a discourse or study about suffering plant. Plant pathology is area of botanical science which deals with the diseases or troubles of the plant thus the field of plant pathology comparises both the art of treating the sick plant and science of understanding the nature of diseased plant. Phytopathology refers to the study of plant disease and covers scientific activity concerned with plant diseases. Therefore disease is defined by various workers in different ways. According to modern conception, disease is an iteraction among host, parasite, and environment. A dictionary meaning is any departure from health, properly marked symptoms, malady, illness, disorders and acoording to Stakman and Harrar (1957) plant disease is a physiological disorder or structural abonormality that is harmful to the plant or to any of its parts or products that reduces the economic value.

Nature and concept of plant diseases:

It is imperative to defined and discus the meanings of certain terms commonly used in phytopathology before we discus the basic principles and concepts of the plant pathology. It is essential to include these definitions and terms in an introductory chapter, since an understanding of these terms is required before the reader can be made aware of the details of the processes involved in plant disease. Phytopathology (phyton-plant, pathos-suffering, logos-knowledge or study) is the study of the diseases of plants and covers the entire field of biological and scientific activity concerned with the understanding of this complex phenomenon. Phytopathology is thus the study of the nature, development and control of plant disease.

Disease is a complex phenomenon is difficult to define in a few words. We have to keep in mind what Locke said in Human Understanding, through definitions will serve to explain the names of substances as they stand for our ideas, yet they leave them not without great imperfection as they stand for things. According to the modern conception, disease is an interaction among the host parasite and environment. A simple dictionary meaning of disease is any departure from health, presenting marked symptoms, malady, illness, disorder. Diseased plants are distinguished by changes in their morphological structure or physiological processes which are brought about by unfavorable environment or by parasitic agencies. Several definitions of diseases in plants have been proposed. Some examples are a series of harmful physiological processes caused by continuous irritation of the plant by a primary agent a harmful deviation from the normal functioning of physiological processes a continuous impairment of metabolism. Stakman and Harrar in 1975 defined plant disease as a physiological disorder or structural abnormality that is harmful to the plant or to any of its part or products that reduces the economic value.

Simple interest disease:

This denotes a disease the increase of which is mathematically analogous to simple interest in money. There is only one generation of disease in the course of one epidemic. A simple interest disease develops from a common source of inoculum, which is the capital is constant and often there is one generation of infection in a season. In the increase of simple interest diseases, the log [1/(1-x)] is potted against time (where x = the proportion of diseased tissue), thus allowing for the fact that with increasing infection, the number of plants susceptible to infection decreases (Fig. 1.1). Soil brone diseases such as vascular wilts and soil borne smuts which infect seedlings and subsequently sporulate in the inflorescences of the mature plants provided that the pathogen does not spread from the smutted heads are examples of simple interest diseases.

Compound interest diseases:

This denotes a disease the increase of which is mathematically analogous to compound interest money. There are several or many generations of the pathogen in the life of the crop, that is the capital is increased by the amount of interest. In plotting the amount of disease against time, the transformation log (x/1-x) is used where x represents the proportion of infected susceptible tissue or of infected plants if the pathogen develops in a systematic manner (Fig. 1.2). Late blight of potato, stem rust of wheat or powdery mildew of wheat are examples of compound interest diseases.

If we know that a particular disease is of the simple interest type, suitable control measures can be applied. For example sanitation affects the simple interest disease more than the compound interest type. If the disease is of the former type, sanitation, including the eradication

of the inoculum of the pathogen form planting material and sites should be insisted upon. If it is of the compound interest type, and the epidemic spreads rapidly and lasts long the infection rate should be reduced by a timely application of fungicides. The stress should shift from sanitation to reducing the infection rate in proportion to the increase in the rate of spread and duration of the epidemic.

Disease can be identified by the visual signs shown by the diseased plants called symptoms, by identifying the causal organism associatd with the host plants. The perticular organism which causes disease is called as pathogen. The ability of the pathogen to cause disease is known as pathogenecity. The chain of events inducing disease is called as pathogenesis. When the causal organism invades it penetrates the host cell. The initial invasion or entry of pathogen into host is known as penetration. When the pathogen reaches the host tissue and established further. The establishment of disease in the host plant is known as infection. The period which require infection and appearance of disease known as incubation period. Resistant is the extent to which the plant is able to prevent the entry or the growth of the pathogen whereas succeptibility is the extent to which the plant is damaged by the spathogen. The effects or changes which are produced externally or internally in the host are known as symptoms.

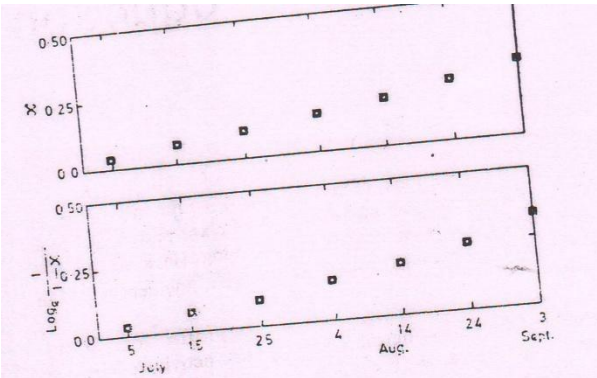

Fig. 1.1 The progress of an epidemic of wilt cotton caused by *Fusarium oxysporum* f. *vasinfectum*. In the upper graph, x is plotted against time; the lower part plots $\text{Log}_e [1/(1-x)]$ against time. (Redrawn from J.E. Van der Plank. *Plant Diseases—Epidemics and Control*, Academic Press, New York and London, 1963, p. 34.)

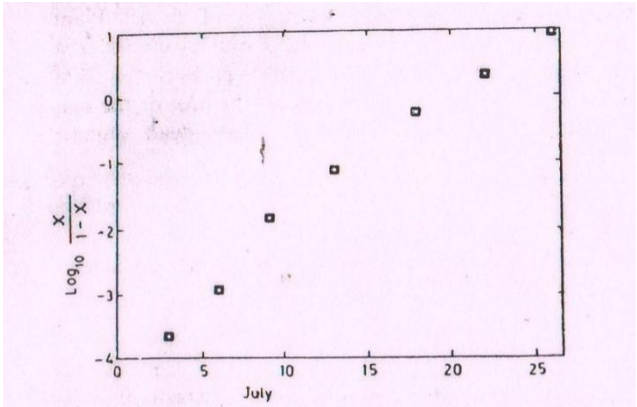

Fig. 1.2 Progress of stem rust of wheat caused by *Puccinia graminis tritici*. (Redrawn from J.E. Van der Plank. *Plant Diseases—Epidemics and Control*. Academic Press. New York and London, 1963, p. 32.)

Causes of disease:

When a parasitic micro-organism enters into the causal complex of a disease, it is commonly considered to be the cause of the disease. However we know that sometimes a variation in the environment also makes a plant liable to infection. The micro-organism is thus not the sole causal factor. After a micro-organism has successfully infected a susceptible plant, the subsequent interaction between the plant and micro-organism (which results in a disease) is also subject to environmental influences. Thus, strictly speaking it is not correct to refer to a

micro-organism as the cause of a given disease since this implies that it is the sole cause. The term causal organism is proper since it implies that the organism is a part of the causal complex. Most of the causal organism is parasites. A parasite is not synonymous with the pathogen. In some cases, organisms are parasitic without becoming causal factors in the disease. Many parasitic establishments do not become pathogenic if the parasitic compensates for the damage done done by its presence. The mycorrhizal fungus is certainly parasitic on the roots of trees but it is not pathogenic. The root nodule bacterium (*Rhyzobium leguminosarum*) is another example. In other cases the by-products of a strict saprophyte are an important part of the causal complex. Leaf tip unjury to oats may be due to the absorption of griseofulvin from soil Brian et. al. in 1951. Milo root rot is caused by a powerful toxin produced by the fungus (*Periconia circinata*) growing in the root zone Oswald in 1955, Leukel in 1948.

Thrower in 1966 modified a definition offered by the British Mycological Society in 1950 and defined a parasite as an organism or virus existing in an intimate association with another living organism from which it derives an essential part of the materials for its existence. In a literal sense a pathogen is any agent which causes damage. According to A guide to the use of terms in plant pathology prepared by the Federation of British Plant Pathologists in 1973, a pathogen is an organism or virus capable of causing disease in a particular host or range of hosts. Most but not all pathogens are also parasites in that they derive the materials they need for existence from a living plant (the host or the suscept) as distinct from saprophytes which derive these materials from dead organic matter.

Importance of plant disease:

The late blight of potato, a disease caused by the fungus, *Phytopthora infestans* is a famous example of what a plant disease can do to change the course of history. In 1845 this disease destroyed the potato crop of Ireland where potato constituted the staple diet of the majority in rural areas. The disease had started in Ireland, England and parts of the continental Europe as early as 1830 and was causing some damage every year, resulting in food shortage. When the late blight of epidemic destroyed the potato crop in 1845 there was famine in Ireland. The demographic data are highly variable (cf. Hampson in 1992) but it was reported that in 1840 the population of Ireland was 8 million which was reduced to 4 million after the famine. Hundreds of thousands perished from hunger and disease. There was large scale migration of the population to other countries including the North American continent where 6 million are reported to have migrated between 1847 and 1854. There are opinions contradicting the belief that the entire catastrophe in Ireland was due to potato famine. Socio-political conditions including the failure of the government to manage the situation were also equally, if not more responsible. But the fact remains that this single disease forced man to realize the importance of plant disease. As a result scientific investigations were taken up the cause of disease was

identified concept of fungus as cause of a disease was finally established and extensive use of chemicals for plant disease control came into existence. The late blight epidemic not only brought the science of plant pathology to limelight it caused many social and political changes in the affected countries. Free trade in England was permitted and import of food grains and other foodstuff was allowed. In order to protect shipping the country had to strengthen its navy which became world's strongest navy.

Wheat rust has been another disease that has appeared in epidemic form from time to time in many countries. This disease has forced the farmers in many parts of the world to change their cropping pattern. Wheat has been replaced by corn (maize) or rye because it was regularly destroyed by rust. This caused change in the food habit of the population in the affected areas. In the last years of the Second World War IN 1943 Bengal had to face a serious famine. One of the reasons to which this famine has been attributed was the loss in yield of the rice crop (major diet of the population) due to attack of Helminthosporium leaf spot which had been affecting the crop for the last several years. Situation was similar to the Irish potato famine but not so catastrophic. In this case also many reasons other than loss of rice crop are listed.

In the middle of the nineteenth century coffee and tea were equally consumed in England because these were available in plenty from such occupied countries as India, Srilanka and Malaysia. Sri Lanka (Ceylon) used to produce the maximum coffee in the world. In 1867 coffee rust attacked the plantastions in Sri Lanka and by 1893 the export of coffee from Sri Lanka had declined by 93 % . The economic crisis forced the planters to cut down coffee plants and take to tea planting. Export of the tea revived the economy to some extent and at the same time consumption of tea increased in England. When coffee rust was spreading in Sri Lanka the science of plant pathology was just developing and control measures for the disease were non known. Tea was also attacked by a blight but by that time chemical control measures were known and the situation did not deteriorate. The system of monoculture in coffee plantation of Sri Lanka was considered as contributory factor in devastations caused by the coffee rust which was not prevalent in coffee growing countries of South America. Coffee rust was first seen in the Western Hemisphere in 1979 in Brazil where it is now spreading. In Brazil coffee trees are often surrounded by others kinds of trees. The decline of coffee cultivation in Sri Lanka gave a boost to coffee industry in Brazil which became a major coffee exporting country in the world. Nearness of this country to USA could be one reason for popularity of this beverages in USA. These instances of plant disease epedemics are worth mentioning because they left their effect not only in the country concerned but also in other countries.

In India wheat rusts had been considered to cause a loss of over Rs. 40 million annually. In the years of epidemics there have been losses amounting to Rs. 500 million or more. Although

introduction of dwarf high yielding varieties has reduced the losses to great extent even now the farmers lose 8-10 % of the expected yield due to rusts. The loose smut of wheat is estimated to cause an average loss of 3 % (about Rs. 50 million) every year. The Molya disease caused by a nematode (cereal cyst nematode) is another example. This disease of wheat and barley prevalent in most parts of Rajasthan, causes a loss of Rs. 30 million in barley and Rs. 40 Million in wheat every year. Different smuts of sorghum are responsible for an annual loss of Rs. 100 million. Five to 75 % loss in chickpea due to Ascochyta blight was reported form Rajasthan during 1982. Wilt of pigeonpea causes 5-10 % loss every year in U. P. and Bihar. At a time when there is shortage of pulses in the countru control of only wilt and sterility mosaic of pigeonpea could increase the production by 15-16 %. Of about 10 % crop losses in the country due to nematodes, there is a loss of about Rs. 20 million every year in coffee alone due to the attack of *Partylenchus coffeae*. Other plant diseases such as red rot and wilt of sugarcane, potato viruses, rice blast and blight, Karnal bunt of wheat, root knot of tomato, eppplant and cucurbits, apple scab, mango malformation, bunchy top of banana, and sandal spike are responsible for huge losses.

In addition to direct loss in yield and monetary returns to the farmers, the plant diseases affect the society in many other ways. When foodgrains are attacked by fungi they may contain toxin (such as aflatoxins) which cause insanity, paralysis, stomach disorders and liver cancer. The money spent on management of plant diseases is also a loss because in absence of diseases this money could be saved. The expenditure on raising the crop before it is attacked by the pathogens is also a waste. When there is less production transport industry may suffer due to lack of goods for transport. Industries that consume raw agricultural materials (cotton, jute, oilseeds, vegetables and fruits for processing) face difficulty in utilizing their installed capacity when there is less production due to plant diseases. In order to make up for the loss of foodgrains and other agricultural products such oilseeds the Governments have to import these commodities which means loss of foreign exchange at the disposal of the Government. Plant disease management requires use of toxic chemicals. Excessive use of such chemicals may lead to environmental pollution affecting human health.

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CHROMATOGRAPHY: A POPULAR TECHNIQUE USED FOR STUDYING CHEMICAL ANALYSIS IN A MIXTURE

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Chromatography is one of the popular laboratory technique used in chemical analysis by separating the components of a given mixture as a result of their differential distribution between stationary and mobile phases. The term" chromatography" have been derived from the Greek word Chroma meaning color and graphein meaning to write. The mixture is dissolved in a fluid solvent (Gas or Liquid) called the mobile phase, which carries it through a system (A column, a capillary tube and a plate or sheet) on which a material called the stationary phase is fixed. Chromatography was first devised by a famous Russian Botanist Mikhail Tswett (1872-1919) in the year 1901 during his research on plant pigments. He developed the technique and coined the term Chromatography, primarily for the separation of Plant pigments such as Chlorophylls, carotenes and xanthophylls. Since these components separate in bands of different colors (green, yellow orange etc.) they directly inspired the name of the technique .Later on Chromatography technique developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s for which they won the Nobel prize in the field of chemistry in1952. They established the principles and basic techniques of partition chromatography and their work encouraged the rapid development of several chromatographic methods: Paper chromatography, gas chromatography and what become known as high performance liquid chromatography. The interaction between the mobile phase and the stationary phase results in the separation of various compounds from the mixture

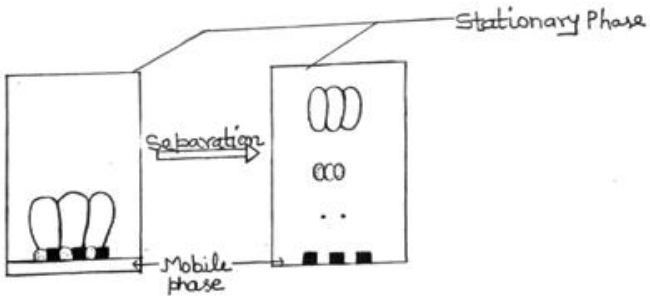

Figure 1: Diagram Shows basic principle of Chromatography

Types of Chromatography- On the basis of functioning /working principles chromatography is of following types-

1. Adsorption chromatography- In this type of chromatography seperation is based on adsorption of chemicals to the surface of a support.

2. Partition Chromatography- Here separation of components from a mixture is based on the partitioning of chemicals into a layer of the stationary phase.

3. Ion- Exchange chromatography- Here separation of ions is based on their binding to fixed charges on a support. It is used for the separation of proteins, RNA and DNA.

4. Gel exclusion chromatography- Here separation of chemicals based on their size and ability to enter a porous support .It is used for the separation of proteins, nucleic acids, polysaccharides and lipids.

5. Affinity chromatography- Here separation of chemicals is based on their interactions with a biologically related binding agent. It is used for the separation of immunoglobulins, cellular enzymes and mRNA

Modes of carrying out chromatography- There are basically three modes that's are generally used /carrying out the process of chromatography. These carrying modes are called as chromatograms. On the basis of these different types of modes that are used in separation, chromatography is divided into following categories.

1. Paper (Called as Paper chromatography)
2. Thin layer (Called as Thin layer chromatography)
3. Column (Called as Column Chromatography)
4. Liqulid (Liquid chromatography/H.P.L.C)

1. Paper Chromatography:

It is a simple method for the separation of smaller molecules from one another. the mixture of the molecules to be separated are applied to the sheets of suitable paper, which are subsequently placed in a vessel which contains a suitable solvent .A distinction is made between ascending and descending paper chromatography according to weather the solvent migrates on the paper from below to above. This technique of chromatography is used for the separation of amino acids, nucleotides and other lower molecular weight metabolic products.

2. Adsorption chromatography:

In this chromatography the solute molecules binds directly to the stationary phase. The components which have more affinity towards mobile phase elutes out first and the components which have more affinity for the stationary phase elutes later no any two components have same

affinity towards mobile phase and stationary phase. Some Solid substances which are known as adsorbents have power to hold molecule at their surface.

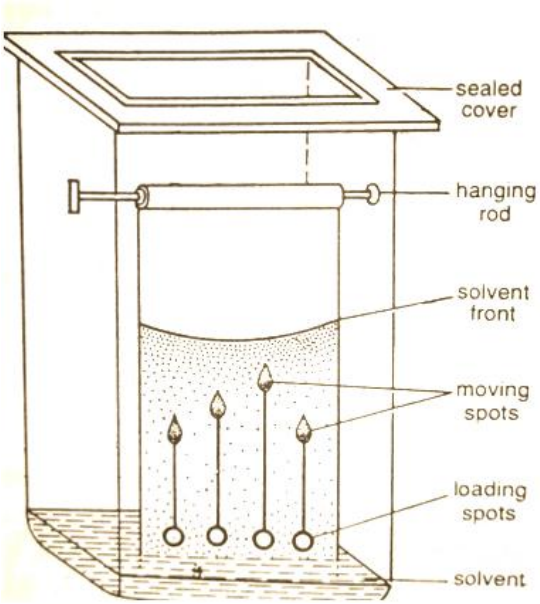

Figure 2: Paper chromatography

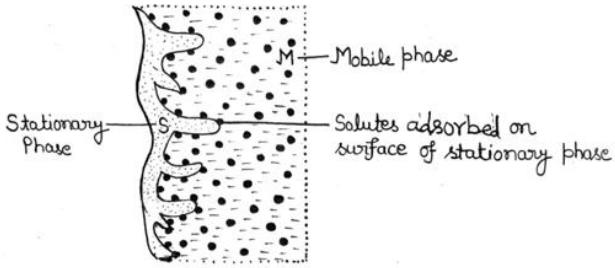

Figure 3: Adsorption chromatography

This holding force is due to weak non ionic attractive forces of the Vander wall and hydrogen bonding which occur only at specific adsorption beds.

Advantages of adsorption chromatography:

This technique retains and separates some compounds that cannot be separated by other methods, like separation of geometrical isomers.

Disadvantages of adsorption chromatography:

- Very strong retention of some solutes.
- It may cause catalytic changes in solutes.
- Solid support may have range of chemical and physical environment that cause nonsymmetrical peaks and variable retention times.

Applications of adsorption chromatography:

- To separate unsaturated hydrocarbons from the mixture.
- To separate and isolate lipids, steroids and fats.
- Use in identification of carbohydrates.
- Use to separate stereo-isomers.

Table 1: Some adsorbents and their possible application

Name of adsorbent	Name of components to be used in separation from mixture
Silicic acid	Steroides, aminoacids and Lipids
Charcol	Peptide sand carbohydrates
Aluminium oxide	Steroides, ester, alkaloids
Magnesium carbonate	Porphyrines
Calcium Phosphate	Proteins, Poly nucleotides
Cellulose	Proteins

3. Column Chromatography:

In column chromatography an insoluble medium is packed into a glass tube. The length and width of this tube affects the separation of molecules .the molecules to be separated is applied to the top of the column and their migration is started by adding a solvent. The characteristic separation which results depends on the choice of solvent and carrier material.

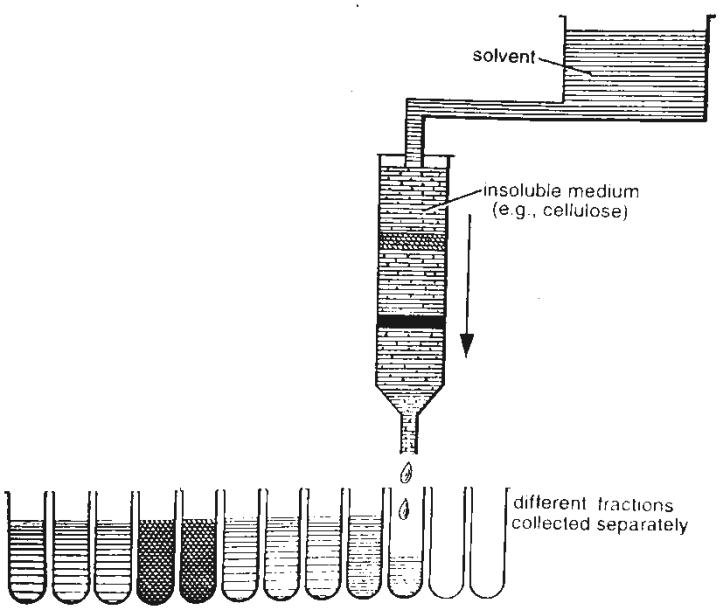

Figure 4: Demonstration of Column Chromatography

A positively charged carrier binds to the negatively charged molecules. Other carrier contains pores which are penetrated by the smaller molecules which are therefore slowed down the solution which flows from the column is collected in small fractions, which contain the separated classes of molecules Column chromatography is important for the separation of mixtures of proteins, that is for the isolation of enzymes such as cytochrome C or RNA Polymerase. Good Column is characterized a maximum good peak and less width the peak. The

efficiency of a column and its subsequent ability to separate or resolve solute zones can be determined in two ways like resolution index and theoretical plates

4. High performance / pressure liquid chromatography (HPLC):

It is the chromatography that results in high resolution (Sharp Peaks) with rapid separation of components of mixture like in a few minutes and needs very small sample material like 0.01-0.1ml. The basic components of HPLC are as pump, injecton, column, dectector, recorder or data system like computer etc. It is used for all types of chromatography like size exclusion, ion exchange and affinity chromatography. Solvents to be used in HPLC should be-

- Filtered through micro filters to remove impurities.
- Should be de gassed to avoid presence of air bubbles.
- HPLC grade refers to the quality of the mobile phase ingredients and solution not the sample. They should be pure.
- Excellent UV-Vis Transparency.
- The solvents commonly used for HPLC are often flammable and moderately toxic. Consequently most of the solvents should be stored in a secure metal cabinet.

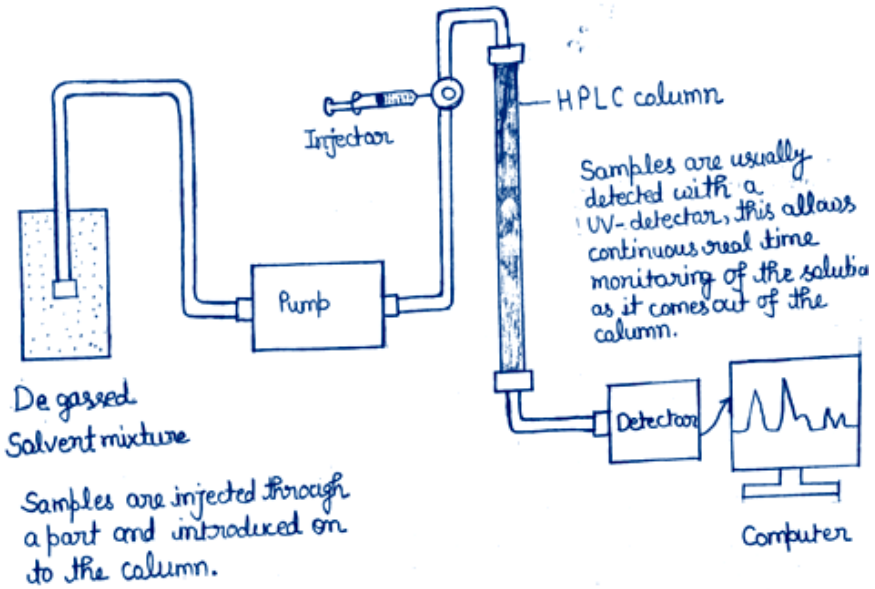

Figure 5: Various components of H.P.L.C chromatography

HPLC columns are normally made of stainless steel and are 50-300mm long with an internal diameter of 2-5mmFor selection of right column knowledge of sample (Number of compounds present, sample matrix, concentration range, solubility etc.) and goal for the separation (weather want to have maximum resolution of all compounds, partial resolution, fast

analysis of a single compound, sensitivity etc) should be well known. The stationary phase packed in the column should be small particle (approx, 3-10 micron) and large surface area.

Applications of HPLC Chromatography:

- Chemistry and Biochemistry research analyzing complex mixtures.
- Used in purifying chemical compounds.
- Quality control to ensure the purity of raw materials.
- Analyzing air and water pollutants.
- Monitoring materials that may jeoparadise occupational safety or health.
- Monitoring pesticides levels in the environment.
- To survey food and drug products.
- To determine the amount of such chemical compounds found in new drugs in pharmaceuticals.

Applications of Chromatography:

Chromatography is such a vital technique ,that is used in various fields like Pharmaceutical industry ,Chemical industry, Food industry ,Forensic science as well as various molecular studies for human welfare .This is also used for analysis of presence of pollutants in environment as well as other such purposes. So it is very useful technique for human kind by various ways.

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SOYBEAN PROCESSING FOR HEALTH SPECIFIC DESIGNER FOODS

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Abstract:

Designer foods are normal foods fortified with health promoting ingredients. Benefits of available designer foods such as designer egg, designer milk, designer grains, probiotics, these are foods enriched with micro and macronutrients. Since ancient times, soybeans have been identified as a high protein source with good amount of oil content. In food industry, soybeans based foods are mentioned as soyfoods. Soy protein, are most exploited but other ingredients, which can be used as ingredient for futuristic foods. Soybean ingredients such as essential amino acid and secondary metabolites such as isoflavone, saponins, phytic acids, phytosterols, trypsin inhibitors and peptides are also providing a good opportunity for the development of health specific designer foods. In the present chapter, health benefits of soy foods, development of designer foods by utilizing nutritional ingredients from soybean is discussed.

Keywords: Soybean, Health benefits, Designer foods, Nutritional, Ingredients, Futuristic foods.

Introduction:

Soybean belongs to the family of Fabaceae (Leguminosae), in the genus, Glycine. Soybean contains majorly vegetable proteins, carbohydrate and unsaturated fatty acids. It contains approximately 78% unsaturated fatty acids [1]. The most part of soybean is not

consumed directly as the bean, but in the form of foods such as tofu, soymilk, tempeh, and miso [2].

The Food and Drug Administration (FDA) recommends that consumers incorporate at least 25 grams of soy protein each day for healthy heart [3]. The unique chemical composition of soybean seed, which includes the number of nutraceutical compounds such as isoflavons, tocopherol, and lecithin besides 20 % oil and 40 % protein, has made it one of the most valuable agronomic crops in the world [4]. And this unique composition of soybean seeds makes it best candidate for future designer foods.

The soy ingredients, soy based food products, soybean based dairy replacer foods and beneficial effects of soybean products consumption on human health are the object of our discussion in the present chapter.

2. Nutritional aspects of soybean

Soybean is recognition for its value in enhancing and protecting health. Soy protein has all the eight essential amino acids. Moderate amount of saturated fatty acids (Palmitic acid & stearic acid) are also found in soybean.

Table 1: Vitamins, minerals and amino acids contents in soybean (per 100g)

Vitamin	**Nutrient Value**	**Minerals**	**Nutrient Value**	**Essential Amino acids**	**Nutrient Value**
Folates	375 µg	Calcium	277 mg	Tryptophan	0.591
Niacin	1.623 mg	Copper	1.658 µg	Threonine	1.766
Pantothenic acid	0.793 mg	Iron	15.7 mg	Isoleucine	1.971
Pyridoxine	0.377 mg	Magnesium	280 mg	Leucine	3.309
Riboflavin	0.870 mg	Manganese	2.517 mg	Lysine	2.706
Thiamin	0.874 mg	Phosphorus	704 mg	Methionine	0.547
Vitamin A	22 IU	Zinc	4.89 mg	Cystine	0.655
Vitamin C	6 mg			Phenylalanine	2.122
Vitamin E	0.85 mg			Tyrosine	1.539
Vitamin K	47 µg			Valine	2.029
				Arginine	3.153
				Histidine	1.097

(Source: USDA National Nutrient data base)

Nutritive value of soybean per 100g: protein 36.49 g, carbohydrate 30.16 g, total fat 19.94g, dietary fiber g, and cholesterol 0 mg. Most of the soy vitamins are required by the body to perform specific functions such as regulation, maintenance, growth and protection [5]. Soybean

is an ideal complement to grain foods and content of essential nutrients in soybean are shown in table 1.

Soybeans are also one of the finest sources of minerals and these minerals are used in our body as tissue building and regulation of body fluids.

3. Anti-nutritional aspects of soybean or soy ingredients

Soybean based foods will protect against the free radicals damage as they contain antioxidants and several anti-nutritional factors (ANFs) associated with them which are biologically active and need to be eliminated prior to human consumption. Fortunately, most of them are thermos labile and destroyed or inactivated by proper heat treatments (eg. Trypsin inhibitor) during usual processing/ cooking. And some by supplemental enzymes (NSP enzymes, phytase), while some are heat stable unaffected by the methods applied now commercially [6].

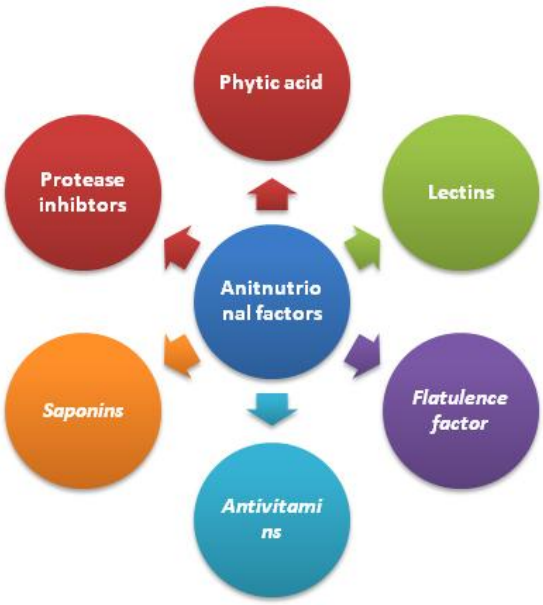

Figure 1: Anti-nutritional factors present in soybean

Soybean products as dairy replacer

Soy dairy free products are a healthy, high-quality source of protein that contain all essential amino acids needed for growth. The FDA has approved a health claim stating that "25 grams of soy protein in a daily diet low in saturated fat and cholesterol can help reduce total and LDL cholesterol that is moderately high to high."

Soymilk

One of the simplest methods for converting soybeans to a high-quality food is to produce a beverage known as soymilk. It is said soymilk and tofu to be invented in China by the legendary An Liu about 2000 years ago [7]. It contains similar amount of protein and fat as

cow's milk, and it is free of lactose and cholesterol. Therefore, it is considered as a healthy beverage and a popular alternative to dairy milk for population that is lactose-intolerant.

Tofu

It is a curd made by coagulating soy protein by mineral salts or acid. It is much similar to the fresh cottage cheese in Western world which was made by coagulating cow's milk. Calcium-coagulated tofu also serves as an important source of calcium, an essential macro mineral. Several major tofu producers in U.S. contain varying amounts of proteins because there is no established standard of identity [8]. Standardizing various varieties of tofu products based on composition will certainly help to further promote the products to consumers.

Soymilk yoghurt and cheese

Soy "yogurt," or cultured soy, is generally found alongside traditional dairy yogurts and in similar flavors and sizes.

Soy cheese is a cheese analog made with tofu. Soy cheese and dairy alternatives are not fermented products [9]. It is much similar to the processed cheese in market. It is a good source of essential fatty acids and contains no cholesterol and little or no saturated fat.

Health benefits on soy process foods ingredients

Soy foods have been consumed for centuries in many Asian countries. Many potential health benefits have been linked to intake of soy products such as prevention and treatment of many chronic diseases [10]. Much of this interest is because the soybean is essentially a unique dietary source of isoflavones [11]. Important bio-active components, found naturally in soybeans are being studied in relation to relieving menopausal symptoms, such as hot flashes, maintaining healthy bones, and preventing prostate, breast cancers, and colorectal cancer.

Effect on heart health

In 1999, the U.S. FDA awarded a health claim for soy protein and coronary heart disease (CHD) based on its cholesterol-lowering effects [12]. The threshold intake for cholesterol reduction was established at 25 g/d, although there is evidence lower amounts may also be efficacious [13]. However, questions have recently been raised about the efficacy of soy protein [14] and, in fact, the FDA is currently reevaluating evidence in support of the health claim, although this reevaluation was undertaken because of the large number of clinical studies published within the past decade.

Soybean oil contains a very high level of unsaturated fatty acid and a significant amount of omega-3 fatty acids [15], which is considered as part of the healthy fat group.

Effect on metabolic syndrome: diabetes

Glyceollins' role in improving glucose homeostasis was reported by Park and colleagues [16, 2] and they concluded that glyceollins act through regulating glucose utilization in

adipocytes and modulating cell function and survival. It was shown that glyceollins could improve insulin-stimulated glucose uptake and decrease triacylglycerol accumulation in 3T3-L1 mouse adipocytes. It was shown that 5μMglyceollins increased basal glucose uptake by 150%. It was proposed that glyceollins improved glucose homeostasis partly by enhancing hepatic insulin sensitivity in type 2 diabetic mice [2,17].

Effect on bone health-Osteoporosis

The well-recognized skeletal benefits of estrogen therapy for postmenopausal women provide a theoretical basis for exploration of the possible skeletal benefits of isoflavones

Proactive effect on Cancer

Soy has also shown potential in reducing the risk of many types of cancer (18;19;20; 21) and, among which, breast and prostate cancers are of particular interest due to their sensitivity to sex steroid hormones. The impact of soy intake and breast cancer risk has been rigorously investigated for two decades [22].

Effect on body weight- Obesity

Obesity is a disorder of energy balance, which became a worldwide epidemic and is associated with hyperinsulinemia, insulin resistance and abnormalities in lipid metabolism and it is one of the most important risk factors in the development of Type II diabetes, cardiovascular disease, atherosclerosis and certain cancers.

Futuristic approach: soy based designer foods

Table 2: Designer foods and their health benefits

Sr.No.	Designer foods	Health benefits
1.	Selenium (Se) enriched foods-Se enriched egg, broccoli and milk	Prevents cardiac muscle degeneration, muscular dystrophy [24], reduce the risk and prevalence of prostate and colon cancer and antioxidant activity [25].
2.	Vitamin D and calcium enriched foods foods - Vitamin D and calcium fortified milk	Lowers PTH levels, reduce bone turnover, prevents the occurrence of overweight and obesity among postmenopausal women [26, 27]
3.	Micronutrient fortified milk, salt fortified with iodine, iron and vitamin A	Improves anemic status and reduces anemia in children and pregnant women [28]
4.	Phytosterols enriched food items- Phytosterols enriched oil	Reduces total cholesterol, very low density lipoproteins and RLP cholesterol [29]

Consumers are becoming more and more interested in healthful foods and designer foods are nothing but normal foods fortified with health promoting ingredients. Designer foods are produced by the process of fortification. The term was introduced in Japan in 1980s for referring processed food containing nutrient conferring of some additional health benefits apart from its own nutritional value [23]. These types of foods are a kind of nutraceuticals. The Europoean regulatory has broad category as nutraceuticals and the definition of a nutraceutical is, "any food or food ingredient which is considered to have a beneficial effect on health". European regulatory includes food for specific health benefit rather than to enhance physiologic function, may include infant formula, processed baby foods (weaning foods), low-calorie foods for weight reduction, high-calorie foods for weight gain, ergogenic foods for athletes, and foods for special medical purposes like the treatment of diabetes or hypertension. A brief account of selected designer foods is given below in table 2.

By the above said definition/s of designer foods and their role in human diets, it is clear that the food should have high nutritive value. Soy foods can be easily fitted within this definition as their potential role in improving health condition and there is much scope in future to develop designer foods by utilizing soybean.

Conclusions:

It is difficult to find a food plant with so many valuable ingredients apart from soybean. Soybean has been utilized for the preparation of number food product with functional value like. Soy foods also have many health benefits such as it play vital role in prevention of prostate cancer, coronary heart diseases and osteoporosis. Soybean ingredients can also be utilised to prepare designer foods to reduce micronutrients deficiency in the developing countries. Hence, soybean is a very good candidate for designing health specified foods for individual groups, so called "Designer foods".

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