ISBN: 978-93-91768-51-5

Advances in Plant Science

Volume III

Editors

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Bhumi Publishing

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ISBN: **978-93-91768-51-5**

Published by:

Bhumi Publishing,

Nigave Khalasa, Kolhapur 416207, Maharashtra, India

Website: www.bhumipublishing.com

E-mail: bhumipublishing@gmail.com

Book Available online at:

https://www.bhumipublishing.com/books/

PREFACE

We are delighted to publish our book entitled ***"Advances in Plant Science Volume III"****. 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 III

CONTENTS

RESPONSES OF PHOTOSYNTHETIC PARAMETERS TO DROUGHT AND REWATERING IN A HIGH YIELDING GROUNDNUT CULTIVAR (*ARACHIS HYPOGAEA* L. CV. K-134)

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Abstract:

Groundnut is an important edible oil crop plant whose quality and yield are greatly affected by drought. An experiment was conducted to evaluate photosynthetic performance of a high yielding groundnut (*Arachis hypogaea* L. cv.K-134) during drought stress by withholding water supply and recovery. During dehydration, as the leaf water potential was dropping progressively with the severity of treatment, the values of leaf area, dry mass accumulation, chlorophyll (Chl) content, net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were declined whereas intercellular CO_2 concentration (C_i) increased in groundnut cultivar. The changes in theses parameters were dependent on stress severity and duration. Rewatering of the plant lead to an almost complete recovery of P_N, E and g_s, indicating that a short-term stress brings about reversible effect in this cultivar of groundnut.

Keywords: Groundnut; drought; photosynthesis; chlorophyll content; leaf area; dry mass

Introduction:

Plants encounter various abiotic stresses due to their sessile nature which include heavy metals, salt, drought, nutrient deficiency, light intensity, pesticide contamination, as well as extreme temperatures. Drought is a major abiotic stress that impairs crop production worldwide, affecting growth rate and development (Begcy *et al.*, 2012, Fahad *et al.*, 2017, Hussain *et al.*, 2018), and has been focus of much research. Water stress primarily reduce the photosynthetic efficiency of plants, due to their negative consequences on chlorophyll biosynthesis, performance of the photosystems, electron transport mechanisms, gas exchange parameters, and many others. A better understanding of the physiology of plants under water stress can help in the development of pragmatic interventions for managing drought. Groundnut (*Arachis*

hypogaea L.) is an important food legume as well as an oilseed crop being grown in 112 countries of the world on about 25 million ha of land. The production of peanut is about 41.1 m ton and is grown mostly in tropics and subtropics of arid and semi-arid regions where the availability of water is a major constraint. Frequent drought of various spells and intensities in these areas results in the productivity of peanut being less than 1000 kg ha^{-1} in more than 35 % of peanut growing countries (Kalariya *et al.,* 2015). Consequently, much research has been devoted to exploring the physiological mechanisams of the drought response in groundnut. The rainfed groundnut occupies a major portion in Rayalseema of Andhra Pradesh encountering the frequent drought spells, as there is erratic and uneven distribution of rainfall, ultimately resulting in poor returns to the farmer. Drought stress effects on photosynthesis have been well demonstrated (Gomes *et al.,* 2008; Farooq *et al.,* 2009; Neto, *et al.,* 2017). Under drought, plants reduce the water vapor loss through reducing the stomatal opening (Xu *et al.,* 2008). However, it also restricts CO_2 entry in the leaf, which may lead to the decrease of the photosynthesis rate (Lawson *et al.,* 2014) and a decrease in primary photochemical processes (Goltsev *et al.,* 2012), which will inhibit plant growth and even reduce dry matter accumulation and yield (Zhou *et al.,* 2019). Therefore, the measurement of photosynthetic performance is considered a standard technique for studies on drought stress, especially, the net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) have been determined to be the most used techniques to discriminate the plant responses to drought (Osakabe *et al.,* 2014; Chen, *et al.,* 2016; Sousa *et al.,* 2017). Consistent information has been reported on water deficit effects on the relative stomatal and non-stomatal limitations to photosynthesis (Tezara *et al.,* 2002; Zhou *et al.,* 2007). However, the contribution of stomatal and non-stomatal factors to photosynthesis impairment and its incomplete recovery upon rewatering has not been fully determined and has therefore been debated for a long time (Lawlor and Cornic, 2002; Gomes *et al.,* 2008). Thus, the present study aimed to determine the photosynthetic performance in groundnut plants when submitted to drought as well as during the rehydration process.

Material and Methods:

Seeds of groundnut (*Arachis hypogaea* L.) cultivar (K-134) were sown in earthen –pots containing 8kg of red loamy soil and farm yard manure (3:1 proportion). Pots were maintained for one month in the departmental botanical garden under natural photoperiod of 10-12 h and temperature 28 ± 4 ^{0}C. One-month-old plants were then divided into four-sets and arranged in randomized complete black design. One set of pots received water daily to field capacity and

served as control (100 %). The remaining three sets received water daily to 75, 50 and 25 % of the field capacity and were characterized as mild, moderate and severe stresses, respectively. After induction of stress, the pots were maintained for another 8 days and the experimental data were collected at different time intervals i.e. on day-4 and 8. Duration of the stress treatment was 8 days, and the plants subjected to severe stress on day-4 and 8 were assessed for recovery 48 hours after re-watering them to the field capacity. For the determination of dry mass, the leaves were dried at 80^{o} C in a hot air oven until a constant mass was formed. The leaf area of the expanding leaf (second leaf from the apex) was measured in a leaf area meter. Leaf water potential was measured using a portable PR-55 psychrometer microvoltmeter with C-52 sample chamber. (Wescor, Logan, Utah, USA). The readings were measured between 8.00 to 10.00 AM. The measurements were the average of twenty discs to obtain a mean water potential for the leaf. The total chlorophyll content was estimated in the leaves according to the method of Arnon, (1949). Rate of photosynthesis, stomatal conductance, intercellular CO_2 concentration and the rate of transpiration were monitored by using a portable photosynthesis system (Infrared gas analyzer: LCA-3) equipped with a Parkinson leaf chamber (6.2 cm^2), (PLC) (Analytical Development Co., Hoddesdon, U.K.). The measurements were made between 8.00 AM to 10.00 AM at photosynthetic photon flux density of approximately 1100 ± 100 µ mol m^{-2} s^{-1}. The leaf temperature was ranged between 30 ± 2°C. The measurements were done in the second leaf (fully expanded) from the top, since this leaf was found to possess maximum photosynthetic capacity (Mekhri *et al.,* 1977). Water use efficiency (WUE) was calculated as the ratio between net photosynthesis and transpiration as suggested by Blum and Sullivian (1986). Means of five individual estimations were taken from both control and stressed plants. The data were analyzed statistically using Duncan's multiple range (DMR) test to drive significance (Duncan, 1955).

Result and Discussion:

Photosynthesis is central to the growth and productivity of plants. Drought can severely inhibit the growth and productivity of plants through affecting some key physiological processes, such as the photosynthesis rate (Neto *et al.,* 2017). From the table 1, it is clear that the leaf water potential decreased at all stress regimes and on all days of sampling. The decrease was significant in all stress treatments except in mild stress treatments on the day-4 and 8. It remained nearly constant in control plants of the cultivar throughout the experimentation. A similar decline in leaf water potential as a result of drought stress is noticed in groundnut (Subramanian *et al.,* 1993; Clavel *et al.,* 2005) and in other legumes (Martinez *et al.,* 2007). The

present investigation also revealed that groundnut maintained high leaf water potentials under prolonged stress treatments on the days-4 and 8 thereby performing various physiological and biochemical processes to continue more efficiently even under low soil moisture condition developed by scarcity of water is an indicative of moisture stress endurance. This may be due to marked osmotic adjustment occurred in growing leaves of groundnut allowing them to maintain higher turgor during periods water stress (Ali Ahamad and Basha, 1998). After 48 hours of re-watering, leaf water potential values recovered completely to the control values. The total chlorophyll content was significantly declined in the cultivar with increase in the intensity of stress and over duration. The decrease in total chlorophyll content in the leaves of water stressed plants was reported in groundnut (Babitha, 1996). The decrease of chlorophyll under water stress may be due to decreased rate of its synthesis or enhanced chlorophyllase activity (Drazkiewicz, 1994). The total chlorophyll content showed an upsurge on rewatering. A similar evidence was also presented by Nandwala *et al*. (1991) and Ramanjulu *et al.,* (1998). Plant dry weight can reflect the plant growth condition and can be considered as an indicator of drought degree. Leaf dry weights decreased significantly at all stress levels except under mild stress conditions where there was no significant change in dry mass accumulation of leaves when compared to control from day-4 to 8. In general, the magnitude of decline in dry weights of leaves was dependent on severity and duration of stress. These results agree with earlier reports in groundnut (Madhusudan *et al.,* 2002, Latha and Reddy, 2007). The decreased dry matter as a result of stress may be attributed to both the reduced leaf area, P_N and chlorophyll content was observed (Table 1), which is in agreement with earlier results (Ramanjulu *et al.,* 1998, Thimmanaik *et al.,* 2002). Although leaf area in stressed plants decreased over controls, there was leaf expansion from day-4 to 8 in all stress treatments indicating cultivar tolerance nature to stress treatments. These results are inconsistent with earlier reports in groundnut (Vorasoot *et al.,* 2004). After re-watering of the severe stressed plants on day-8, leaf area was restored to 78% and chlorophyll content to 85%.

Insufficient photosynthesis is the main cause of crop productivity and yield decline under drought, and the decrease of photosynthetic capacity can be caused by stomatal and non-stomatal factors (Sengupta *et al.,* 2013). As an adaptation to drought, plants adjust the relationships among water, transpiration, and photosynthesis through changing the stomatal opening in order to maximize CO2 assimilation and prevent water content loss, and thereby reduce levels of tissue damage (Li *et al.,* 2017) However, this restricts CO2 entry in the leaf, progressively decreasing the photosynthetic capacity (Ashraf and Harris, 2013). The decrease in P_N and g_s under water stress has been reported in several crop species i.e in groundnut (Subramanian *et al.,* 1993;

Chakraborty *et al.,* 2016) and in other plants (Thimmanaik *et al.,* 2002; Liberato *et al.,* 2006; Khanna-Chopra and Selote, 2007; Gomes *et al.,* 2008; Jia *et al.,* 2020). Similarly in the present study, there was gradual reduction in P_N and g_s in groundnut cultivar with the increase of stress severity. Decline in CO_2 assimilation rate in plants during water stress could be attributed to stomatal (decreased stomatal conductance) and/or non-stomatal (biochemical dysfunctions) responses of a plant species (Gomes *et al.,* 2008; Farooq *et al.,* 2009). Strong correlation between stomatal conductance and photosynthetic rate seems to represent an adjustment of stomatal conductance to match the intrinsic photosynthetic capacity (Sheshashayee *et al.,* 1996) as showed in the present study. The occurrence of unaltered/increased intercellular CO_2 concentration levels and reduced stomatal conductance under water stress conditions could be taken as an indication of mesophyll limitation to photosynthesis (Thimmanaik *et al.,* 2002). However, the heterogeneity of leaf photosynthesis as a consequence of patchy-stomatal closure which can appear when the plants are submitted to short-term water stress could affect the Ci calculation (Cornic and Briantais, 1991) and thus the validity of the mesophyll limitation to photosynthesis. A decrease in P_N without a corresponding decline in Ci has usually been interpreted as a documentation of non-stomatal effects of water stress on photosynthesis. In the present study, the Ci values were almost unaltered under mild stress and only slightly increased under moderate stress. However, they were significantly increased under severe stress, indicating decreased carboxylation efficiency (Kicheva *et al.,* 1994). On re-watering Ci attained almost normal values. The increased Ci under water stress might may be due to the effects of stress on the CO_2 fixation machinery or to stomatal control through the alternation in the stomatal aperture (Samuel and Paliwal, 1993). Under water stress, decreased rate of transpiration was evident from the earlier reports in groundnut (Subramanian *et al.,* 1993) and other plants (Liberato *et al.,* 2006). Similar to the earlier reports, in the present study rate of transpiration decreased under water stress conditions. The stomatal control of transpiration is a mechanism used by different species to restrict loss of water and to overcome periods of drought and this to an extent ameliorates the stress onset and helps to maintain photosynthesis. The E declined correspondingly with the decline in P_N and g_s (Table 1). The reduction in water loss by stomatal behaviour is one of the adaptive responses maintaining a high water use efficiency as the drought develops. The obtained WUE value in water-stressed groundnut cultivar was lower than controls at all stress regimes on all days of sampling. These results are in agreements with the earlier reports (Thimmanaik *et al.,* 2002) and imply inefficient use of water during water stress.

Table 1: Effect of water stress on the groundnut leaf water potential (-M Pa), leaf area (cm^2), leaf dry weight (g $plant^{-1}$), total chlorophyll content (mg g^{-1} FW), net photosynthetic rate, P_N (µ mol CO_2 m^{-2} s^{-1}), stomatal conductance, g_s (m mol H_2O m^{-2} s^{-1}), intercellular CO_2 concentration, C_i (m mol m^{-2} s^{-1}), transpiration rate, E (m mol H_2O m^{-2} s^{-1}), and water use efficiency, WUE (m mol CO_2 mol^{-1} H_2O)

Parameter	Day	Control	Mild	Moderate	Severe	Recovery after 48 h
Leaf water potential	04	0.83a (100)	0.87a (104.52)	0.93b (111.72)	0.99c (119.94)	0.89a (107.23)
	08	0.85a (100)	0.91a (107.53)	1.12b (131.76)	1.25c (147.05)	0.87a (102.35)
Leaf area	04	22.04a (100)	22.81a (103.49)	21.28a (96.55)	20.80a (94.37)	22.01a (91.06)
	08	27.13a (100)	26.84a (98.93)	24.99a (92.12)	23.45b (86.43)	21.28c (78.43)
Leaf dry weight	04	0.342a (100)	0.331a (96.78)	0.307a (90.02)	0.268b (78.54)	0.316a (92.35)
	08	0.506a (100)	0.479a (94.65)	0.437b (85.76)	0.349c (69.00)	0.381c (75.26)
Total chlorophylls	04	1.524a (100)	1.427a (93.64)	1.293b (84.88)	1.084c (71.15)	1.446a (94.87)
	08	1.638a (100)	1.506a (91.95)	1.287b (78.57)	1.021c (62.35)	1.403b (85.68)
P_N	04	21.69a (100)	20.05a (92.46)	16.91b (77.98)	14.42c (66.50)	21.56a (99.40)
	08	21.77a (100)	19.60a (90.02)	16.08b (73.90)	12.59c (57.85)	20.35a (93.48)
g_s	04	592a (100)	502b (84.78)	411c (69.52)	319d (53.92)	592a (100)
	08	627a (100)	518b (82.60)	409c (65.19)	273d (43.60)	618a (98.56)
C_i	04	248a (100)	247a (99.60)	249a (100.40)	254a (102.42)	249a (100.4)
	08	248a (100)	248a (100)	251a (101.21)	271b (109.27)	249a (100.4)
E	04	8.8a (100)	8.4a (95.02)	7.3b (83.40)	6.0c (68.79)	8.8a (100)
	08	8.9a (100)	8.1b (90.84)	7.0c (78.64)	5.6d (63.58)	8.8a (98.87)
WUE	04	2.46	2.38	2.32	2.40	2.45
	08	2.45	2.41	2.29	2.24	2.31

The increase of leaf water potential values in rehydrated groundnut stressed plants to the same level shown by control plants was corroborated by the behaviour of gas exchange variables (P_N, g_s and E), which presented a gradual process of recovery on rewatering (Table 1). This is in agreement with earlier results of Rocha and Moraes, 1997, where in *Stryphnodendron adstringens* seedlings without irrigation for thirty days, total recuperation of water potential and gas exchange variables occurred in 48 h. Similar reports were recorded in mulberry plants (Ramanjulu *et al.,* 1998). Conversely, the P_N may not recover completely after stress in coconut plants due to non-stomatal factors (Gomes *et al.,* 2008). Most likely the severity of water stress and drought tolerance capacity of given plant species or cultivar are important for recovering the photosynthetic capacity.

In the light of present results discussed, we conclude that the drought stress treatments negatively affected photosynthesis and all gas exchange parameters (P_N, g_s and E). The decrease in photosynthesis was due to water stress was attributed to both stomatal and non-stomatal components. Although each parameter seemed to be affected during drought, the plant could regain a full functional capacity after 48 h after rehydration, which showed the short-term recovery of structural and functional components of this processes.

The mean values (n=5) in a row followed by different letter for each plant species are significantly different ($P \leq 0.05$) according to Duncan's multiple range (DMR) test. Figures in parentheses represent per cent of control

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SPECIES LEVEL RELATIONSHIP IN GENUS *ELEOCHARIS* R.BR.FAMILY; CYPERACEAE

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Abstract:

The *Cyperaceae* is a large and successful family having small and inconspicuously reduced flowers mostly adapted to wind pollination. The family is worldwide in distribution, the plants living in the damp, swampy or marshy localities of the temperate and cold regions (Hutchinson, 1959). *Eleocharis* R.Br. is the cosmopolitan genus of family Cyperaceae. The species of *Eleocharis* R.Br. has less known in India. The other aspect of the life history were subsequently studied by different worker (Schnarf, 1931; Schneider, 1932; Padhye and Makde 1982; Hinchliff and Roalson, unpublished Data 2009). Subject of rigorous phylogenetic analysis of genes *Eleocharis*, as such, Species-level relationship within the genus is not well understood. Wilson, Royal Botanic Gardens Sydeney, As the first phylogenetic study of *Eleocharis* R.Br. to attempt sorting these Species level relationship. This paper marks an important first stepin understanding the evolution of this unusual and biologically significant genus *Eleocharis* R.Br.The genus *Eleocharis* R.Br. has become to study the rigorous taxonomical charactersuch, species level relationship with in the genus is well understood. Which is used for the determination of evolutionary relationship in the genus *Eleocharis* R.Br. of Cyperaceae family? Author collected 06 *Eleocharis* R.Br. plants from different region of Bhandara district. Where studying anatomical character for species level relationship in *Eleocharis* R.Br. plant.

Keywords: Species Level Relationship, *Eleocharis* R.Br.

Introduction:

In India Cyperaceae is represented by 28 genera and about, 320 species. Cyperaceae is the second largest family among monocotyledons and stands next to the grass family (Goetghebeur, 1998).

The genus *Eleocharis*, R.Br. appears to be natural. Based on some anatomical criteria, Rikli's 1895 divided the genus into Chlorocharis and *Eleocharis*s.str. However, Rikli‟s cleavage

has not been confirmed by anatomical study of Metcalf (1971). As regard to this subdivision into infrageneric grouping among the modern Cyperologists viz. Clarke (1893, 1902).

The *Eleocharis* R.Br. genus is comparatively small with over 120 species distributed throughout the world and represented by about 16 species in India. Clarke (1893) reported 22 taxa, 18 species by Karthikeyan *et al.* (1989). Khan (2015) reported 22 taxa including 15 species, 4 subspecies and 3 varieties. The genus is very near to Scirpus (s.l.), Fimbristylis and Bulbostylis and is mostly characterized by the more dilated style base, articulated with ovary and persistent on fruit and bladeless sheaths. *Eleocharis* is distinguished from Fimbristylis and Bulbostylis in having hypogynousbristles while from Scirpus by dilated articulated persistent style base.

The genus *Eleocharis* R.Br. occurring in a wet environment like swamps, lakes and river margins. *Eleocharis* R.Br. is generally found in wet place often where there is strong seasonal variation in water level. Their Arial parts are formed by simple not ramified stalks that end in a spiciform inflorescence formed by numerous very inconspicuous flowers. Their subterranean parts are formed by root and stem (called rhizome and stolon depending on its form). Growth forms included herbaceous annuals and perennials, which can be caespitose, mat forming or rhizomatous and grow from height of less than 1 cm to greater than 3 cm. All species of *Eleocharis* R.Br. lack leaf blade entirely, having instead reduced leaf sheath at the base of there photosynthetic stems. *Eleocharis* R.Br. is morphologically uniform with respect to some distinctive feature, inflorescence reduced to one spikelet, base of the style persistence's a tubercle.

Svenson (1957) published a details series of monographs on *Eleocharis* R.Br., describing numerous species and delimiting many infrageneric taxa in the process, using morphological data. Ugemuge (1986) collected *Eleocharis* R.Br. species from Nagpur district of Maharashtra State was mentioned in "The Flora of Nagpur District". The need to revise the supraspecific classification in section *Eleocharis* R.Br. has also been pointed out by Roalson and Friar (2000). Khan (2015) in his book "Cyperaceae of Western Ghats, West Coast and Maharashtra" gave a very valuable data of *Eleocharis* R.Br. of Maharashtra which is useful for floristic study of Cyperaceae.

About 85 species of Cyperaceae have been dealt in this flora. Following 09 species of *Eleocharis* R.Br. distributed in Western Ghats, West coast and in central part of Maharashtra state.

1. *E. acutangula* (Roxb.) Schult 2. *E. atropurpurea* (Retz.) Presl. 3. *E. congesta* D. Dan
4. *E. dulcis* (Burm.F.) 5. *E.equisetina* Presl. 6. *E. geniculata* (L.) Roem&Schult

7. *E. mohamadii* W.Khan 8.E.retroflexa (Poir) 9. *E. spiralis* (Rottb) Roem&Schult.

The species like *E. ochrostachys, E.swamyii, E. tetraquetra* are less known but they are today difficult to found in Maharashtra.

Materials and Method:

The present investigation deals with the study of species levelrelationship in *Eleocharis* R.Br. of the family Cyperaceae on the basis of its taxonomic consideration. The taxa for this investigation were collected from Bhandara district of Maharashtra state. Provisional identification of collected plant was done with the help of Cooke‟s Flora of presidency of Bombay (1908), Sharma *et al.* Flora of Maharashtra (1996). Species level relationship carried to understanding the evolution of this unusual and biologically significant genus *Eleocharis* R.Br. of Cyperaceae family. The author has collected the *Eleocharis* R.Br. taxa from different regions area of Bhandara district. The Voucher species collected in the subsequent season are deposited in the herbarium of research institute. The filed diary also maintained by author.

Taxonomic Character

***Eleocharis* geniculate (Linn.)Roem&Schult.**

Biome- Densely, tufted annual, 5 - 20 cm tall. **Ecology**- Species grows in Marshy place in Margin of pond and lake and it grow in place of wet land. **Stem**- Angular, 0.4 - 0.5mm wide, striates. **Sheaths**- 2.5 - 4cm long, purplish near the base.**Blades**- absent or reduced to 1.5 - 3 mm long subula**. Infl**.- with a single, terminal, spikelet, Inv. Bract represented by 3 - 6 empty gl. At the basethe lowest one often with 3 - 5 nerved keel, spikelet ovoid, or sub globose, 4 - 6 x 2 - 3.5 mm much wider than stem, greenish or straminious tinged with brown or purple, obtuse. **Glumes**- broadly obovate or almost suborbicular, 1.5 - 2 x 1 - 1.2 mm; keel uninerved ending below the hyaline apex; sides nerveless, with narrow hyaline margins tinged with brown purple near rounded apex, muticous. **Stamens**-3 : anthers linear, ca0.5 mm long muticous. **Hypo. Bristles**- 7 – 9 glistening purplish-brown, or ferrugineous, loose, exceeding the nuts, retrorselybarbellate. Style 2- fid, much shorter than stigma, stigma hairy. **Nut** - biconvex, obovate, 0.8-1.1 x 0.5-0.6 mm smooth, shining, purplish to finally black with tumor at apex. Flower and fruits- October to February **Pollen** Hetropolar, asymmetrical tricolpate, spherical, size 42.5 µ range 40 µ to 45µ rather large, folded prolate, spheroidal granular surface.

***Eleocharis atropurpura* (Retz.)Presl**.

Biome – Densely tufted, glabrous, annuals, 4 - 40 cm tall; **Ecology**- species grown in morshy place where the sprinkling of water done, It grow in place of tank, ditches, rice fild, river

bank. **Stem**-angular, 0.4-0.5mm thick, erect, striate; **Sheaths** - Glabrous, 2.5 - 4 cm long, purplish; blades reduced to 1.5 - 3 mm long, subula. **Infl.-** reduced to a single terminal spikelite, subgloboseor ovoide, 3 - 6 x 2 - 3.5 mm, greenish tinged with brown, acute or subacute; involucre of bract represented by 1-3 empty glum at the base, with some what thick 3- nerved keel; rha. wingless. **Spikelet** - ovoid, greenish. **Glumes-** narrowly elliptic-oblong, 1 - 1.1 x 0.5 - 0.8 mm, thinly membranous,with a uninerved keel ending below the hyaline apex; sides nerveless, hyaline, red- brown, banded at the upper half, obtuse at apex, muticous. **Stamens** - 1; linear anther, ca 0.5 mm long, muticous.**Bristle-** Hypogenous 3 - 5, shorter than or as long as nuts, tightly appressed to nuts, retrorselybarbellate, glistening white. **Nut-** biconvex, obovate, 0.6 - 0.8 x 0.3 - 0.4 mm, smooth, shining, purplish to finally black shorter stiptat, tumorous at apex. **Style** 2- fid, hairy, shorter than the stigma, glabrous. **Flower and fruits**- November to February Pollen shape- oval. 1-colpate, colpus distal **Pollen**hetropolar asymmetrically monocalpate to bicalpate, spherical size 42.5 µ range 40µ to 45µ, rather large, prolate, spheroidal, exine 1.5 µ thick, faintly reticulate surface. biconvex, obovate, smooth, tumorous apex, 1× 0.6 mm. shining stipitate.

***Eleocharis acutangula* (Roxb.)Schult**.

Biome- Perenial, with short rhizome; stolon long 2 - 3 mm thick, rooting at nodes. **Ecology**- Common in shallow stagnant water in pond, tanks, rice fields, ditches, marshes and margin of pond and tanks, often forming large patches. **Stem-**triqueterous 30 - 75 cm long, 3 - 5 mm thick, solid not transversely septate. **Leaves sheaths**- membranous, 5 - 17 cm long, oblique at mouth, purplish toward base. **Spikelet** - cylindrical, acute at apex, 1.5 - 3.5 cm long as broad as the stem many fid.Bract replace by glumes at the base. Glumes comparativly loosely imbricate, broadly oblong or oblong ovate, obtuse- acute at apex, 4.5 - 3 mm, not keeled, scarious towards margins; midvein prominent with many faint veins on both sides. **Bristles** hypogynous, 6 linear, slightly broader towards apex, ca 2.5 mm long, subequal, as long or slightly exceeding the nut, retroselyscabrid. **Stamen** 3, anthers linear, oblong, ca 2 mm long connective appendage very minut, dark brown.Style 3 – fid, with ca 0.5 mm long conical base. **Nut** – obovoid, slightly compressed, constricted to a conspicuous neck below the apex, ca 2 x 2.5 mm, yellowish brown, with persistence style base dark brown; epidermal cell conspicuous, in ca 15 vertical raw on either face, tranversly oblong. **Flower and fruits-** July to May.**Pollen** shape- prolate. No aperture observed. Hetropolar, asymmetrical, monocolpate, spherical, oval size 47.5 µ range 45µ to 50µ, rather large, spheroidal, Exine 2µ thick Exine surface reticulate thinner.

Achene- turgid, biconvex, ovate, striate, constricted below, 0.8 × 0.6 mm, tumor at apex shining.Gregarious by marshy bank of pond, lake, pantrop.

***Eleocharis chaetaria* (Roem&Schult).**

Biome- Dilicate annual amphibious species fresh water usually C3 and C4 Carboxylation plant. **Ecology**- species are weeds in rice fields, species are wet grassland, coastal marshes and swampy areas. Herb, usually cespitose often rhizomatous, ascending and caudex like with very slender hair like stems. **Stem** – angular, 3 - 25 cm long, often curved; tip often touching the soil, often viviparous, **Culms** sometimes solitary, terte 3-5 angled or more strongly compressed in cross section, spongy with internal air cavities and incomplete transverse septa or sometime hollow with complete transvers septa.**Leav. Sheath-** purplish toward the base, basal 2 per culm: ligules absent: blades absent or awn (tooth) at apex. **Bracts** absent rarely proximal scale of spikelet resembling short bract.**Spikelet**– ovoid, subcompressed, 3.4 x 2.3 mm, few (4-8)-fid, greenish tinged with purpal or wholly purplish. **Glum** – hyaline, fruiting one loosely imbricate, oblong- ovate, obtuse, ca 2.75 x 1.75mm, not keeled, broadly hyaline margined. **Bristle**– hypogynous 6, unequal; longest ca 0.5 mm long, shorter to longer than nut, whitish.Stamens – 2 (-3); anther. Muticous**Style** – ca 3 mm long, conical at base**. Nut** – obovoid, trigonous, ca 1.1 x 0.7 mm, with 3 acute angles projecting from the apex, yellowish; epidermal cell very prominent, pitted; persistant style base pyramidal, deccurent on the three acute shoulder of nuts. **Flower & fruit** –September to December. **Achene**- Biconvex, Plano-convex or Trigonous to Subterete.

***Eleocharis dulci* (Burm F.):**

Biome-Perenial 15- 150 cm tall, rhizome short; stolons slender, ending in subglobose tubers.**Ecology-** species grow in marshy places along lake side (Yadow and Sardesai 2002) and in fallow marshy fields in kerala. Comman along margin of tank and lakes.**Taxonomy**- Grass like and rush like herbs temperate zone, rounded stem, edible tuber belonging to sedge family Cyperaceae. **Stem**- terete, fistular, 3 - 6 mm broad, faintly striate, distinctly transversely septate, compressed, glabrous**. Leaves Sheath** – glabrous, often purpulish; blades reduced or absent. **Inflorences** – of single, elongated spikelet terminated by stem. Involucre of bract of 2 glume like. **Spikelets**- wider or narrower than cylindric, 1.5 – 4cm x 4 - 6 mm long, almost as broad as to slightly wider or narrower than the stem sessil, terete, greenish or stramineous, often tinged with red, many fid, rhachilla wingless. **Glume -** oblong with almost parellal margin, much longer than broad 4.5-7 x 2- 2.5 mm strongly keeled ; keel ending below the hyaline obtus apex; sides nerveless or faintly striat, hyaline on margin, reddish brown Lineolate. **Bristles-**

hypogynous 6, retrorselyspinulose from base to top, flate near base longer than nut or reaching the top or little exceeding the conical apex or twice longer than the nut Stamens sub equal 3: anther linear, ca 1.5 mm long. Style– bifid, much longer than the stigma, glabrous Nut- biconvex, elliptic, 2- 2.2 x1.2 -1.5 mm, subturgid on faces, smooth with costate angles (with a single rib on each edge) and a ring on the top but at the base of ca 1 mm long style base, epidermal cell minute, indistinct. Flower and fruit- October to December.**Pollen-** Hetero polar, asymmetrically, bicolpate to tetracolpate, oval size 40µ range to 42µ.Exine 2 µ thick, exine surface smooth, thinner.**Achene**- biconvex ovate, straite, tumor at apex shining.

E. geniculata, (linn.) **Roem.&Sechult)**

E. atropurpura **(Retz.) presl.**

E. acutangula **(Roxb.)Schult**

E. retroflexa **Subspecis** ***chaetaria*** **(Roem&Schult.)**

E. dulci **(Burn F.)**

E. setifolia **(A.Rich.)Raynal.**

Eleocharis **setifolia (A Rich.) Raynal**.

Biome-Delicate tuberiferous perennial; tuber witish, knotty (under 1mm broad).**Ecology**- it grow in marshy place along the margin of lake and inside of rice field near the lake where

sprinkling of water done. Also, in the wetland and rice paddy field. **Stem**- filiform, 5 - 12 cm tall, under 1mm thick, tetragonous leafless, channeled on each side of stem. **Leaves** – reduced to sheath; sheaths 0.8 - 1.6 mm long, whitish to pale-brown. **Inflorences**– of single spikelet terminated by stem.Involucre of bract of 2 unequal glum, which are longer than fertile ones.Spikelet, ovoid,-oblong, terete 2-5 x 1-1.5 mm, greenish tinged, with red brown, many fid. **Glum**- ovate to ovate or ovate lanceolate, 0.6 -1 x 0.5 -1 mm, with brown vertical bond along the uninerved keel, notched at apex, muticous. **Bristle–**hypogynous, absent or rudimentary.Stamen – 1 anther ca 0.5 mm long. Nuts - obovoid, obscurely trigonous, 0.5 - 0.4 mm, strongly 3 - costate, smooth, pale brown to gregish-green.**Style**- basedecurrent on the shoulders on nuts.**Flower and fruit**- October to November.

Result and Discussion:

In the present study species level relationship of genus *Eleocharis* R. Br. of cyperaceae family is discussed on the basis of taxonomical character.The author has collected six *Eleocharis* R.Br. taxa from different regions of Bhandara district are of follows,

E. geniculata (Linn.) Roem&Schult.E. atropurpura, (Retz.)Presl.

E. acutangula (Roxb.) Schult. *E. retroflexa* Sub species chaetaria, (Roem&Schult)

E. dulci (Burm F.)E. setifolia, (A. Rich.) Raynal.

Morphological characters found in *Eleocharis* R.Br. is variable to one another, but they look like similar one.For most families of flowering plant, the morphological data are recorded at the flowering time. Because of most Cyperaceae cannot be reliable identified when in flower. In this way fruiting time is given for all species by season, sometime qualified by early, mid or late or by month. The fruiting time has been interrupted broadly to include the period when the fruit is more or less fully formed, but not yet ripe. The fruiting period provided cover the entire range of taxon.Quite a difference between fruiting period in different part of the range of the species may well occur, especially for widespread species with extensive elevation rang (Simpson, D. A. & C. A. Inglis, 2001).

On the basis of taxonomic point of view, character found in *Eleocharis* is mentioned from taxanomic data. **Plant** species of *E. geniculata, E. atropurpura is terrestrial, E. acutangula, E. dulci* is fully aqutic fresh water, *E. chaetaria, E. setifolia* is amphibians in habitat.

Roots of *E. geniculata*, *E. atropurpura*, *E. chaetaria*, *E. setifolia* are fibrous. *E. acutangula*, *E. dulci* is rhizomatous with tuber or bulb. Water chestnut plant is leafless, due to the absence of leaves, photosynthesis in plant is carried out by the culms or stems.

Flower which are very small and occur on the tips of the culms. Flowers are usually produced before the plant reaches its height of vegetative growth. The plant has elongated stolon with a tuber attached to its bottom. The plant produced two type of tuber, the first type for propagation and the second for storage, its nut is delicious.

Stem of *E. geniculata, E. atropurpura* angularstraite, erect, 5-20 cm tall E. acutangula, E. dulci spongystraite slender 20-40 cm tall. *E. chaetaria, E. setifolia* tuft slender 2-8 cm tall.*Eleocharis* R.Br. species show variation in stem architecture. Mature stem diameter varies by nearly two order of magnitude from less than 0.5 mm in the narrow. Mostly aquatic species are 2 cm in the largest often grow emergent aquatic, but may also grow terrestrially in close proximate to water or in seasonally inundated habitats(Hinchiff, E. & E. H. Roalson, 2009).

Leaves sheath obliquely obtuse, glabrous. Blades reduced in *E. geniculata, E. atropurpura*. Leaves sheath flat, slender, acute and membranous in *E. acutangula*and *E. dulci*. Leaves sheath absent, blades reduced in *E. chaetaria, E. setifolia*.

Glumes 3-6 broadly abovate, membranous, obtuse *E. geniculata*, E. atropurpura.Glume, oblong, ovate, loosely imbricate, keel distinct in *E. acutangula,* E. dulci.Glume solitary, compressed, elliptic lowest fertile, glume upward in *E. setifolia, E. chaetaria.*

Stemen, 1-3 muticose, style linear, at the base persistent hypogynous bristles.

Style -2 fid, hairy shorter than nut in *E. geniculata*, *E. atropurpura*Style - 3 fid hairy short in *E. acutangula, E. dulci*.Style 2-3 fid longer base depressed conic paired in *E. chaetaria, E. Setifolia*

Monocolpate pollen grains present in *E. acutangula, E. dulci,* but mono-tricolpate pollen grains found in *E. geniculata, E. atropurpura, E. chaetaria,* and *E. setifolia*.

Nut biconvex, obovate, shining tumor at apex in *E. geniculata, E. atropurpura, E. chaetaria, E. setifolia,* but in *E. dulci, E. acutangula* nut turgid, ovate constricted below.

From the **above data**, *Eleocharis* R.Br. species are grouped, their characters also intermingled to one another. These grouped are evolved from different elevation range.

Classification of *Eleocharis* R.Br. is unusually difficult because relatively few macroscopic characters are provided by the simple structures, characteristic of the genus. Undoubtedly much evolutionary convergence has occur in most vegetative and reproductive structures (Roalson, E. H. and E. A. Friar, Gonzalez – Elizando and Tena Floras, 2000).

Eleocharis R.Br. includes some extremely difficult species complexes. These indicate that the above supraspecific taxa are probably monophyletic.

Some species like *E. acutangula, E. dulci* often proliferate from spikelets, often on arching or horizontal clums especially when growing as submerged or floating aquatic, because many such plants reproduced entirely asexually and have no normal spikelet or achenes. It is often impossible to identified them to species, least aquatic from of species such as *E. geniculata, E. atropurpura, E. chaetaria, E. setifolia* are very hard to distingused, because of some proliferation in the spike. The submerged plant may be entirely vegetative which may have spike.

E. acutangula, E. dulci having a rhizome tuber is not found in *E. geniculata, E. atropurpura, E. chaetaria,* E. setifolia.They are mostly extraterrestrial in habit.

Above all species of *Eleocharis* R. Br. show same character like hypogynous bristal, But in cases *E. chaetaria, E. setifolia* the hypogynous bristal reduced to small 6 toothed cup , where called by Nees a disc understood the obpyramidal 3-(or several) toothed Gynophore of scleria and many species of fimbristylis. The stames are entirely within the disc of *Eleocharis* R.Br. entirely without the disc of *E. agutangula, E. geniculata*.

Conclusion:

From the above data species level relationship found morphologically. But due to difference in stem architecture and is due to different elevation rang and different in there metabolic activity. The marshy and aquatic plant of *Eleocharis* R.Br. like *E. chaetaria,* E.setacea, similar to that of E.geniculate, *E. atropurpura*. On the other hand plant is fully aquatic like *E. acutangula* and *E. dulci*. But differ in stem artitecher in compare to *E. geniculata, E. atropurpura, E. chaetaria, E. setifolia*.

The *Eleocharis* R.Br. species found to be diffident elevation range, so that there metabolic activity and morphology is different. But phytochemically they are evolved to some aspect the *E. chaetaria* and *E. setifolia* is found to be evolved from taxa of E.geniculate, *E. atropurpura* but in fully aquatic in habitat. The *E. acutangula*and *E. dulci* they are separately evolved from due to elevation range. The individual of the species *E. geniculata* and *E. atropurpura* are placed as a sister to the *E. chaetaria* and *E. setifolia*. This group of species is differ by there morphological characteristic.

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ANTIMYCOBACTERIAL ANEFFICACY OF NEWELY SYNTHESIZED NICKEL OXIDE NANOPLATELETS OBTAINED FROM GREENERY *ANNONA RETICULATA* LEAF EXTRACT

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Abstract:

The present study revealed that, nickel oxide (NiO) nanoplatelets (NPs) were synthesized by a simple, cost-effective, eco-friendly and low-temperature successive ionic layer adsorption and desorption method by using an aqueous *Annona reticulata* leaf extract (ARLE) to form an *Annona reticulata* leaf silver nanoparticle (ARLENiONPs) annealed for 300 °C for 1h, scratched from the supporting substrate i.e. glass and envisaged for the antimycobacterial, antioxidant and cytotoxic activity studies. The structural investigation of synthesized NiO NPs was carried out with the help of X-ray diffraction (XRD) and Raman shift spectra. Reflection planes i.e. (111), (200), (220), and (311), obtained from the interatomic spacing based on individual ring diameter, of selected area electron diffraction (SAED) pattern and Raman shift peaks at 550 cm^{-1} and 1095 cm^{-1}, which were due to the one and two phonon longitudinal optical modes of Ni-O oscillation, corroborated for the XRD analysis i.e. NiO formation. Platelets-type morphology of NiO was corroborated from the FESEM and TEM measurements. Fourier transform infrared spectrum suggested capping of the phyto constituents, probably polyphenols which might appear from the ARLE. The results obtained from the antimycobacterial activity assays suggested that these green ARLENiONPs were more potent against three *Mycobacterium* species like *M. tuberculosis* (MTCC-300), and. Minimum inhibitory concentration value of the ARLENiONPs was within the range of 10-20 µg/ml.

Keywords: *A. reticulata*; Silver Nanoparticles; Antimycobacterial; MIC.

Introduction:

Nowadays, the versatile ability of material science and nanotechnology to prepare nanostructures of particular size and shape is likely to lead to the development of new drugs.

Therefore, the syntheses of novel nanoparticles of metal oxides/ chalcogenides have received great attention due to their unique physical, chemical and effective biological properties in various fields, for example, medicine. Since nanoparticles and bacterial pores can be smaller in size, thus, they have a unique ability to cross the cell membrane. There, however, lies a strong challenge in preparing nanomaterials stable enough to restrict bacterial growth significantly while in a nutrient medium. Antibacterial activity is related to compounds that locally kill bacteria or slow down their growth, without being, in general, toxic to surrounding tissues.Most current antibacterial agents are chemically modified natural compounds (Nussbaum *et al.,* 2006; Hajipour *et al.,* 2012). The reason why nanoparticles offer improved properties to classical organic antibacterial agents and why they are an effective one lies in their high surface-area-to-volume ratio, resulting of new chemical, optical, electrical, magnetic, electro-optical, and magneto-optical properties, quite different from their bulk counterparts (Whitesides, 2005). Present investigation, in the plant contain is a valuable source for new antitubercle agents. The present article focuses on how synthesized AgNPs can be targeted on three different Mycobacterium species to develop novel ant tuberculosis agents. The literature reports that many plant based NPs show better results as compared to the chemical based NPs (Prathap *et al.*, 2014; Muniyappan and Nagarajan, 2014).

Materials and Method:

Collection of pathogen

The Mycobacterium tuberculosis (MTCC 300), Mycobacterium pheli (MTCC1723), Mycobacterium avim (MTCC 1724) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh (PB), India, were sub cultured and maintained into Lowenstein-Jensen media.

Synthesis of NiO NPs

Analytical reagent grade (AR) chemicals like 0.1 M nickel chloride (NiCl2).6H2O, 1 M urea (NH2CONH2) and aqueous ammonia (25-28%) (to maintain a highly alkaline pH) were used as received for the synthesis of NiO NPs. The ultrasonically cleaned glass substrate was used as a supporting medium for forming NPs of NiOOH in thin film form. Growth of NPs in a thin film over the glass substrate was achieved by SILAR method. Vertical dipping of the cleaned on-glass substrate into the freshly prepared reaction mixture of nickel precursor, having the 12 pH for 25 s, formed the basis of SILAR process. In the second stage, the same glass plate was immersed into a hot water (90˚C) for 20 s, to result in one complete cycle. The process of

fabrication continued up to the thirty cycles of SILAR to get a uniform, well-adherent and relatively thicker film of NiOOH. After completion of the reaction, greenish precipitate of NiOOH was formed on the glass substrate which was annealed at 300 °C for 90 min in a muffle furnace to get black-coloured NiO thin films which were then scratched with a knife of fine and clean edge and used in structural, chemical and biological studies.

The Biosynthesis of NiONPS

A. *reticulata* is a high amount of alkaloids containing belonging to the family Leguminoceae. The leaves of *A. reticulata* used in this study were collected from the Swami Ramanand Teerth Marathawada University Nanded campus (Maharashtra, India). The leaves were primarily cleaned with Millipore Deionized (DI) water, washed and dried by pressing with blotting paper. They were then shade-dried and chopped into small pieces. 10 g of leaves in 100 ml of distilled water were microwave-irradiated for 5 min, and the extract was filtered through Whatman filter paper and stored at 4ºC for further experiments. A 100 ml portion of aqueous 0.1 M NiONPS was move to a conical flask, and 10 ml of aqueous *A. reticulata* extract was added with vigorous magnetic stirring for 10 min. The reduction of NiO+ ions into NiONPS at room temperature was completed in 30 min, as observed by the changed color of the liquid extract from greenish-yellow to dark brown to indicate the formation of NiONPs (Fig. 1). For the purification of ARLE NiONPS, the reduced solution was centrifuged at 5000 rpm for 15 min. The supernatant liquid was discarded, and the residue was dispersed in distilled water. The samples were centrifuged about five times to wash distilled water off any nonessential materials from that had been absorbed onto the surface of the NiONPs.

Minimum inhibitory concentration (MIC)

The MIC determination for the antimycobacterial activity of ARLENiONPs was studied by employing a microdilution method by Lowenstein-Jensen medium (Broth). The inoculum was prepared by using the method (Juan, L. *et al.*, 2010). The ARLENiONPs to a concentration of 20µl against mycobacterium species. Serial dilutions were studied by by adding culture broth to reach concentrations ranging from 20 µl of each dilution was distributed in 96-well microtitre plates, along with a sterility control and growth control (containing culture broth plus DMSO without antimicrobial substances). Each test and growth control well was inoculated with 5 µl of a bacterial suspension (102 CFU (colony focusing unit) per ml or 98 CFU per well). The present study were performed in equal, and the micro-dilution trays were incubated at 36ºC for 18 h. Bacterial growth was detected by measuring the optical density (ELISA reader, Thermo Multiscan EX Ref:51118170) and by adding an equal concentration of ARLENiONPs and solvent. The microtitre plates were again incubated at 36 ºC for 30 min, in those wells where

bacterial growth occurred, the ARLENiONPs changed from yellow to brown shown in table no.1.

The antibacterial activity of test drugs

The antimycobacterial activity of three *Mycobacterium tuberculosis*was performed against biosynthesized ARLENiONPs. The three *Mycobacterium* strains of are resistant to most new antibiotics resulting in new research into the well-known nickel oxide-based nanomaterial's including ARLENiONPs.

Table 1: Minimum inhibitory concentration (MIC) of ARLENiONPs, NiONPs and the standard tested against three *Mycobacterium* species (µg / ml)

			Minimium inhibitary concentration
Sr.No.	Test drug	Concentration	M.tuberculosis
1.	NiONPs	20 µl	5
2.	ARLENiONPs	20µl	4
3.	Rifampicin	20 µlg/ml	4

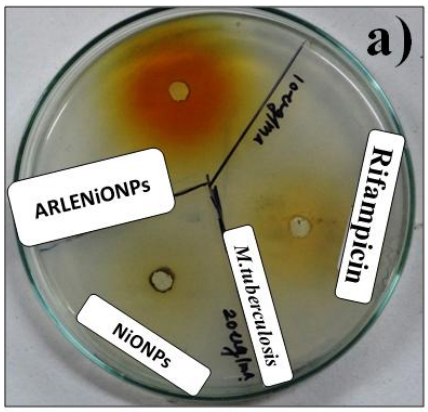

Figure 3: Zone of inhibition shown by a) ARLENiONPs against *M.tuberculosis*, Standered drug Rifampicin and NiONP against *Mycobacterium* species

Conclusion:

In present investigation green route synthesis of bio-functionalized NiO using *A. reticulata* plant extracts in the presence of sunlight to produce ARLENiONPs. These synthesized ARLENiONPs are good antimycobacterial agent. Still date, there have been no reports on the as antimycobacterial agent and cytotoxic study of ARLENiONPs from *A. reticulata*. *M.*

tuberculosis against ARLENiONPs causes the 99.9% cell death. A direct connection between green approach ARLENiONPs stability and antimycobacterial effects were also demonstrated. The present investigation suggest that apart from its usual biogreenery and biocompatible properties, bio-aspects of green synthesized ARLENiONPs are also a ecofriendly,easilysynthesized, less toxic, targeted oriented potential TB agent.

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PHYTOCHEMICAL ANALYSIS AND ETHNO MEDICINAL USE OF *CALOTROPIS PROCERA* L AND *EUPHORBIA HIRTA L.*

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Abstract:

Calotropis procera L.(Rui) is an important ethnomedicinal plant from the family Asclepiadaceae found in this region. There are two species of *Calotropis*, namely, *Calotropis procera L.* and *Calotropis gigantea* Land another plant *Euphorbia hirta L.* plant belongs to Euphorbiaceae family which are found in Poladpur all plant parts of both plants parts such as root leaves and stem flower and seeds having medicinal properties. Attempts have been made to give complete phytochemical constituents and HPTLC profile of leaf extract of *Calotropis procera* L and *Euphorbia hirta L.* ethanolis used for sonication. The densitometric analysis shows fingerprinting, Rf values, peaks of densitogram and chemical variation. Therefore HPTLC fingerprint analysis shows different peaks of these two selected plant leaf extracts. HPTLC fingerprint analysis profile of plant will be helpful for drug identification, adulteration and also act as biomarker for plant in pharmaceutical industry.

Keywords: *Calotropis procera* L. Asclepiadaceae, Euphorbiaceae *Euphorbia hirta* L.HPTLC.

Introduction:

The peoples are aware about using chemical and synthetic medicines cosmetics and give more preference to use of herbal products. India recognize more than 2500 plants species which have medicinal value, However, large flora is waiting for their medicinal properties. The use of medicinal plants as a source of medicine and human substances has been in vogue since antiquity India has rich heritage of use of plants as medicines and near about 805 medicines obtained from plants. The use of medicinal plants as a source of medicine and human substances has been in vogue since antiquity. India has a rich heritage of using plants as medicines and about 805 medicines have been obtained from plants.

In India, there are 2,500 plant species with documented medicinal value. Raigad district of the Kankan region is very well known for its huge biodiversity of flora and fauna. The area has a forest situated in its surrounding mountains. The Sahyadri hills have a vast reservoir of natural resources, including a wealth of vegetation and traditional knowledge of medicinal plants. Raigad district of Kankan region is very well known for its huge Biodiversity of flora and fauna. The main range of sahyadri, spurs and valleys form important botanical pockets of high biodiversity.The area has forest situated on its surrounding mountains. Sahyadri hills has a huge reservoir of enormous natural resources including vegetation wealth and traditional knowledge of medicinal plants The last two decades Pharmaceutical industry has made massive investment in research throughout the world to discover new drugs. Plants have effectively passed the taste of commercial screening (Dubey, 2003).

Different parts of the plant *C. procera* L. possess a varied number of pharmacological properties, such as fruits, flowers, roots, leaves, and latex. In traditional Indian medicine, this plant has been used to treat a variety of ailments, including leprosy, ulcers, tumors, hemorrhoids, and rheumatism (Warrier *et al.,* 1994). Many of the relevant pharmacological activities of *Calotropis procera* L are related to or to its latex. In chemical analyses of two crude extracts of latex of *C. procera,* various compounds have been identified, such as active cardenolides, proteolytic enzymes, alkalides, and carbohydrates (Seiber *et al.,* 1982), apart from steroids, terpenes, and organic carbonates (Gallegos-Olea *et al.,* 2002). Cysteine proteases (Dubey; Jagannadham, 2003; Singh *et al.,* 2010) and umaosmotin (Freitas *et al.,* 2011) are the most recently identified non-latex of *C. procera.* L. indicating the presence of a protein with papain inhibitor activity (Ramos *et al.,* 2010). *Euphorbia hirta L.* is a well-known medicine for inflammation of respiratory tract and for asthma as it has a special reputation for causing bronchial relaxation. It can also be used as diuretic and purgative action.

These secondary metabolites are synthesized naturally in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds, etc. i.e. any part of the plant body may contain active components making them rich sources of different types of phytochemicals. Mostly, these phytochemicals are secondary metabolites like flavonoids, steroids, alkaloids, resins, fatty acids, tannins and phenol compounds, etc. (Wadood, 2013). These compounds are extracted from different parts of the plant. The amount of phytochemical compounds differs significantly from species to species and even from plant to plant, depending on the age and different ecological and climatic conditions. In recent years, phytochemicals which have unknown pharmacological activities have been widely investigated as a source of phytomedicines (Yadav, 2011).

Material and methods:

Extraction of Plant Material:

About 10 gm powder of each selected plant leaf was extracted and separated using 70% ethanol in a Soxhlet Extractor (Borosil) for about six hours. After extraction the extracts were evaporated to dryness. The dried extracts were dissolved in 5 ml ethanol and filtered using Whatman filter. The filtered extracts were later used for further phytochemical and HPTLC analysis.

Preliminary phytochemical analysis:

Primary phytochemical analysis of ethanolic extract of *Calotropis procera* L and *Euphorbia hirta L.* were done as follows.

Procedure for alkaloids: 2ml of extract is taken and added 2ml of Wagner's reagent, a brownish precipitate indicating the presence of alkaloids.

Cardiac glycosides: 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. Deep reddish brown colour at the interface of the steroid ring indicates the presence of cardiac glycosides.

Flavonoids: 2ml of extract is treated with 2 ml of 10%lead acetate. Yellowish green colour indicates the presence of flavonoids.

Saponins: 2ml of extract is dissolved with 2ml of Benedict's reagent. Blue black ppt indicates the presence of saponins.

Tannins: 2ml of extract is treated with 0.1% of ferric chloride. Brownish green indicates the presence of tannins.

Terpenoids: (Salkowski test) 2ml of extract is dissolved with 2ml of chloroform and concentrated sulphuric acid is carefully added to form a layer. A reddish brown colour indicates the presence of terpenoids.

Anthraquinones: 1ml of extract is boiled with 10% HCL for a few minutes in a water bath. It is filtered and allowed to cool. Equal volume of CHCl3 is added to the filtrate, a few drops of 10% Ammonia is added to the mixture and heat. Formation of rose pink colour indicates the presence of anthraquinones.

Glycosides: The extract is hydrolyzed with HCL solution and neutralized with NaoH solution. A few drops of Fehling's solution A and B are added and red precipitate indicates the presence of glycosides.

Reducing sugars: The extract is shaken with distilled water and filtered. The filtrate is boiled with drops of Fehling's solution A&B for a few minutes. An orange red precipitate indicates the presence of reducing sugars.

HPTLC analysis of Extracts:

HPTLC profiling of leaves extracts of *Calotropis procera* L and *Euphorbia hirta L.* were done by using CAMAG HPTLC system with WIN CATS software and phytochemical analysis of leaf extracts of selected plants leaves is done as per method described by Wagner and Kirtikar (1995), Harborne and Khandelwal (2005).

HPTLC profiling was done by using CAMAG HPTLC System with WIN CATS software two microliters of the ethanolic extract was applied (band length –8.0 mm) on a percolated TLC aluminium sheets of silica gel G60 F254 of 200 μm thickness plate- 05 x10cm (Merck, Mumbai) using Linomat V TLC applicator (Camag, Muttenz, Switzerland) equipped with a 100-μL syringe. Prior application, the plate was pre-washed with methanol AR and dried at 60°C. TLC plates were developed using the mobile phase Toluene: Ethyl acetate: Diethyl ether (6:3:1) in a Camag HPTLC twin-trough chamber (10 x10cm). The chamber was saturated with filter paper for 15 minutes and plate equilibrium was carried out for 10 minutes. Plate was developed up to 85.0 mm and dried under a stream of air. Separated bands were quantified by HPTLC densitometric scanning using Camag TLC Scanner 4 in the absorption mode (multi wavelength Scanning) operated by WinCATS software (version 1.4.8). After scanning the spectra and tables thus obtained were analysed to interpret the results.

Observations and preliminary phytochemical results:

Preliminary phytochemical analysis of ethanol extracts of selected plants leaves in presence of following compounds. Table No 1 Different phytochemical compounds present in selected leaf extracts

Sr. No.	Phytochemical compound	*Euphorbia hirta. L.*	*Calatropis procera*.L
1	Alkaloid	+	-
2	Saponins	+	+
3	Tannin	-	+
4	Cardiac Glycosides	-	+
5	Glycosides	+	-
6	Flavonoids	+	-
7	Reducing Sugars	+	+
8	Terpenoids	+	-
9	Anthraquinones	-	-

In the present research work preliminary phytochemical analysis of ethanol extracts of *Calatropis procera* shows presence of Tannin, Saponins, Cardiac Glycosides, Reducing Sugars and Anthraquinones. Tannin, Terpenoids Alkaloids Glycosides was absent.In *Euphorbia hirta. L*. Alkaloid, Flavonoids *s*aponins Glycosides Flavonoids Reducing Sugars Terpenoids were present and Anthraquinones. Cardiac Glycosides Tannin is absent.

Collection of Plant material:

The leaves of *Calotropis procera* L and *Euphorbia hirta L.* were collected from Poladpur district Raigad and brought to laboratory for further analysis. The *Calotropis procera* L and *Euphorbia hirta L.* leaves washed gently with running tap water to remove surface dust and pollutants. The leaves were dried under the shade. The dried plant material was made of powder using a mixture grinder.

Morphology of *Calotropis procera L*:

Erect shrub, leaves ovate, obovate, umbellate cyme flower, corolla lobe truncate with a recurved spur at the base, The follicle is boat-shaped, recurved, and cottony pubescent; the seed is ovoid, compressed, and silky white. The search for environmentally friendly prototypes to replace chemically synthesised drugs is rapidly increasing. Thus, a lot of research has been focused on the plant species mentioned in traditional medicinal systems. The pharmacological activities of *C. procera L.* have been used in the past to cure several diseases in human beings, such as colds, fevers, leprosy, asthma, rheumatism, eczema, indigestion, diarrhoea, elephantiasis, skin diseases, and dysentery (Al-Rowaily *et al.,* 2020). In Saudi Arabia, the decoction of above ground parts is being used to treat fever, joint pain, muscular spasms, and constipation (Mossa *et al.,* 1991). The plant is also used to treat neuropsychiatric disorders in Burkina Faso (Kinda *et al.,* 2020). The medicinal attributes of *C. procera* L. can be credited to secondary metabolites and cardio tonic substances present in the plant (Hagaggi and Mohamed, 2020). The extracts of plant parts of *C. procera* L. exhibited strong antipyretic, analgesic, antidepressant, and neuromuscular blocking activity (Garabadu *et al.,* 2019) and latex contained anticancer activity (Tenzin *et al.,* 2006).

Table 2: Rf value of the peak formed of *Calotropis procera*. L. leaf extract

Peak	Start rf	Start height	Max rf	Max height	Max%	End rf	End height	Area	Area %	Assigned substance
1	0.09	0.2	0.13	17.8	3.25	0.15	0.0	432.4	2.66	unknown
2	0.19	0.0	0.22	30.1	5.49	0.25	2.7	542.0	3.34	unknown
3	0.28	0.3	0.32	21.6	3.94	0.34	3.2	424.8	2.62	unknown
4	0.35	3.5	0.38	37.6	6.80	0.40	6.7	739.7	4.55	unknown
5	0.40	6.9	0.45	236.7	43.11	0.50	0.1	5114.0	31.48	unknown
6	0.59	0.0	0.63	13.6	2.47	0.66	3.3	342.3	2.11	unknown
7	0.66	3.4	0.72	46.0	8.38	0.77	0.2	1560.0	9.60	unknown
8	0.77	0.3	0.80	11.2	2.03	0.83	0.0	236.3	1.45	unknown
9	0.86	1.4	0.96	134.7	24. 53	1.00	3.3	6852.6	42.19	unknown

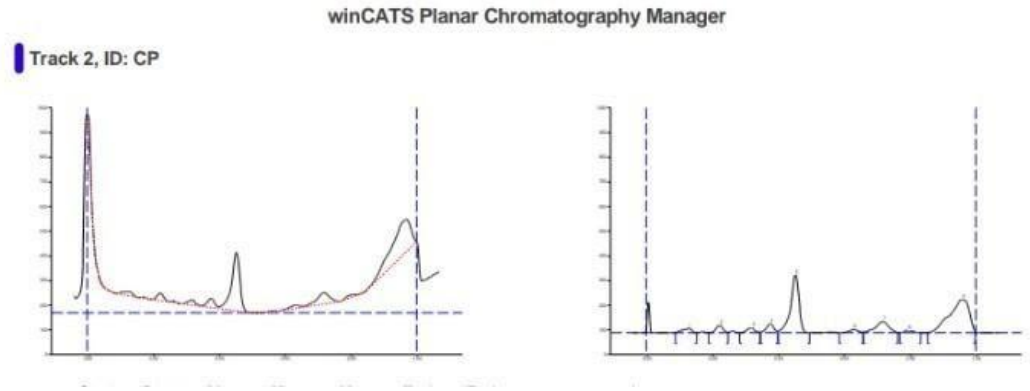


Figure 1: Chromatogram of *Calotropis procera* L. leaf extract

The HPTLC analysis obtained high resolution and shows different peaks. The leaf extract *of Calotropis procera* L. was run along with the standard and it was perceived to validate the presence of phytochemical compounds from the chromatogram after derivatization. The result of the selected plant extract is given in table 2. Ethanol is used as a solvent. Rf values and different wavelengths were obtained. Table 2 shows the picture plate at UV254nm. The graphic representation shows different peaks of polyvalent phytoconstituents. The Rf value starts from 0.15 to 1.00, in which the highest concentration of phytoconstituents was found to be 24.53, and the maximum percentage of stars from 3.22 to 24.53 %, and the maximum height from 17.8 to 134.7. The secondary metabolites glycoside and alkaloid are hazardous to human health, when they are used with any intention, such as to kill the person or blind the person.

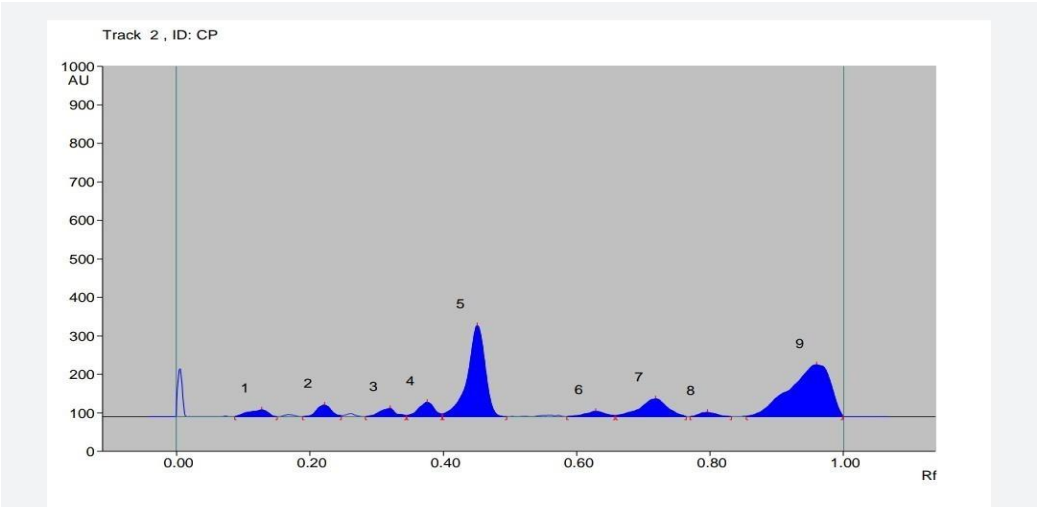
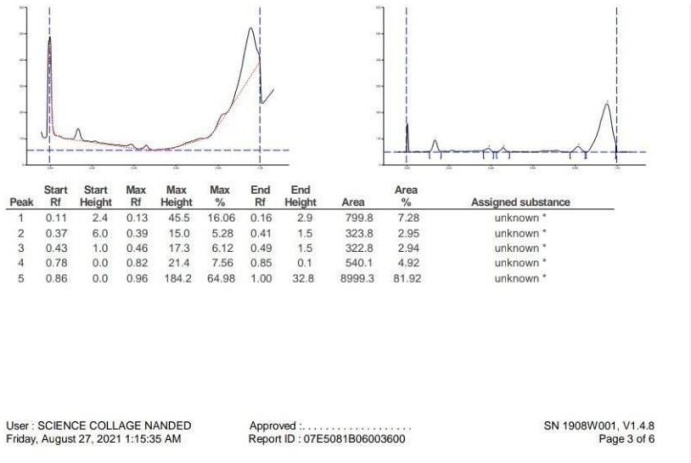


Figure 2: HPTLC Chromatogram of *Calotropis procera*.L. leaf extract

Morphology of plant*:*

Euphorbia hirta L. or Birhat Dhugdhika is an annual herb with milky latex, medicinally important plant from the family Euphorbiaceae with 1600 species. Harborne (1988) stem hispid with yellowish crisped hairs leaves unequal, sided and cordate at base. Cyathia many, crowded in small axillary, sub sessile cyme.fruits globose hispid. Seeds ovoid, trigonous transversely rugose reddish brown. It is also called asthma herb and pill bearing spurge (Rastogi, 2002). The stem sap is used in treatment of eyelid style and leaf poultices is used in swelling and boils (Williamson, 2005). Latex is applied on lower eyelids, like *surma*to cure sores, Root exudates exhibits nematicidal activity against juveniles of Meloidogyne incognita and also used for snake bite. The whole plant shows sedative, antispasmodic antifertility antifungal and antimalarial properties, antidiarrheal activity, antibacterial, anthelmintic, antifungal (Galvez, 1993). The powdered *E. hirta* showed a galactogenic activity in guinea pigs before puberty by increasing the development of the mammary glands and induction of secretion.

Further research is going on to find out more activities in constituents of *E. hirta*.There are many other traditional uses of *E. hirta* in Ayurveda which serves as the basis for further studies. In pharmacological study of *Euphorbia hirta* L may contain s afzelin, quercitrin, myricitrin, rutin, gallic acid, quercetin, euphorbin-A and ephorbin-B, euphorbin-C, euphorbin-D, β-amyrin, 24-methylenecycloartenol, β-sitosterol, heptacosane, n-nonacosane Hung 92012)shikmic acid, tinyatoxin, choline, camphol, and quercitol derivatives containing rhamnose, and chtolphenolic acid (Abubakar, 2009).



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.11	2.4	0.13	45.5	16.06	0.16	2.9	799.8	7.28	unknown *
2	0.37	6.0	0.39	15.0	5.28	0.41	1.5	323.8	2.95	unknown *
3	0.43	1.0	0.46	17.3	6.12	0.49	1.5	322.8	2.94	unknown *
4	0.78	0.0	0.82	21.4	7.56	0.85	0.1	540.1	4.92	unknown *
5	0.86	0.0	0.96	184.2	64.98	1.00	32.8	8999.3	81.92	unknown *

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Figure 2: Chromatogram of *Euphorbia hirta* L leaf extract

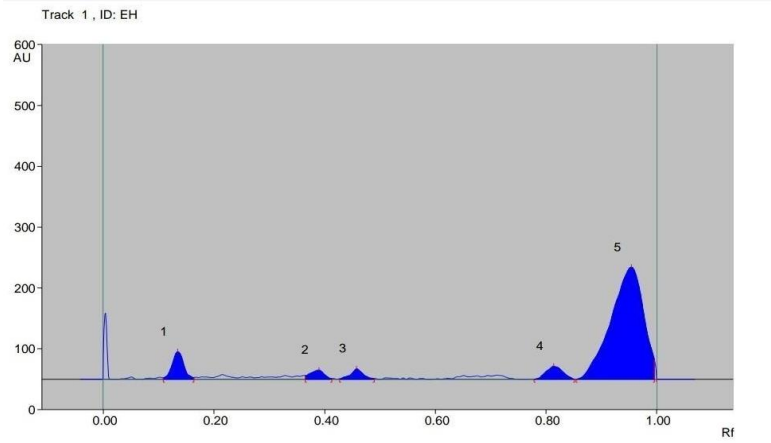

Figure 3: HPTLC Chromatogram of *Euphorbia hirta* L. leaf extract

Table 3: RF Value of leaf extract of *Euphorbia hirta* L Leaf at UV254nm

Peak	Start Position	Start Height	Max position	Max height	Max %	End Position	End Height	Area	Area %
1	0.11Rf	2.24AU	0.13Rf	45.5AU	16.06%	0.16Rf	2.9AU	799.8AU	7.28%
2	0.37 Rf	6.0 AU	0.39Rf	15.0AU	5.28%	0.41Rf	1.5AU	323.8AU	2.95%
3	0.43 Rf	1.0AU	0.46Rf	17.3 AU	6.12%	0.49Rf	1.5AU	322.8AU	2.94%
4	0.78 Rf	0.0AU	0.82Rf	21.4AU	7.56%	0.85Rf	0.1AU	540.1AU	4.92%
5	0.86 Rf	0.0AU	0.96Rf	184.2AU	64.98%	1.00Rf	32.8AU	8999.3 AU	81.92%

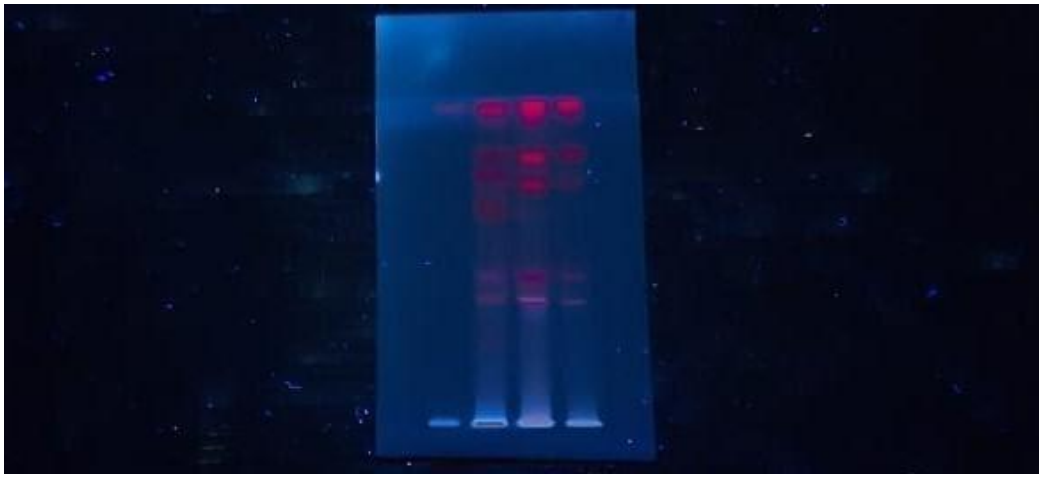

Figure 4: HPTLC profile of *Euphorbia hirta* Lplant ethanol extract at 366 nm

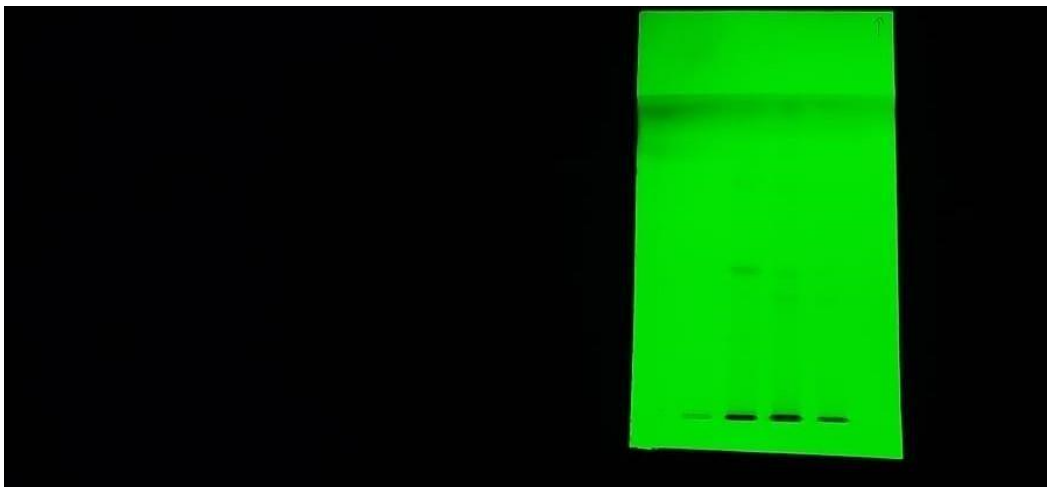

Figure 5: HPTLC profile of *Euphorbia hirta* Lplant ethanol extract at 254 nm

Material and Methods:

Preliminary phytochemical analysis of leaf extracts of *Euphorbia hirta L* is done as per method described by Wagner, Kirtikar (1995), Harborne, Khandelwal (2005) and Eike ReichEike (2006). HPTLC profiling was done by using CAMAG HPTLC System with WIN CATS software.

Result and Discussion:

The HPTLC analysis obtained high resolution and shows different peaks. The leaf extractof *Calotropis procera* L. was run along with the standard and it was perceived to validate the presence of phytochemical compounds from the chromatogram after derivatization. The result of the selected plant extract is given in table 2. Ethanol is used as a solvent. Rf values and different wavelengths were obtained. Table 2 shows the picture plate at UV254nm.The graphic

representation shows different peaks of polyvalent phytoconstituents. The Rf value starts from 0.15 to 1.00, in which the highest concentration of phytoconstituents was found to be 24.53, and the maximum percentage of stars from 3.22 to 24.53 %, and the maximum height from 17.8 to 134.7. The secondary metabolites glycoside and alkaloid are hazardous to human health, when they are used with any intention, such as to kill the person or blind the person.

The HPTLC analysis obtained high resolution and shows different peaks leaf extract of *Euphorbia hirta L* was runs along with the standard and perceived to validate the presence of phytochemical compounds from chromatogram after derivatization. The result from HPTLC fingerprint scanned at wavelength 366 nm for *Euphorbia hirta L* shows polyvalent phytoconstituents and corresponding ascending order of Rf value are from 0.11to 0.86 in which highest concentration of the phytoconstituents was found to be 64.98 % .This is recorded in Table No.3 ethanol is used as a solvent Rf value and different wavelength were obtained in picture plate at UV254 nm. The graphical representation shows different peaks of polyvalent phytoconstituents.TheRf value starts from 0.11 to 0.86 in which highest concentration of phytoconstituents were found and maximum percentage starts from 16.06 to 64, 98% and maximum height from 45.5AU to 184.2AU control.

The peak retention in ethanol extracts and is found with Rf start with 0.11,0.37,0.43,0.78,0.86 and end with 0.13,0.39,0.46,0.82, 0.96 and maximum percentage is 16.06%,5.28%6.12%7.56%,64.98% in Table no.3.These studies have shown that it is more versatile than ordinary TLC methods as the spots are well resolved. The HPTLC method is simple, rapid, accurate, reproducible, selective and economical for quality and quantitative determination of plant material.Khandelwal (2005)

Conclusion:

The study of the HPTLC fingerprint profile of *Calotropis procera* and *Euphorbia hirta is* useful to determine the quality of crude drugs. It is also useful for separation of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and cardiac glycosides, bioactive products that are used to understand biochemical and physiological mechanisms in plants.

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CHECKLIST OF MOSSES FROM SATARA DISTRICT (WESTERN GHATS)

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Abstract:

Bryophytes including plant which are commonly known as mosses, hornworts and liverworts. They are second largest group of plant and show wide range of distribution. Mosses are most evolved group of bryophytes because presence of stem, leaves and root like structure. Satara district of India comprises unique topographical condition hence is rich in bryophyte. Satara district divided in two part Western part and Eastern part. In present Work preliminary checklist has been prepared which revealed the occurrence of 9 genus and 12 species of mosses were reported first time from Satara district.

Keywords: Mosses, Diversity, Satara.

Introduction:

India encompasses Eastern Himalaya and Western Ghats as two biodiversity hot spots. The Western Ghats also locally known as the Sahyadri Hills, are formed by the Malabar plains and the chain of mountains running parallel to India's western cost, about 30-50 km inland. They cover an area of about 1,60,000 km^2 and stretch for 1,600 km from country's southern tip to Gujarat in the north, interrupted only by the 30 km Palghat Gap (Verma *et al*. 2011). It is a major tropical evergreen region, well known for its rich, diverse and unique flora and fauna.

Bryophytes including plant which are commonly known as mosses, hornworts and liverworts. They are second largest group of plant and show wide range of distribution. They classified under three different lineages, are Hepaticae, Anthocerotae and Musci. Currently, about 2480 taxa of bryophytes are reported from India, Comprising about 722 taxa of Liverworts in 128 genera and 52 families, 36 taxa in 6 genera and 2 families of Hornworts and about 1623 taxa in 342 genera and 52 families of mosses (Afroz Alam *et al.*, 2011).

Mosses are differentiated into stem, leaves and root like structure, rhizome with multicellular rhizoids. Stem erect or creeping, leaves with or without costa, seta usually present;

capsule never elevated on gametophytic tissue; peristome usually present, capsule dehiscing mostly by operculum. Protonema filamentous. In many species the capsule opens by a lid; peristome present in most forms, arising from only two or three concentric layers of cells; some walls of these cells are absorbed during growth; the teeth consists of thickenings, laid on the tangential walls of the component cells and are usually articulated.

Material and Methods:

The specimens were collected during June 2012 - September 2019. A knife and forcep are used foe peel of specimen from bark of rock. Collected specimens were washed kept on wet brick till identification while some are preserved in 4% formalin. Morphological and anatomical characters were studied under stereoscope and compound microscope. Identification of specimens was done by standard literature.

Result and Discussion:

In present investigation *Fissidens curvato-involutus Hyophila rosea, Entodon laetus Erpodium biseriatum, Gymnostomiella vernicosa* were first time reported from study area. Satara district were divided in two part Eastern and Western they show climatic difference in both. While we collecting the bryophytic species they show maximum diversity in Western part (Ajinkyatara, Kas, Mahableshwar, Pateghar, Pateshwar, Pasarani Ghat, Valmiki) as compare to Eastern part (Ramacha Dongar, Wardhangad, Dahiwadi, Jarandeshwar, Kartikswami Dongar, Mahimangadh,). Western part of Satara district shows high rainfall and humidity which supports the growth of bryophytes.

Present investigation was based on existing literature of mosses from Maharashtra. The first work on mosses of Maharashtra was carried out in 1897 Birdwood (Magdum *et al* 2017). Sedgwick (1910, 1911, 1913), Blatter (1909), Dixon (1909), Chaudhary *et al.*, (2008).Dabade in 1988, 1998 give greatest contribution in the mosses flora of Mahableshwar. Magdum *et al.* (2017) included 128 species of mosses belonging to 11 orders; 26 families and 59 genera from Western Ghat

The preliminary assessment of bryophytes was conducted from Satara district. These investigations are helpful to knowing the status of Mosses flora in the study area. It also helps in conservation and making aware about their usefulness.

Table 1: Checklist of Mosses from Satara district

Sr. No.	Name of the species	Occurrences	Habitat
1.	***Funaria hygrometrica***	Ajinkyatara, Bhekawali, Dahiwadi, Jarandeshwar, Kartikswami Dongar, Kas, Mahableshwar, Mahimangadh, Pateghar, Pateshwar, Pasarani Ghat, Ramacha Dongar, Wardhangadh	Coticolous
2.	***Gymnostomiella vernicosa***	Khandala, Mahableshwar	Rupicolous
3.	***Macrimitrium sulcatum***	Pateghar, Pateshwar, Mahableshwar	Coticolous
4.	***Erpodium biseriatum***	Pateghar, Pateshwar, Mahableshwar	Coticolous
5.	***Bryum coronatum***	Ajinkyatara, Pateghar, Pateshwar, Pasarani Ghat, Ramacha Dongar, Wardhangad	Coticolous
6.	***Stereophyllum anceps***	Ajinkyatara. Kas, Pateghar, Pateshwar	Coticolous
7.	***Stereophyllum wightii***	Mahableshwar, Pateghar, Pateshwar	Coticolous
8.	***Entodon laetus***	Ajinkyatara, Mahableshwar, Pateghar, Pateshwar	Coticolous
9.	***Hyophila involuta***	Ajinkyatara, Mahableshwar, Pateghar Pateshwar	Coticolous
10.	***Hyophila rosea***	Mahableshwar	Terricolous
11.	***Hyophila walker***	Valmiki	Terricolous
12.	***Fissidens curvato-involutus***	Ajinkyatara Kas Pateghar, Valmiki	Terricolous

Coticolous- epiphytic on tree trunk, Rupicolous -On stony wall, Terricolous - On moist ground

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PLANT BIOSTIMULANTS IN AGRICULTURAL SCIENCES: AN OVERVIEW

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Introduction:

Definition:

A plant biostimulant can be defined as any chemical formulation or microbial inoculant used among plant population aiming towards enhancement of nutritional efficiency, resistance towards different abiotic stress as well as different qualitative and quantitative traits irrespective of nutritional composition Kauffman *et al.* (2007) first defined the term "biostimulant". Scientists explained the term "biostimulant" to be any substance besides fertilizers, applied in minimum quantity and is responsible for promotion of plant growth and development. The main aim of scientists for stating "minute quantities" was to differentiate biostimulants from other soil amendments as biostimulants are used in very less quantities in comparison to other amendments. Different Plant Growth Promoting Regulators including "Biofertilisers" and "biocontrol agents"are also applied for enhanced outcome of Agricultural and Horticultural crops. In November 2012, biostimulants gained its importance in academic area after the 'First World Congresson the use of Biostimulants in agriculture' which was organized in Strasbourg.

On the basis of Agricultural applications, biostimulants can be categorized into different types. These include different beneficial microbes in the form of inoculants like *Azospirillum, Azotobacter* and *Rhizobium.* Biostimulant also includes some beneficial microbes including Arbuscular Mycorrhizal Fungi (AMF) other than the Nitrifying bacteria. Different naturally active diversified bioactive substances including humic and fulvic acids, vegetal protein hydrolysates, Sea Weed Extracts (SWE) *etc.* are also included under the category of plant biostimulants.

The overall productivity of crop plants depends upon the genetic regulation of the growth and development which is enhanced and improved by the application of biostimulants.

General concepts:

Prof. V. P. Filatov first proposed the "biogenic stimulant" theory in 1933 in USSR (Sukhoverkhov, 1967). He inferred that the different biological substances extracted from different plants and other organisms under exposure to different abiotic stressors drastically affect the biological metabolism and bioenergetic processes of the applied plants. Blagoveshchensky (1945, 1955, 1956) considered biogenic stimulants as organic acids having stimulating effects because of their dibasic nature leading towards enhancement of enzymatic activity in plants. Herve's (1994) pioneering review provides the first real conceptual approach to biostimulants. Herve suggested that biostimulants should function at low doses, be ecologically benign and have reproducible benefits in agricultural plant cultivation. They discussed the concept of biostimulants as "pre-stress conditioners," their effects being manifested in improved photosynthetic efficiency, reduction of spread and intensity of some diseases and in better yields. Du Jardin (2012, 2015) provided the first in-depth analysis of plant biostimulant science with an emphasis on biostimulant systematization and categorization on the basis of biochemical and physiological function and mode of action and origin. Several significant scientific meetings in the field of biostimulants have been held over the past ten years and have contributed greatly to our understanding of conceptual and methodological development of different biostimulant theories. There is huge prospect towards further research.Biostimulant mode of action can be best determined using molecular microarray analysis to identify gene changes in transcript levels (Gates *et al.,* 2012). Conan *et al.* (2015) proposed identification of the bioactive compounds responsible for the plant growth response by means of a metabolomic profiling of biostimulant products and analysis of their physiological effects through transcriptomic and metabolomic strategies.

Terminologies:

- **Biogenic stimulators:** Among living organisms, the different tissues on exposure to different stress, undergoes a cascade of different biochemical reactions due to the formation of different substances which are non-specific in nature affecting the overall growth and development of organisms. These substances are referred to as biogenic stimulators (Filatov, 1951a).
- **Biostimulants:** These include substances containing plant hormone which are responsible for stimulation of growth when applied externally (Schmidt, 1992).

- **Organic biostimulants:** These are products which are beneficial for the growth and development of plants which are not rich in nutrients. These include different natural products including vitamins, sea weed extracts, humic acids, hormone free plant metabolites, marine algae *etc.* (Kumar and Shivay, 2008).
- **Agricultural biostimulants:** These include different diversified formulations of different compounds which are applied to soil and different plant parts for the regulation and enhancement of different physiological processes. These chemicals affect the plant physiology by involving in different biochemical pathways than applied nutrients for the improvement of quantitative, qualitative and shelf life.
- **Phytostimulator:** This can be defined as microbial formulation with the ability for the production and alteration of PGR concentration (Martínez-Viveros *et al.,* 2010).
- **Biostimulant plant growth promoters**: These are natural, organic, eco-friendly products which are comprised of phytohormones for the stimulation of plant growth and development (Huang, 2007).
- **Metabolic enhancer**: These are non mineral substances which are applied externally in minute quantities for the stimulation of metabolism in plants (Doak *et al.,* 2005).
- **Biostimulant microorganisms**: This includes class of micro-organisms encompassing "Plant Growth-Promoting Microorganisms (PGPMs)" and "Both biocontrol microorganisms (BCMs)". They are responsible for enhancement of plant growth and development of defence mechanism against different pathogens during all phenological stages (Sofo *et al.,* 2014).
- **Plant strengthener**: These are class of "borderline" chemicals which are applied in Agriculture for development of resistance to different biotic stress and are responsible for improving plant strength (Torre *et al.,* 2013).

Categories of plant biostimulants:

Humic acids and fulvic acids

Humic Substances (HS) generally include components of soil organic matter available naturally. These are formed by degradation of residues of different plants, microbes and animals as well as by the metabolism of different soil micro-organisms. Fulvic acids are a group of naturally occurring organic acids which are also constituent of humus. These are highly rich in different nutrients which are responsible for better growth of plants. The nutrient rich substance promotes maximum delivery of nutrients to plants. This promotes better seed viability and improved plant growth. These are responsible for retaining the soil fertility through different

physico-chemical and biological properties. The biostimulation activity of Humic Substances (HS) has been aimed towards mitigating different stress. It has been reported that in maize seedlings grown by hydroponics, humic substances with higher molecular mass enhance the rate of metabolism thereby responding to different stress and humic substance modification (Schiavon *et al.,* 2010).

Protein hydrolysates and other N-containing compounds

Chemical and enzymatic hydrolysis of proteins obtained from different plant and animal residues as well as Agro-Industrial biproducts (Halpern *et al.,* 2015). The direct effects of protein hydrolysates on plants basically include the regulation of genes responsible for synthesis of enzymes responsible for alteration of assimilation and uptake of Nitrogen. These biostimulants have significant role towards improvement of overall fertility of soil and enhancement of activity and biomass of soil microbial population. The availability of nutrients as well as their uptake by the root system is dependent upon the chellation and combining activities of different peptides and amino acids.

Seaweed extracts and botanicals

The fact that seaweed extracts have biostimulant activity has been recently reported. Commercially seaweed extracts are used as alginates, polysaccharides, laminarin and carrageenans. Plant growth is also promoted by different micro and macro nutrients, betaines, hormones and sterols (Craigie, 2011). These biostimulants can be used as foliar applications, as nutrient media in hydroponics as well as in soil.It has been reported that the polysaccharides of the sea weed extracts are responsible for retention of water, aeration of soil and formation of gel (Craigie, 2011). Biostimulants have important role on germination of seeds, growth and development till maturity of crop plants due to the presence of phytohormones.

Chitosan and other biopolymers

Chitosan is a derivative of chitin obtained by deacetylation which is produced naturally as well as synthetically in industries. Since chitosan oligomers are polycationic in nature, therefore they combine with cellular constituents like DNA, constituents of cell wall and cell membrane. Besides, they combine with specific receptors thereby leading to activation of defense genes and plant defense elicitors.

Beneficial elements

Beneficial elements as biostimulant can be used as fungicides when applied in the form of inorganic salts (Deliopoulos *et al.,* 2010). They help in plant growth by enhancing nutrient use efficiency, improving abiotic stress tolerance. Therefore they can be applied both as fungicides as well as nutrient rich fertilizer.

Beneficial fungi

There are two types of mutual interactionsbetween fungiand plant roots. These include symbiotic relationship as well as parasitism. Reports infer that, the application of mycorrhiza is increasing with rising trend for the promotion of sustainable agriculture. This is basically due to its benefits of symbiotic relationship, moisture balance, protection from biotic and abiotic stress as well as enhanced nutrient use efficiency. For the utilization of benefits of such symbiotic associations, different plant cultivars and crop management practices should be applied along with the interactions with different micro-organisms.Biostimulant also includes different fungal-based products including *Trichoderma* spp. which can be applied for the promotion of nutrition efficiency, tolerance to biotic and abiotic stress (biopesticides and biocontrol agents), enhancing crop yield and improving the crop quality. These products can be exploited by biotechnological industries for the commercial production of enzymes

Beneficial bacteria

Beneficial bacteria affect different bio-geochemical cycles of plants thereby increasing the nutrient use efficiency, promotion of tolerance to different stress and induction of different morphological changes. On the basis of the agricultural applications, biostimulants can be categorized into2 types including (i) mutualistic endosymbionts of the type *Rhizobium* and (ii) mutualistic, rhizospheric PGPRs (‘plant growth-promoting rhizobacteria’) (Berg *et al.*, 2014).

Common features of biostimulants:

The nature of biostimulants is diverse

Biostimulants can be single substance including glycine betaine, as well as composite form in groups including seaweed extracts. Biostimulants can be natural products as well as synthetic in nature like artificial phenolic compounds including nitro-phenolates. Some biostimulant like microbial inoculants might include single strains (*e.g. Bacillus subtilis* inoculant) or multiple microbial strainsresulting in synergistic effects.

The physiological functions are diverse

The function of biostimulants is influenced by different molecular mechanisms including scavenging ROS (Reactive Oxygen Species) as well as enhanced formation of auxin transporters.

Mechanism/mode of action

The “mechanism of action” encompasses all the biochemical events while “mode of action” indicates characterization of bioactive molecules with a specific biochemical action on treated plants (Aliferis and Jabaji, 2011). Mode of action of plant biostimulant includes binding

of receptor site and thereby activation of downstream reactions. By identifying the target site of binding of the biostimulant molecules, new insecticidal molecules with better mode of action can be designed **(**Rattan, 2010).

The "mechanisms of action" of biostimulants basically includes positive effects on the overall productivity of plants by enhancing the different biological processes of plants. Besides, they are also responsible for "switching on" different genes responsible for tolerance to different abiotic stress, modification of architecture of plants as well as phonological stages (Khan *et al*., 2009; Sharma *et al.*, 2012).

Penetration into tissues, translocation, transformation in plants

Different radiolabelled amino acids are used for the detection of the mode of penetration of amino acids and peptide based biostimulants into plant tissues (Maini, 2006) and mathematical modeling (Kolomazník *et al*., 2012; Pecha *et al*., 2012). Besides these also illustrates the mechanism of transport from the point of penetration in the leaves till the distant tissues (Kolomazník *et al*., 2012; Pecha *et al*., 2012). Biostimulants must beeffectively soluble in water or other suitable solvents. This is the initial condition for efficient application of biostimulants as well as forrelevant penetration of ingredients into internal plant tissues.

Gene expression, signaling and hormone interactions

A collection of different molecular methods (including microarrays and omics technologies) has been applied for detecting active compounds in biostimulants.The above mentioned technologies alter the gene expression of biostimulants and thereby change their applications (Jannin *et al*., 2013). The entire process of signal transduction includes secretion of signaling molecules (ligands) followed by their transport and binding with specific receptors leading to initiation of cellular responses and thereby destruction of the ligand molecules (Zhao *et al*., 2005; Wang and Irving, 2011). In general, water soluble compounds are specific for membrane- mediated action. On the other hand, lipid soluble compounds are specific for cytosol mediated action. The bioactive compounds present in biostimulants are responsible for displayof signaling activity in plants or induction of signaling pathways. Different types of amino acids (Forde and Lea, 2007; Arbona *et al.,* 2013) and peptides (Ivanov, 2010) act as signaling molecules for the control of overall growth and development of plants (Ertani *et al.,* 2009; Mochida). Signaling of different peptide molecules is relevant in consideration of different biological and cellular mechanisms (Schiavon *et al.*, 2008).

Role of plant biostimulants:

Effect of biostimulants for agronomic and physiological traits of crops

In the different types of primary and secondary chemical reactions, the biostimulants regulate the activities of different bioactive molecules which are in turn responsible for regulation of different growth attributes (Calvo *et al.,* 2014). Gelatin hydrolysate can be considered as an important biostimulant which is considered as an uninterrupted source of Nitrogen.The different ligand moieties in various biologically active peptides are responsible for elucidation of signaling pathways by stimulation of different internal plant hormones.

Implications of biostimulants for abiotic stress tolerance

There are different effects of biostimulants towards mitigation of different abiotic stress. Reports infer the mitigation of adverse effects of drought in tomato cultivars by the application of different phytohormones. These were applied either in foliar form through drenching which in turn increased the efficiency of transpiration. Application of microbial inoculants of *Rhizobium* spp. and *Azospirillum* spp., enhanced the resistance of plants towards salinity stress (Cordovilla *et al*., 1999; Hamaoui *et al.,* 2001). There are reports which state that inoculation of *A. brasilense* to maize seedlings in greenhouse conditions can withstand the adverse conditions of drought stress (Casanovas *et al.,* 2002). Under dry conditions, inoculation of different bacterial inoculants including *Pseudomonas* spp. and *Bacillus* spp. to different plantshave been reported to stimulate the overall growth and development of plants. There are certain hormones like compounds produced by different bacteria which have major role towards tolerating the effects of droughts (Döbbelaere *et al.* 1999). For instance, the microbial inoculation of white clover (*Trifolium repens*) along with the natural auxin synthesizing strains of *Pseudomonas putida* and *Bacillus megaterium*, promoted enhancedbiomass of root and shoot as well as increased moisture content during drought stress (Marulanda *et al.* 2009).

Implications of biostimulants for improving nutrient use efficiency

The application of biologically active natural substances as well as microbial inoculants can be considered as a valuable tool for theincreased availability of soil nutrients, uptake by plant and their assimilation (De Pascale *et al*., 2017). Enhancing efficiency of nutrient use especially with respect to Nitrogen and Phosphorous is relevant both economically as well as ecologically. There are significant reports inferring improved growth attributes in greenhouse tomato especially at both sub-optimal and optimal regimens of Nitrogen (112 and 7 mg L-1, respectively). It was reported that, *Trichoderma virens* GV41 enhanced Nitrogen Use Efficiency (NUE) of lettuce which in turn stimulated the overall uptake of available nitrogen in the soil.

Implications of biostimulants for enhancing produce quality

Scientists have reported that the overall metabolism of plants can be modified by the use of different microbial and non-microbial inoculants (plant biostimulants) (Colla *et al*., 2015). These in turn lead towards the production and accumulation of different secondary metabolites being highly essential for human diet. Reports infer that spent mushroom substrate on biofortification by *Trichoderma harzianum* has shown drastic improvement in terms of different biochemical properties like TSS, carotenoid content, polyphenol content and so on. The mineral composition (especially including Potassium, Phosphorous, Calcium, Magnesium, Iron, Manganese as well as Zinc) of the treated mushroom was also enriched. Scientists have reported that use of phosphite in the nutrient media led to enhanced deficiency of phosphate. This has been concluded to favor the production and accumulation of some biomolecules including glucosinolates and flavonoids thereby improving the overall defense mechanism of the treated plants and mitigating the nutritional stress.

Toxicological and ecological aspects

Ecological safety is one of the primary concerns towards sustainable crop production with improved quality and quantity by all Agricultural workers, consumers and so on (Jannin *et al.,* 2012). Biostimulants are considered to be eco-friendly as they are biodegradable, with no toxic effects, polluting free and harmless to different organisms. Therefore, the different biological materials including seaweed extracts can be recommended for use (Michalak and Chojnacka, 2014). Biostimulants may also lead towards reduction of the amount of ecologically hazardous agrochemicals (Kolomazník *et al.,* 2012) as well as reducing the application of inorganic fertilizers and pesticides. Therfore, for sustainable agriculture, biostimulants can pave the path for an eco-friendly technology (Vijayanand *et al*., 2014).

Applications of biostimulants in agricultural sciences (biostimulant paper 10: role in horticulture):

Vinković *et al.* (2012) inferred that tomato plants treated with biostimulants had significantly higher concentrations of macronutrients in plant dry matter than control plants. Biostimulants including protein hydrolysates are responsible for synthesis of amino acids in plants which in turn affect the overall metabolism of plants, stimulating effects like plant growth promoting substances, increasing uptake of nitrogen and thereby improving crop performance. Sanchez-Andreu (2009) used humic acids commercially produced from lignites to iron deficient hydroponically grown young tomato plants. Besides, scientists have also proved the development of stress tolerance nature among tomato plants by the application of plant biostimulants (Grabowska *et al*., 2015).

Kwiatkowski and Juszczak (2011) inferred better plant growth, suppressed weed population and enhanced herb yield of sweet basil plants (22%–31%) in their research by the application of 3 biostimulants (Asahi SL, Bio-algeen and Tytanit). The application of these biostimulants has been reported to increase the growth attributes and in turn helps the plant to mitigate stress due to transplantation.

In garlic, application of humic acids increased bulb yield, diameter of clove and by reducing weight loss of bulb so as to improve the post-harvest quality (Abdel-Razzak and El-Sharkawy, 2013). Observed improvement of yield and yield contributing traits on application of Radifarm biostimulant (0.25%) to different garlic plants grown in- vitro.

Table 1: Application of Biostimulant Extracts for development of Abiotic stress tolerance in different crops

Crop	Biostimulant Extract	Developed trait
Wheat	*Azospirillum brasilense*	tolerance to drought
	Azospirillum brasilense	resistance to osmotic and salt stress
	Azotobacter chrococcum	tolerance to salt stress
	Azotobacter chrococcum	resistance to heat
	Protein hydrolysates	tolerance to heavy metals
Rice	Humic acids	tolerance of oxidative and drought stress
Maize	*Azotobacter chrococcum*	resistance to salt stress
	Melatonin	tolerance to chilling injury
	Sea Weed Extract	tolerance to cold
Barley	Protein hydrolysates	homeostasis of ions
Chick pea	*Azospirillum brasilense*	tolerance to salt stress
Faba Bean	*Azospirillum brasilense*	tolerance to salt stress

Challenges and future prospects:

There are many challenges faced by biostimulant industries. Till date, the biostimulant products had been produced from naturally available raw materials especially waste matter exclusively on the basis of observation with no reference data. The mode of action of most of the recently used biostimulants is not yet understood by the researchers (Khan *et al.,* 2009). The active ingredient of the plant raw material can be affected by various external and biological factors (Sharma *et al.,* 2012).

- This has also been reported that the different –omics technologies will be complicated to illustrate the mode of action of the biostimulants and in optimization of their applications (Jannin *et al*., 2012).
- Therefore, scientists have proposed the integration of different inter disciplinary methods with omics technologies along with biochemical analysis which can be used for identification of active ingredient and their mode of action (Lee *et al.,* 2012).
- The collaboration of experts from different branches including chemistry, plant physiology, engineering, marketing and Agricultural Sciences is a solution to the above mentioned problems (Jannin *et al.,* 2012).

Conclusion:

The recently developed biostimulants are composed of different raw materials having different origins produced by different technologies and have wider biological activity and safety. There is a discrete commercial perspective for the rationalization of biostimulants into distinct category of products. Reports infer that research is going on for the scientific advancement of biostimulants. Hence, the use of different biological substances obtained from different organisms on exposure to different stressors can alter the metabolism and bioenergetics of human beings, plants and animals.

There are requirements of local and time based adaptability of solutions especially in Agricultural and horticultural sectors. Steps should be taken to monitor the influence of biostimulants. Well defined plans to promote optimal use of biostimulants should be implemented. Assessment of long term effects through ecological services and biogeochemical cycles, by integrating in the decision-making process on the farm. On consideration of global climate change, ecological concerns and hike in population, the above mentioned methods are significant for achievement of sustainable food production.

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MUCUNA PRURIENS L.: A REVIEW

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Abstract:

Mucuna pruriens Linn. is a popular Indian medicinal plant. *Mucuna* belongs to the leguminous family. *Mucuna pruriens* is known as "Khajkuhilee" in Marathi. They have been used in traditional Ayurvedic Indian medicine for a long time to treat diseases. The plant has been reported as traditionalMedicinal uses ofthe treat neurological disorders, blood purification, diuresis, diabetes, gout, rheumatism and CNC stimulant. The pharmacological research of this plant has a variety of activities, such as anti-microbial, antioxidant, Antiprotozoal effect and Antivenom activityetc. In view of the many important discoveries of this plant recently, acomprehensive description of the taxonomy, nutritional constituent's phytochemical composition andsome pharmacological activities.

Keywords: *Mucuna pruriens*, Taxonomy, Traditional, Antioxidant activities, Antiprotozoal and Phytochemistry.

Introduction:

Since ancient times, medicinal plants have been used as sources of medicine in almost all cultures. Widespread use of herbs and health preparations was described in ancient texts such as the Vedas, Bible and Brahma jnana. It extracted from commonly used traditional herbs and medicinal plants, which can be traced back to the emergence of natural products with medicinal properties (Bukke *et al.,* 2011). According to the WHO (1991), traditional medicine is a synthesis of the treatment experience of several generations of indigenous medical systems. Herbs only constitute traditional medicines that mainly use medicinal plant preparations for treatment. The earliest recorded evidence of herbal use in Indian, Chinese, Egyptian, Syrian Greek, and Roman texts date back to about 5,000 years ago.

Mucuna pruriens belongs to the legume family, commonly known as cowhage plants.It is a popular Indian medicinal plant and has been widely used since ancient times. Ayurvedic medical systems usually appear in tropical regions and are used for various purposes of

traditional medicine in some countries (Rajeshwar *et al.*, 2005). It is widely distributed in most areas of the subcontinent, appearing in the form of shrubs and hedges. It is found in the dry deciduous low forests of the entire Indian plains. It grows naturally, from the lower Himalayas to the entire tropical plains of India.

Taxonomy of the genus and species:

Mucuna belongs to the leguminous family andtaxonomy and photo plate of *Mucuna pruriens* Linn. Shown in fig.1. This is the second largest flowering plant family, containing 600 genera and about12000 species (Evans, 2002).

Taxonomy of *Mucuna pruriens* Linn.

Kingdom: Plantae

Division: Angiosperms

Class: Dicotyledon

Sub class: Rosidae

Order: Fabales

Sub Family: Fabaceae

Genus: Mucuna

Species: *pruriens* L.

Figure 1: Habit of *Mucuna pruriens* Linn.

Common names

English - Cowhage; Marathi - Khajkuhilee; Hindi - Kaunch; Tamil - Poonaikkali;
Telugu - Duradagondi; Gujarati - Kavach; Kannada - Nasugunnee; Assam - Banar;
Malayalam - Naikuna; Panjabi - Kawach; Urdu - Konch.

Traditional medicinal uses of *M. pruriens*

Root: used to treat neurological disorders. Traditionally, root decoction is used for bloodpurification and diuresis.

Stem and Leaves: In the Ayurvedic medical system, the whole plant has been used to treatgout, diabetes, rheumatism, tuberculosis, cough andcancer (http://www. tropicalforages.info).

Seed: This is used as a CNC stimulant like coffee beans. It has multiple functions, such astreatment of Perkinson's disease importance, diuretics, worms, nerve tonic and aphrodisiac. Sometimes, it has antidepressant activity.

Toxicology: This plant does not have such a toxicological consensus, but it has somelimitations. Due to the presence of tryptamine and levodopa, it is toxic to humans andanimals. Trypsin enzyme activity has been inhibited by seeds. The undried leaves have ahairy substance that can

relieve itching. According to Ayurveda, the roots of *M. pruriens* are bitter, emollient, thermogenic, stimulant, aphrodisiac, laxative, diuretic, laxative, diuretic, insect repellent, antipyretic andtonic (Natarajan *et al.,* 2012). All parts of *M. pruriens* have valuable medicinal values inthe traditional medical systems of India and West Africa for example, the flowers, seeds and leaves of *M. Pruriens* are used to combat snake bites (Chikagwa *et al.,* 2009), it is also used as an aphrodisiac and uterine stimulant.In the *Mucuna pruriens* screening, there are alkaloids, anthraquinones, reducing sugars, flavonoids, tannins, cardiac glycosides, saponins, steroids and phenols (Minari, 2016). It is used to treat many diseases, such as urinary tract, nerve andmenstrual disorders, edema, fever, constipation, ulcers and tuberculosis (Katzenschlager *et al.,* 2004) and elephantiasis and other helminths (Oudhia, 2002). Traditionally, powdered seeds of *M. pruriens* have been found to increase the general mating behaviour of rats, thereby increasing the sexual activity of rats (Amin *et al.,* 1996).

Pharmacological properties of M*ucuna pruriens* (l.)

1. Antimicrobial activity

M. pruriens is also used to extract antibacterial properties of plant metabolites against phytopathogenic bacteria and fungi. The methanol extract shows high antibacterial activity against *Pseudomonas syringae, Xanthomonas brassica, Pseudomonas marginal, Pseudomonas aeruginosa,* and against, *Fusarium oxysporum, Rhodotorula solanacearum, Penicillium dilatatum* (Cauliflower, 2011). Aphrodisiac activity (Shukla and Mahdi, 2010) demonstrated that for men with decreased sperm count andmotility, oral administration of 5g *Mucuna pruriens* seed powder once a day can seminal fluid peroxide levels and improve mental stress, while increasing motility and sperm count.

2. Antioxidant Activity

Antioxidant activity in vitro tests showed that the whole plant ethyl acetate and *M. pruriens* methanol extract contain a large number of phenolic compounds showed high antioxidant and free radical scavenging activities. These plant extracts are an important source of natural antioxidants and may help prevent the progression of various oxidative stresses (Kumar and Muthu, 2010; Satheesh *et. al.,* 2010). The methanol extract of *M. pruriens* (MEMP) is measured in the presence of 1,1- diphenyl-2-picryl-hydrazyl radical. Ethyl acetate and MEMP plants contain a large number of phenolic compounds, which have high antioxidant and free radical scavenging activities (Kumar *et al.,* 2010).

3. Antiprotozoal effect

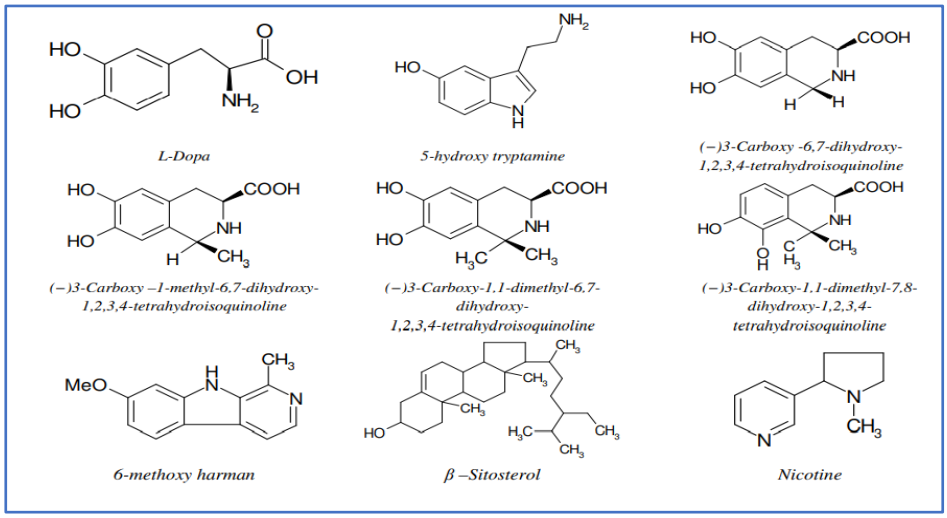
The methanol extract of *Mucuna pruriens* leaves treated in a 200mg/L plant extract bath can eradicate Lichthyophtirius infections (90%) in gold fish and significantly reduce fish mortality caused by parasites. In vitro studies have shown that the mortality rate of test parasites in 150mgL of *M. pruriens* extract is 100% (Ekanem *et al.*, 2004).

4. Antivenom activity

Fung *et. al.* (2010) studied the antivenom activity of seeds, in which *Naja sputatix* venom reduced the neuromuscular and inhibitory effects of Rat pre-treated with *M. pruriens* seeds.

Phytochemistry:

According to reports, the main component of the plant is L-dopa in the seeds (Bell, and Janzen, 1971; Daxenbichler, 1971). Alkaloid components (Mehta and Majumdar, 1944; Santra and Majumdar, 1953), namely prurienidine, prurienine, mucunadine, and mucunine, come from seeds (Majumdar, 1953). The numberof amino acids in this plant are reported (Pant *et al.*, 1974; Niranjan, and Katuyar, 1979). Epoxy fatty acids, namely cis12, 13-epoxyoctadec-trans-9-cis-acid, cis-12, 13-epoxyoctadectrans-9-enoic acid (Hasan *et al.*, 1980). According to Dr. Duke's phytochemical and ethnobotanical database, *Mucuna pruriens* contains many different phytochemicals, such as L-Dopa, 3-carboxy-1-methyl, 6,7-dihydroxy-1,2,3,4-tetrahydroiso Quinoline, 5-hydroxytryptamine, 6-methoxy harman,3-carboxy-1,1-dimethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline, β-Sitosterol, 3-carboxy-1, 1-dimethyl-7, 8-dihydroxy-1,2,3,4 tetrahydroisoquinoline and Nicotine. Recently, threenew lipid derivatives extracted from the n-hexane extract of Mucuna pruriens seeds havebeen reported, namely (Z)-Triactont-5,7,9-triene; (Z)-Docos-2,4,6 -trien-1,8-diol and (Z)-Docos-5-en-1-oleic acid (Misra, and Wagner, 2006). This plant is a source of minerals (Misra and Wagner, 1993) reported the isolation of four 1,2,3,4 tetrahydroisoquinolinealkaloids from seeds (Misra and Wagner, 2004).



Conclusion:

Mucuna pruriens L. used in traditional Ayurvedic Indian medicine for a long time to treat diseases. This plant has important medicinal properties and wide range of pharmacological activities.Due to presence of L-dopa, especially antimicrobial, antioxidant, antiprotozoal effect and antivenom activityas well as its traditional use in the treatment. Further evaluation is needed to explore the hidden and its practical applications for the beneficial of mankind.

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BIODIVERSITY OF CYANOBACTERIA OF SOME REGIONS OF AHMEDNAGAR DISTRICT OF MAHARASHTRA STATE, INDIA

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Introduction:

Cyanobacteria are morphologically diverse group of photo-synthetic prokaryotes, having unique cosmopolitan distribution. They found in extreme environments such as rocky shores, hot springs, drought, desiccation, osmotic salinity, etc. (Sinha and Häder, 1996; Zehr *et al*., 2000; Kalib, 2002; Saha *et al*., 2003). Cyanobacteria occupy a central position in global nutrient cycling especially due to their inherent capacity to fix atmospheric N_2 through nitrogenase enzyme (Sinha *et al*., 1995; 1997).

Until past few decades of research, cyanobacteria were of academic interests and were mostly ignored as nuisance but now are proved as potential organisms for much biotechnological utilization (Richmond, 1990; Sundararaman and Sekar, 2001; Thajuddin and Subramanian, 2005). Cyanobacteria are one of the potential groups of organisms, which are useful to mankind in various ways. Cyanobacteria constitute a vast potential resource in varied applications such as food, feed, fuel, fertilizer, medicine, industry and in combating pollution (Thajuddin and Subramanian, 2005).

Cyanobacteria, belonging to the order Chroococcales, and families Osillatoriaceae and Nostacaceae occur ordinarily as planktonic forms (Thajuddin and Subramanian, 2005). Cyanobacteria also occupy a variety of terrestrial environments. Soil is one of the most potential habitats for algal growth particularly in moist or waterlogged conditions. They play a significant role in maintaining soil fertility and in soil reclamation. Waterlogged rice field is ideal habitats for these cyanobacteria which are capable of nitrogen fixation .These include, species of *Anabaena*, *Aulosira*, *Calothrix*, *Cylindrospermum*, *Gloeocapsa*, *Nostoc*, *Rivularia*, *Scytonema* and *Tolypothrix* (Venkataraman, 1975).

Therefore, for several reasons, estimation and conservation of cyanobacterial biodiversity from yet unexplored habitats has become very important, which need to be initiated with

systematic survey followed by establishment of pure culture collection and their characterization. In present study efforts have been made to study the biodiversity of cyanobacteria of some regions of Ahmednagar district of Maharashtra state (India).

Materials and Methods:

Area of collection:

Ahmednagar district is one of the largest districts of Maharashtra State with an area of 17,035 sq. km (Ahmednagar Gazetteer) occupying more or less central part of the State. It is divided into 14 tehsils viz. Akole, Sangamner, Rahata, Shrirampur, Kopargaon, Newasa, Rahuri, Ahmednagar, Shevgaon, Pathardi, Jamkhed, Parner, Shrigonda and Karjat. The district is divided into Northern and Southern regions for the convenience. The Northern region is mainly of hilly Sahyadri ranges and low land areas, with the main rivers, Mula and Godavari and its tributary Pravara. This includes Kopargaon, Rahata, Akole, Sangamner, Shrirampur, Rahuri, Newasa, Shevgaon and Pathardi. This region is from Mula-Godavari-Pravara river basin. The Southern region includes Shrigonda, Karjat, Jamkhed, Parner and Ahmednagar. This region belongs to the Ghod-Bhima-Sina river basin (Pradhan and Singh, 1999).

In order to get maximum number of cyanobacterial species, field visits to different locations of Ahmednagar, districts were given in the month of July to September in the year 2021. The soil samples were collected from Pathardi, Shevgaon, Newasa, Rahuri, Rahata and Shrirampur tehsils of Ahmednagar district. Fresh biomass was collected from Bhandardara region of Ahmednagar district. For collection of samples, various locations with varied climatic conditions were purposefully selected to achieve the target of variation in the species. For collection of soil samples, irrigated area was preferred. The fresh biomass was collected from the rivers as well as from stagnant water of the various regions.

Method of collection:

The soil samples from 5-10 cm deep soil layers were collected using the scalpels. The scalpel was inserted into the soil and moved to make the circle and then lifted up with the soil. Random collections were made from different spots in a given locality in order to get the maximum collection. Soil samples were collected in polythene bags of size 6 x 4 inches. Each bag was tied with the help of rubber band and labeled. The information regarding location, habitat, date of collection, temperature and soil type was written on the labels at time of collection. The soil samples collected from well irrigated, occasionally irrigated and dry areas were preferred in order to get maximum diversity.

Fresh biomass from various places was also collected. For the collection of fresh biomass plastic bottles with screw caps were used. The fresh biomass was collected from the rivers and stagnant water bodies from different locations. For the water samples, locality water was also collected for the temporary storage of the samples. The sample bottles were labeled using the permanent marker. Other details of the collection sites were noted in the notebook at the time of collection.

The algae attached to the substratum were also collected. The attached algae were scrapped using the scalpels so as to get the attaching organs intact and stored in the polythene bags. These labeled plastic bags and bottles with collected biomass were brought to laboratory and used for isolation, identification and further experiments.

A total 2500 soil samples and different water samples were collected from the study area. The soil samples were collected from several crop fields, like rice, sugarcane, jowar, wheat, cotton, onion and fallow lands.

For the purpose of the identification of the collected algae, it is necessary to preserve a portion of the collection in some form. For this purpose, liquid preservation was made by immersing the portion of collected samples in 4% formalin, which is a general preservative and is good enough to make observations on gross morphology. The collected soil samples were shade dried and stored in the wide mouth screw cap glass bottles. These dry soil samples were used for the isolation of the unialgal cultures. For the water samples, the portion of the water samples was stored in the glass bottles in locality water. Remaining water samples were used for the isolation of the unialgal cultures after identification.

Identification of the algal samples:

For identification, micro-preparation of the algal samples was made and examination of the samples was done under the microscope. Morphometric studies were by using ocular and stage micrometer. The identification of the collected algal samples was carried out using monograph. The identification of the isolated taxa was carried out by following the monographs and keys of Prescott (1951), Desikachary (1959) and Anand (1990). The identified taxa were arranged as per the system of classification followed by Desikachary (1959). During identification, the recorded dimensions were compared to the descriptions of the algae given in the monograph and standard publications. Other field collected data which was recorded at the time of collection was also used for the correct identification.

After identification, the micro-preparations were subjected to the photomicrography. The microphotographs of all the identified algae were carried out using the Micron made trinocular research microscope at 40x and 100x magnifications.

Establishment of cultures:

1. Nutrient media

The different culture media namely BG-11 (Rippka *et al*., 1979); Fogg's medium 1949; Jacobson, 1951); Allen and Arnon's medium (Allen and Arnon, 1955); CFTRI medium (Venkataraman and Becker, 1984) and Zarrouk's medium (Zarrouk, 1966) were used for the rich growth of cyanobacterial species. These media were separately used in different sets.

2. Glass wares

All the glass wares used for the experiments were of the borosilicate glass. The glass wares were soaked overnight in water with liquid soap. All the glass wares were washed with tap water to remove the traces of liquid soap and placed in the hot air oven at 80^{o}C for 2 hours for drying.

3. Preparation of stock solutions for nutrient media

The nutrients and other chemicals used for preparation of stock solutions for different media were obtained from HI Media, Qualigens, E-Merck and Sigma Company. Glass distilled water was used for the preparation of stock solutions of nutrient constituents and for the preparation of the nutrient media. For preparation of stock solutions, the nutrient constituents are presented in. The desired quantity of nutrient constituents for 10X stocks were weighed accurately on Contech make single pan digital balance and dissolved in small quantity of distilled water individually. The final volume (100 mL) was made by adding distilled water in volumetric flask. From this stock solution 10 mL quantities required for preparation of 1000 mL medium. The stock solution of the micronutrient was made by adding 100 X concentration of each nutrient separately in small quantity of water and finally all the dissolved nutrients were mixed to make final volume of 100 mL with distilled water. From this stock solution 1 mL solution required for preparation of 1000 mL medium. All stock solutions were stored at 0-4^{o}C in refrigerator.

Preparation of culture media:

1. BG-11 (Rippka *et al.,* 1979)

The required stock solutions of nutrient components for BG-11 medium were added. The stock solutions of different minerals were added in distilled water (volume less by 100 mL than that of final volume). The pH of the medium was adjusted to 7.1 with 0.1 N NaOH or 0.1 N HCl.

2. Fogg's Medium (Fogg, 1949**;** Jacobson, 1951)

Required quantity of macro nutrients and micro nutrient stock solutions were added in distilled water (volume less by 100 mL than that of final volume). The stock of Fe-EDTA was prepared separately; 26.1 gm of EDTA in 268 ml of 1 N KOH was taken and 24.9 gm $FeSO_4$. $7H_2O$ was added to it and contents were dissolved by stirring. The solution was aerated for 16-18 hours and Fe-EDTA was stored in amber colored reagent bottle (Jacobson, 1951). The pH of the medium was adjusted to 7.5 by drop wise addition of 0.1 N NaOH or 0.1 N HCl.

3. Allen and Arnon medium (Allen and Arnon, 1955)

Allen and Arnon medium was prepared as described by Allen and Arnon (1955). The required stock solutions of nutrient components of Allen and Arnon medium were added. The stock solutions of different minerals were added in distilled water (volume less by 100 mL than that of final volume). The pH of the medium was adjusted to 8.2 with 0.1 N NaOH or 0.1 N HCl.

4. Zarrouk's medium (Zarrouk, 1966)

The required stock solutions of nutrient components of Zarrouk's medium were added. The stock solutions of different minerals were added in distilled water (volume less by 100 mL than that of final volume). The pH of the medium was adjusted to 9.2 with 0.1 N NaOH or 0.1 N HCl.

5. CFTRI medium (Venkataraman and Becker, 1984)

The required stock solutions of nutrient components of CFTRI medium were added. The stock solutions of different minerals were added in distilled water (volume less by 100 mL than that of final volume). The pH of the medium was adjusted to 10.0 with 0.1 N NaOH or 0.1 N HCl.

6. Preparation of solid medium

By using the above mentioned liquid media, solid media were prepared by adding 2 % agar-agar (HiMedia, Mumbai, India) powder. The flask was kept in microwave oven to digest the agar.

7. Sterilization of media, glass wares and equipments

The plugged culture tubes containing medium was wrapped in a paper to prevent wetting of plugs due to condensation of steam during and after autoclaving. The medium was sterilized at 105 kPa pressure and 121^{o}C for 20 minutes. The required glass wares and equipments such as scalpels, forceps, scissors, petri dishes, and surgical blade holders were wrapped in the paper and sterilized in autoclave. Distilled water was also sterilized in the conical flask.

8. Inoculation

All the operations were carried out under laminar airflow fitted with ultra violet (UV) tube and HEPA filter. The sterilized culture tubes, flasks, petri dishes, forceps and surgical blades were kept in the laminar air flow and exposed to UV for 30 minutes to sterilize surfaces of all glassware and instruments prior to transfer operation.

9. Culture conditions

All the cultures were maintained in the culture room at temperature 28±2°C under 8-h light/16-h dark photoperiod with a photosynthetic photon flux density of 40 μmoles^{-2}S^{-1} provided by cool white fluorescent tube lights (Philips, India).

Isolation of cyanobacterial species:

The dry soil samples were spread in petri dishes and moistened with sterilized distilled water and cultures were incubated in light. When the visible growth of algae begins to appear in the cultures, these cultures were used for the isolation of unialgal cultures. Initial screening of algal samples was carried out which were found in the moistened soil under the microscope and then the filamentous forms were selected for the isolation of unialgal cultures.

For isolation, small amount of the algal material was picked with the help of nichrome loop and transferred to the test tubes containing sterile distilled water. The tube was shaken, this helps to break the clumps and separate the filaments from each other. This suspension was serially diluted and the drop of the suspension was placed at the one end of the agar plates and slants containing different media as described above and the liquid was drawn over the agar surface. The same suspension was used for streak plate. The loop full suspension was used for the streaking on the agar surface. After inoculation the cultures were incubated at controlled conditions as described above.

For isolation of algae from the collected water samples, the water samples were serially diluted and the diluted suspension was placed at one end of the agar plate and the same procedure was followed for the isolation as for the soil suspension. The loop full of suspension was used for streaking on the agar plate. After inoculation the cultures were incubated at controlled conditions as described above.

On incubation for about two weeks the growth was observed on the agar surface. The successive transfer to the liquid medium was carried out in the test tubes and again drop of the suspension from these test tubes was placed at the one end of the agar plate and liquid was drawn over the agar. The filamentous forms exhibited radial spreading growth on the agar surface. The

filaments at the peripheral portions were picked up with the nichrome loop and washed in the sterilized distilled water. Then the filaments were inoculated in the liquid medium. This procedure was repeated still the unialgal cultures were established. The cultures were incubated at the above mentioned controlled conditions.

After one month of incubation the cultures were observed under the research microscope to check the contamination of the other algal forms. Then the unialgal cultures were transferred to the culture bottles with 50 mL of the liquid culture media.

Isolation of bacteria free cultures:

For isolation of bacteria free cultures, the filaments were washed in the sterile distilled water and then transferred to the liquid media supplemented with antibiotics alone or in combination 2 or 3 different antibiotics (cefotaxime, kanamycin, penicillin or ampicillin) with different concentrations for 10-20 minutes. Then the filaments were again washed with the sterile distilled water for 30 minutes and then transferred to the agar plates or culture bottles containing liquid media.

Results and Discussion:

Cyanobacterial flora of study area

Total 2500 samples were collected from various localities of the Ahmednagar district. In all, 54 blue green algal species belonging to 26 genera were identified. Their taxonomical characterization was made by using standard literature following Desikachary (1959). Patil and Satav (1986) reported 66 blue-green algal species from Western Maharashtra. Shinde (1995) reported 21 blue-green algae from soils of Pravaranagar area (Maharashtra). Following are the different genera of cyanobacteria found in various samples of soil and fresh biomass collected from different locations.

1) *Anabaena* sp.; 2) *Nostoc* sp.; 3) *Oscillatoria* sp.; 4) *Westiellopsis* sp.; 5) *Phormidium* sp.; 6) *Hapalosiphon* sp.; 7) *Scytonema* sp.; 8) *Lyngbya* sp.; 9) *Calothrix* sp.; 10) *Microchaete* sp.; 11) *Cylindrospermun* sp.; 12) *Tolypothrix* sp.; 13) *Anabaenopsis* sp.; 14) *Rivularia* sp.; 15) *Stigonema* sp.; 16) *Spirulina* sp.; 17) *Microcoleus* sp.; 18) *Westiella* sp.; 19) *Camptylonema* sp.; 20) *Nostochopsis* sp.; 21) *Scytonematopsis* sp., 22) *Aulosira* sp.; 23) *Chroococcus* sp.; 24) *Gloeocaspa* sp.; 25) *Aphanocapsa* sp.; 26) *Aphanothece* sp.

Table 1: Percentage of cyanobacterial species of soil samples of Ahmednagar district

Sr. No.	Name of Cyanobacterial species	Total no. of soil samples in which sp. occurred	Percentage of cyanobacterial species
1	*Anabaena* sp.	1236	49.44
2	*Nostoc* sp.	978	39.12
3	*Oscillatoria* sp.	1025	41
4	*Westiellopsis* sp.	1356	54.24
5	*Phormidium* sp.	1012	40.48
6	*Hopalosiphon* sp.	530	21.2
7	*Scytonema* sp.	645	25.8
8	*Lyngbya* sp.	836	33.44
9	*Calothrix* sp.	917	36.68
10	*Microchaete* sp.	421	16.84
11	*Cylindrospermum* sp.	280	11.2
12	*Tolypothrix* sp.	640	25.6
13	*Anabaenopsis* sp.	475	19
14	*Rivularia* sp.	230	9.2
15	*Stigonema* sp.	460	18.4
16	*Spirulina* sp.	295	11.8
17	*Microcoleus* sp.	436	17.44
18	Westiella sp.	153	6.12
19	*Camptylonema* sp.	174	6.96
20	*Nostochopsis* sp.	193	7.72
21	*Sctonematopsis* sp.	278	11.12
22	*Aulosira* sp.	267	10.68
23	*Chroococcus* sp.	134	5.36
24	*Gloeocapsa* sp.	193	7.72
25	*Aphanocapsa* sp.	276	11.04
26	*Aphanothece* sp.	167	6.68

After the collection of algal samples from study area, it was noticed that Ahmednagar region is rich in cyanobacterial flora. Among all the species found, *Westiellopsis prolifica* is the

dominant with 54.24% abundance followed by *Anabaena* (49.44%), *Oscillatoria* (41%) and *Phormidium* (40.48%). The dominance of these species might be due to their tolerance to salinity (Jha *et al*., 1987). The blue-green algal genera like *Cylindrospermum, Nostoc, Anabaena, Wollea, Aulosira, Scytonema, Nostochopsis, Plectonema, Calothrix, Hapalosiphon* and *Westiellopsis* were investigated from salt affected soils of Uttar Pradesh and Maharashtra (Singh, 1961; Madane and Shinde, 1993; Shinde, 1995). In the present study, cyanobacterial population in the water samples collected was very less. Species of cyanobacteria were commonly found in moist soil.

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MEDICINAL PLANTS: THEIR ROLE IN INVIGORATING LIFE

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Introduction:

The natural merchandise now-a-days symbolise protection in assessment to the synthetics that are appeared as risky to human and environment. Although herbs have been priced for their medicinal, flavouring and fragranttraits for centuries, the artificial merchandise of the present-day age exceeded their importance, for a while. However, the blind dependence on synthetics is over and those are returning to the naturals with desire of protection and security. Out Of the 2,50,000 better plant species on earth, greater than 80,000 are medicinal. India is one of the world's 12 biodiversity centres with the presence of over 45000 exceptional plant species. India's variety is unrivalled because of the presence of sixteen xceptional agro-climatic zones, 10 plant life zones, 25 biotic provinces and 426 biomes (habitats of precise species). Of these, approximately 15000-20000 vegetation has suitable medicinal value. The Ayurveda gadget of medication makes use of approximately seven-hundred species, Unani seven-hundred, Siddha six hundred, Amchisix hundred and current medication round 30 species. The tablets are derived both from the complete plant and from one-of-a-kind organs, like leaves, stem, bark, root, flower, seed, etc. Some tablets are organized from excretory plant product which includes gum, resins and latex. Even the Allopathic gadget of medication has followed some of plant-derived tablets (Table: medicinal florautilized incurrent medication) which shape a vital phase of the current pharmacopoeia. Some vital chemical intermediates wished for production the current tablets also are acquired from flora (Eg. diosgenin, solasodine, b-ionone). Not only, has that plant-derived drug given a strong market place global wide, however additionally flora stay a vital supply for brand new tablets.

Ayurveda, Siddha, Unani and Folk (tribal) drug treatments are the foremost structures of indigenous drug treatments. Among those structures, Ayurveda is maximum advanced and extensively practised in India. Ayurveda courting returned to 1500-800 BC has been an indispensable a part of Indian culture. The time period comes from the Sanskrit root Au (existence) and Veda (knowledge). As the name implies it isn't always best the technology of

remedy of the unwell however covers the complete gamut of happy human existence regarding the physical, metaphysical and the religious aspects. Ayurveda recognises that except a stability of frame factors one has to have an enlightened country of consciousness, feel organs and thoughts if one must be flawlessly healthy. Ayurveda with the aid of using and huge is a revel in with nature and not like in Western medication, among the ideas eludes medical explanation. Ayurveda is gaining prominence because the herbal gadget of fitness cares all over the world. Today this gadget of drugs is being practised in international locations like Nepal, Bhutan, Sri Lanka, Bangladesh and Pakistan, even as the conventional gadget of drugs in the different international locations like Tibet, Mongolia and Thailand look like derived from Ayurveda. Phytomedicines also are getting used more and more and more in Western Europe. Recently America A Government has installed the "Office of Alternative Medicine" on the National Institute of Health at Bethesda and its aid to opportunity medication consists of primary and carried out studies in conventional structures of drug treatments which include Chinese, Ayurvedic, etc. with a view to investigate the feasible integration of powerful remedies with contemporary-day drug treatments.

Green vegetation synthesises and maintains numerous biochemical products, many of which can be extractable and used as chemical feed shares or as uncooked fabric for numerous clinical investigations. Many secondary metabolites of plant are commercially important and locate use in some of pharmaceutical compounds. However, a sustained deliver of the supply fabric regularly will become tough because of the elements like environmental changes, cultural practices, numerous geographical distributions, labour cost, choice of the superior plant inventory and over exploitation through pharmaceutical industry.

The small fractions of flowering flora which have to this point been investigated have yielded approximately a hundred and twenty healing dealers of recognized shape from approximately ninety species of flora. Some of the beneficial plant pills encompass vinblastine, vincristine, taxol, podophyllotoxin, camptothecin, digitoxigenin, gitoxigenin, digoxigenin, tubocurarine, morphine, codeine, aspirin, atropine, pilocarpine, capscicine, allicin, curcumin, artemisinin and ephedrine among others. In a few cases, the crude extract of medicinal flora can be used as medicaments. On the alternative hand, the isolation and identity of the lively concepts and elucidation of the mechanism of movement of a drug is of paramount importance. Hence, works in each aggregate of conventional medicinal drug and unmarried lively compounds are very important. Where the lively molecule can't be synthesised economically, the product need to be acquired from the cultivation of plant material.

Quality control requirement of new preparation of traditional medicines-

Quality manipulates requirement of recente ducation of conventional medicines- Quality manages requirement of new steering of traditional medicines

1. Prescription and its basis
2. Literature and research information of physico-chemical characteristic involved with remarkable
3. Preparation generation and its research references
4. The draft of the remarkable favoured and clarification of medicinal fabric, and medicament.
5. Literature and test information of initial stability for scientific research
6. The opinions of remarkable detection and hygiene favoured detection of the steering for scientific research
7. Property and specification of the packing fabric of the medicament, format draft of the label and accomplished instructions.

The standard scheme for pleasant evaluation of botanicals - The standard scheme for pleasant evaluation of botanicals as advised through Kraisintu (1997) is as follows:

I. Assessment of crude plant materials

1. General description of the plant
2. Parts used
3. Production of crude drugs-cultivation, harvesting, post-harvest handling, packing, storage.
4. Quality specification: Chemical or chromatographic identity, overseas natural count limit, ash content, acid insoluble ash content, water soluble extractive, alcohol soluble extract, moisture content, lively constituent content, microbial limit, pesticide residue limit, heavy steel limit, probable contaminants, adulterants.

II. Assessment of completed products

1. Tablets: Weight variation, disintegration time, identity of preservatives and lively ingredients, dedication of extractives in diverse solvents, microbial limit, heavy metals.
2. Solutions: pH, identity of preservatives and lively ingredients, alcohol content, microbial limit, Sodium Saccharic content.
3. Infusions: Weight variations, identity of preservatives and lively ingredients, dedication of extractives in diverse solvents, microbial limit, heavy metals, Borax.

III. Chemical Standardisation strategies: TLC/HPTLC, HPLC, GLC, FTIR

IV. Chemical Markers: Specification for uncooked materials, pleasantguarantee in proceduremanipulate, standardisation of product, acquiringbalance profiles, unmarried marker vs. fingerprint.

V. Parameters of assay validation: Linearity, limits of quantification and detection, precision, robustness, recovery. Complex and variable mixtures, desire of compounds to quantify, tough pattern education, loss ofnatural reference requirements, loss of strategies with ok tolerances through analytical chemistry requirements are a number of the demanding situations in Chemical Standardisation of plant drugs. International scheme for pleasant guarantee of pharmaceutics: International scheme for pleasant guarantee of pharmaceutics entailsthe subsequent trendy practices. GAP: Good Agricultural Practice GLP: Good Laboratory Practice GMP: Good Manufacturing Practice GCP: Good Clinical Practice GALP: Good Analytical/Automated Laboratory Practice.

International scheme for quality assurance of pharmaceutics:

International scheme for quality assurance of pharmaceutics involves the following standard practices.

GAP: Good Agricultural Practice

GLP: Good Laboratory Practice

GMP: Good Manufacturing Practice

GCP: Good Clinical Practice

GALP: Good Analytical/Automated Laboratory Practice.

Quality must be constructed into the complete system starting from the choice of propagation cloth to the very last product accomplishing the consumer. It is consequently a control gadget wherein all steps worried withinside the business utilisation system should be nicely and strictly managed to supply the favoured best merchandise. The necessities for ISO 9000 certification should be brought and employees educated in order that organizations may want to introduce the right structures wanted for certification. The manager of the best of the uncooked substances, completed merchandise and of techniques is an absolute necessity, if one is to supply items for global markets and human use. Monographs should be organized for every product to encompass all specs developed. Modern analytical strategies should be significantly used to increase identification and best parameters. The equipment and techniques utilized in industries should be demonstrated to conform to global requirements. It is vital that the processed merchandise observe countrywide and/or global specification. There are International Standard Organisation Specification (ISO) for a lot of the goods. In addition, nations and customers will have their very own necessities. Hence the goods will be tailor made to comply to

the customers' necessities. Sometimes the necessities of the customers are greater stringent and specific, stressful the software of excellent production techniques. Associated with best control is the compliance with modern excellent production practices. WHO necessities of excellent production practices should be brought in each undertaking as maximum growing nations fall very brief of GMP. Without GMP merchandise cannot be predicted to be of required requirements and best. The idea of protection is nearly non-existent in lots of growing nations. Safety necessities with recognize to buildings, equipment and team of workers should be brought and if possible, protection manuals should be organized so that it will recognition the eye of the control and team of workers on those issues. Stringent necessities are being brought currently to protect the surroundings, to lessen pollutants because of use of artificial substances and to preserve the biodiversity. Hence eco-audit techniques might be required for shielding environmental damage. Organic manufacturing will lessen the dangers of infection of merchandise and the surroundings with artificial chemicals. In reality ISO 14000 necessities may also should be met withinside the destiny if the customers insist on eco-labelling.

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ANTICANCER PROPERTIES OF PLANTS USED IN *'DASHAMOOLA'* – AN AYURVEDIC COMBINATION OF TEN PLANTS

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Abstract:

Cancer research is still progressing with probes for novel drugs which precisely targets cancer cells. Plant-derived drugs such as Taxol, Camptothecin, Vinca alkaloids contributed a lot to cancer therapy. More comprehensive investigations for better phyto formulations against cancer are a need today. A variety of incredible plant formulations are available in Ayurvedic tradition, which solicits immense consideration. The plants used in Ayurvedic practices are of enormous medicinal value, so they should be thoroughly evaluated to understand the potential of the same against various diseases. Dashamoola or Dashamoolam is one of the most valuable phyto formulations derived from the dried roots of ten plants. In this review, the anti-cancer properties of the individual plants in the category of Dashamoola are described. All the plants have reported anti-cancer properties against various cell lines.

Keywords: Dashamoolam; Anti-cancer drugs; *Aegle marmelos* Corr.; *Premna integrifolia* Linn.; *Oroxylum indicum* Vent.; *Gmelina arborea* Linn.; *Stereospermum suaveolens* DC.; *Desmodium gangeticum* (L.) DC.; *Uraria picta (Jacq.) DC*; *Solanum xanthocarpum* Schrad & Wendl.; *Solanum indicum* Linn.; *Tribulus terrestris* Linn**.**

Introduction:

Cancer is still a significant threat to the highly advanced human population, a leading cause of death. Nearly 10 million deaths were reported in the last year due to cancer (https://www.who.int/news-room/fact-sheets/detail/cancer.). According to the records, approximately 1.4 million people from India get diagnosed with cancer every year (Jain *et al.*, 2019). The alarming rates make this situation a scenario of 'unbelievable growth of our cells itself'! The scientific realm is constantly searching for an efficient drug that specifically targets the cause and not detrimental to healthy cells. So many plant-derived drugs and semi-synthetic

analogs are available as standard pharmacophores against cancer such as Taxol (Slichenmyer and Von Hoff, 1991), Camptothecin (Wall and Wani, 1996), and Vinca alkaloids (Moudi *et al.,* 2013). Hence plant resources have to be thoroughly studied for developing more efficient drugs.

The Historical and Cultural roots of Ayurveda connect the intimate relationship between humans and nature. The belief "Entire universe and the human body are one" is the principle of Ayurveda (Jaiswal and Williams, 2017). The Sanskrit word "Ayurveda" originates from Ayu (life) and Veda (Knowledge), known as Science of Life (Mathpati, 2020). The use of numerous precious plants is described in Ayurvedic scripts. Screening of those plants for their medicinal property can contribute a lot in the field of anticancer therapy also.

It has been reported that antioxidants scavenge the free radicals can act as good anti-cancer agents (Lopaczynski and Zeisel, 2001). A variety of incredible formulations are available in our Ayurveda which has antioxidant and free scavenging activity seeks immense attention. In this review, *Dashamoola* plants are analyzed for their antitumor properties.

***Dhashamoola:* A formulation of roots of ten plants**

Dashamoola or Dashamoolam is the most incredible phyto formulation derived from dried roots of ten plants. The root word 'Dasha' means ten and, 'Moolam' means the root is a Sanskrit terminology. The magical concoction of this formulation resolves various disease conditions especially related to nerves, muscles, bone, joints, and lungs. The ten plants have their unique properties to derive an efficient formulation. These plants are categorized into two groups;-

1. Brihat Panchamoola -obtained from large trees
2. Laghu Panchamoola -obtained from smaller shrubs

The plants including Bilva, Agnimantha, Kashmiri, Shyonaka, and Patala are known as BrihatPanchamoola and, the LaghuPanchamoola includes Saivan, Kantakari, Shalaparni, Prishniparni, and Gokhru (Pandey, 1998) (Table 1).

***Aegle marmelos* Corr.**

Aegle marmelos plant has a significant ethnomedical and ethological importance among indigenous people of India (Dutta *et al.,* 2014).

Petroleum ether fraction of the stem bark can repress in-vitro proliferation of the leukemic K562, T-lymphoid Jurkat, B- lymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDA- MB-231 cell lines (ampronti *et al.,* 2003*).* The cytotoxicity of *A. marmelos* essential oil was studied against four cancer cell lines such as human pancreatic (PSN-1), colon (LoVo), lung (H157), and ovarian (2008) cancer cells, and obtained IC50 of 5.6 µg/ml, 6.5 µg/ml, 6.7 µg/ml and 2.3 µg/ml, respectively(Pant *et al.,* 2019). The aqueous extract

of fruit pulp inhibited MCF-7 cell lies with a IC50 value of 47.92 µg/mL concentration (SP *et al.,* 2018).

Table 1: Plants included in 'Dashamoola'

Sanskrit Name	Common Name	Botanical Name	Family	Parts Used in Ayurvedic formulations
Bilva	Indian Bael	*Aegle marmelos* Corr.	Rutaceae	Root, Bark, Leaves, Fruits, Root-bark.
Agnimantha	Agnimantha	*Premna integrifolia* Linn.	Verbenaceae	Root, Bark, leaves, roots bark.
Syonaka	Broken bones tree, Indian Trumpet Flower	*Oroxylum indicum* Vent.	Bignoniaceae	Root, seeds, latex, seeds oil
Kasmari	Beechwood, Gmelina, Goomar teak, Kashmir tree	*Gmelina arborea* Linn.	Verbanaceae	roots, stem, stem bark, fruits, leaves, flowers
Patala	Patala	*Stereospermum suaveolens* DC.	Bignoniaceae	Root bark, bark, flowers, seeds, leaves.
Saliparni	salparni	*Desmodium gangeticum* (L.) DC.	Fabaceae (papilionaceae)	Roots, whole plant.
Kantakari	Yellow Berried Nightshade	*Solanum xanthocarpum*Schrad&Wendl.	Solanaceae	Whole plant, roots
Brhati	European black nightshade, Black nightshade	*Solanum indicum* Linn.	Solanaceae	Roots, Fruits, Flowers, Leaves
Goksura	Bindii	*Tribulus terrestris* Linn.	Zygophyllaceae	Fruits, Roots, Whole plant

Gupta *et al*. (2016) analyzed the effect of *A.marmelos* bark extract on DMBA and croton oil-induced papilloma genesis in Swiss albino mice, and Agrawal *et al*. (2011) evaluated the chemopreventive potential of the *Aegle marmelos* fruit hydroalcoholic extract on mouse skin

tumorigenesis initiated by DMBA and promoted by croton oil. In both cases, a significant reduction in tumor incidence, tumor burden, tumor multiplicity, and the cumulative number of papillomas, along with a significant increase in the average latent period observed (Agrawal *et al.,* 2011; Gupta *et al.,* 2016). Treatment with 50% ethanolic extract of *A. marmelos* leaves on Dalton's Lymphoma Ascites (DLA)-bearing mice increased their mean survival time and life span at the same time reduced tumor volume and packed cell volume (Chockalingam *et al.,* 2012). Jagetia *et al.* (2005) observed the same result in Ehrlich's ascites carcinoma bearing mice. George *et al.* (2014) stated the antitumor properties of petroleum ether extract against Dalton's lymphoma ascites, and Ehrlich's ascites carcinoma.

Hydroalcoholic extract of leaves showed a decrease in decreased IL-1b, IL-6, Bcl-2, and c-jun and an increase in p53 and IL-4 expression in N-methyl N-nitrosourea induced hepatocarcinogenesis in Balb/c mice (Verma *et al.,* 2013).

Gangadevi and Muthumary (2008) isolated taxol producing endophytic fungus *Bartaliniarobillardoides* from *A. marmelos* and proved the cytotoxic effect of fungal taxol against various cancer cells viz., human breast cell BT220, human colon H116, human intestine Int407, human lung HL251, and human leukemia HLK 210.

Subramaniam *et al.* (2008) isolated and characterized 1-hydroxy-5,7-dimethoxy-2-naphthalene-carboxaldehyde from ethyl acetate fraction of *A.marmelos* and proved its inhibition on HCT-116 colon cancer cell tumorxenografts and angiogenesis in nude mice through activation of tumor necrosis factor-A (TNF-A), TNF receptor (TNFR)- associated death domain (TRADD), and caspases.

Vijayakumar (2019) synthesized gold nanoparticles from an aqueous extract of fruit pulp and proved its inhibition on MCF-7 cell lines.

***Premna integrifolia* Linn.**

Sridharan et al. (2011) assessed the anti-tumor effect of *Prema integrifolia* on Ehrlich Ascites Carcinoma cell lines and observed a decrease in the Ascites fluid volume, packed cell volume, and viable cell counts and an increase in the mean survival time of tumor-bearing animals. Silver nanoparticles synthesized from aqueous leaf extract caused cytotoxicity against a cervical cancer cell line (SiHa) (Singh *et al.,* 2019).

***Oroxylum indicum* Vent.**

Decoctions prepared using the stem bark of *O. indicum* has been used to cure nasopharyngeal cancer by Tangkhul Naga tribes of Northern Manipur (Mao,2002). Lambertini *et al.* (2004) reported its antiproliferative effect on two breast cancer cell lines MCF-7 and MDA-MB 231 by performing Trypan Blue exclusion method. Petroleum ether hot extract of stem bark

manifested cytotoxicity of MDA-MB 231 cell lines in a concentration-dependent manner (Kumar *et al.,* 2012). Moirangthem *et al.* (2013) reported the cytotoxic potential of petroleum ether extract of o.indicum on HeLa cell lines.

Four flavonoids, viz. Baicalein, Baicalein-7-O-glucoside, Chrysin, and Baicalin are the major phytochemical constituents identified from the seeds of the plant (Chen *et al.,* 2005; Chen *et al.,* 2003). Baicalein, one of the important flavonoids was reported to inhibit the proliferation of human colorectal cell line CT-26 (Lalou *et al., 2013)* and Leukemic cell line HL-60 by inducing apoptosis and cell cycle arrest in the G2 and S phase of the cell cycle (Roy *et al., 2007).* Chen *et al.* (2001) evaluated the effect of baicalein and baicalin in human prostate cancer cell lines LNCaP and JCA-1 and observed that the compounds inhibit cell proliferation, induce apoptosis, and arrest cell cycle at the G1 phase. Majumdar *et al.* (2010) identified flavonoid compounds such as Baicalein, Oroxylin A, and Chrysin asproproteinfurin inhibitors.

Nagasaka *et al.* (2018) isolated Chrysin, from the ethanolic extract of stem bark and studied its role in transcriptional regulation of the p 53 gene through the luciferase assay system. It was found that treatment with chrysinupregulated p53 protein levels and reduced cell viability of the MCF 7 cell line. They also discovered chrysin as a non-canonical inducer of the ATM-Chk2 pathway even in the absence of DNA damage. Weak cytotoxic activity against A549, HepG2, and SW480 human cancer cell lines were reported with chrysin with IC50 values of 40.88 ± 3.85, 50.55 ± 2.59, and 91.60 ± 4.27 μM, respectively (Wu *et al., 2019).*

***Gmelina arborea* Linn.**

Cytotoxic effect of ethanolic leaf extracts of *Gmelina arborea* was tested against Colon cancer (COLO 201), Gastric cancer (HT- 29), and Human oesophageal cancer (TE-2) cell lines using MTT assay and a prominent inhibitory effect with IC 50 values 20±0.15 mg/ml, 12±0.32 mg/ml and 16±0.05mg/ml were observed (Punitha *et al., 2012).*

N'gaman *et al.* (2014) analyzed the effect of hydroacetonic crude extract obtained from *Gmelina arborea* leaves against six cancer cell-lines such as MDA-MB 231, MDA-MB 435, B16F10, Caco-2, C6, and SNB75 and they showed strong inhibition with IC50 (mg/ml) values 0.246,0.379, 0.246, 0.250, 0.304 and 0.404 respectively. Ghareeb *et al.* (2014) isolated and characterized seven flavonoid compounds from *G.arborea* leaf methanolic extract and studied their cytotoxic effect towards liver-carcinoma cell line (HepG-2) via Sulphorhodamine-B assay. Three compounds of that (compound 1,2 and 7) showed cytotoxic activity with IC = 3.38, 8.98, and 15.70 μg/ml respectively.

Anti-tumor properties of methanolic extract of stem bark against 7,12-dimethylbenz(a) anthracene (DMBA)-croton oil-induced skin tumorigenesis in male BALB/c mice were evaluated and a significant reduction in the number of papillomas and delayed onset of papilloma development was observed (Lawrence, *et al.,* 2016).

***Stereospermum suaveolens* DC.**

Silver and gold nanoparticles from aqueous root bark extract have antiproliferative effects on human lung adenocarcinoma cells A549 with IC50 values 33.81 ± 0.72 and 52.97 ± 0.73 µg/mL (Francis *et al.,* 2018).

***Desmodium gangeticum* (L.) DC.**

Srivastava *et al. (*2013) identified 2(hydroxymethyl) phenyl hexopyranoside, commonly known as Salicin in methanolic leaf extract of *Desmodium gangeticum*. The anticancer properties of the plant have mainly been associated with the presence of the same.

High levels of prostaglandins are associated with many tumors including gastric carcinoma. Salicin, conventionally extracted from willow bark tree is a compound capable of inhibiting cyclooxygenase enzymes COX 1 and COX 2 involved in prostaglandin synthesis, like that of aspirin, but with lesser side effects (Husain *et al.,* 2001; Sabaa *et al.,* 2017).

In-vivo studies in Ehrlich ascites carcinoma bearing Swiss albino mice found that treatment with salicin extracted from *D.gangeticum*, at a dose of 100 and 200 mg/kg body weight, reduced the tumor volume, tumor weight, viable tumor cell count, and increased the life span of mice (Srivastava *et al.,* 2013; Srivasthava *et al.,* 2015).

Uraria picta (Jacq.) DC

In-vitro studies in A549 human lung cancer cell lines with methanolic extract of *Urariapicata* proved a significant decrease in cell viability and increase in apoptosis. In-vivo studies with tumor-bearing Immunocompromised C57BL6 mice rats showed a reduction in tumor volume in a dose-dependent manner (Chipa, *et al.,* 2021)

***Solanum xanthocarpum* Schrad&Wendl.**

Bhutani *et al.* (2010) extracted two alkaloids solamargine and solasonine from *S. xanthocarpum* and analyzed its cytotoxicity on HCT 116 human colon cancer cell line and both the compounds were strongly cytotoxic in nature.

Gold nanoparticles synthesized from aqueous leaf extract of *S. xanthocarpum* induced apoptosis of C666-1 nasopharyngeal cancer cell line (P. Zhang *et al.,* 2018). Different fruit extracts showed cytotoxicity towards lung cancer cell line -HOP-62 and leukemic cell line THP-1 (Kumar and Pandey, 2014).

***Solanum indicum* Linn.**

It has been reported that methanolic extract of *S. indicum* has antitumor properties against human non-small lung carcinoma (H1975), prostate carcinoma (PC-3 and DU145), colorectal carcinoma (HCT116), and malignant melan (A375) with IC 50 values 9.03 µg/ml, 8.48 µg/ml, 11.18 µg/ml, 17.58 µg/ml, 27.94 µg/ml respectively(W. J. Syu *et al.,* 2001).

Chiang *et al. (*1991) fractionated and characterized five compounds from ethanolic extract of *S. indicum* and proved its cytotoxicity against Colo-205 (colon), KB (nasopharynx), HeLa (uterine cervix), HA22T (hepatoma), Hep-2 (laryngeal epidermoid), GBM8401/TSGH (glioma) and H1477 (melanoma) cell lines.

***Tribulus terrestris* Linn.**

The aqueous extract of *Tribulus terrestris* fruits inhibited cell proliferation in human liver cancer HepG2 cells in an incubation time and concentration-dependent manner through inducing apoptosis, G0/G1 cell cycle arrest, and inhibited NF-κB activity (Kim *et al., 2011).*

Methanolic and saponin extracts from leaves and seeds of *T.terrestris* were used to assess the cytotoxic and apoptosis potential towards MCF7 breast cancer cell lines and reported that cytotoxicity towards MCF -7 occurs through the intrinsic apoptotic pathway by upregulating the expression of Bax and p53 genes and downregulating the expression of Bcl-2. Induction of extrinsic apoptotic pathway by upregulating FADD, AIF, and caspase 8 genes were also found (Patel *et al.,* 2019). Angelova *et al.* (2013) also studied the effect of ethanolic and saponin extracts from leaves towards the MCF-7 breast cancer cell line and also observed anti-cancer effects in a dose-dependent manner. At the same time treatment with a non-cancerous cell line, MCF10A didn't show any dose-dependent effect. Ethanolic fruit extract of *T. terrestris* also reported as a potent Topoisomerase II inhibitor thus can be used in cancer therapy (Kumar *et al.,* 2011).

Aqueous extracts of the root and fruit of *T. terrestris* have tested for their chemopreventive potential in DMBA induced papilloma genesis in male Swiss albino mice and observed a significant reduction in tumor volume, tumor incidence, and the cumulative number of papillomas observed (Kumar *et al.,* 2006). It has been also reported that methanolic extract of the whole plant has cytotoxicity towards rat kidney proximal tubular epithelial cell line (NRK-52E) with IC 50 value 160 µg/mL (Abudayyak *et al.,* 2015).

Goranova *et al.* (2015) studied the mechanism by which saponins isolated from Tribulusterestris extract show antitumor activity against MCF 7 breast carcinoma cell lines, and

observed a reduction CXCR4 and CCR7 genes which are involved in metastasis progression and decrease in BCL 2 expression which is involved in apoptosis regulation.

Célia Cristina *et al.* (2021) prepared standard extract and saponin enriched extract from T.terrestris and evaluated its anti-tumor activity towards different cell lines such as glioblastoma (U251), melanoma (UACC-62), breast cancer (MCF7), doxorubicin-resistant high-grade ovarian serous adenocarcinoma (NCI-ADR/RES), renal cell carcinoma (786-0), large cell lung carcinoma (NCI-H460), high-grade ovarian serous adenocarcinoma (OVCAR-03), rectosigmoid adenocarcinoma (HT-29) and chronic myelogenousleukemia (K562). Both the extracts had a promising anti-tumor effect on all cell lines, especially saponin enriched extract with the highest activity towards kidney (786-O) cell line with GI50 of 2.91 µg/ml.

Cytotoxicity of methanolic fruit extract of *Tribulus terrestris* was evaluated in Dalton's Lymphoma ascites (DLA) and Ehrlich's Ascites Carcinoma (EAC) cells, a dose-dependent inhibition was observed with (IC50) values 380 ± 2.12 and 420 ± 5.43 µg/ml for DLA and EAC cells, respectively. In-vivo studies with Swiss albino mice also showed considerable anti-tumor activities (D. M. K *et al.,* 2014).

Conclusion:

Dahamoolam plants have immense medicinal value against various diseases. All plants have an inhibitory effect on various cell lines. Among the ten plants, *Aegle marmelos and Tribulus terrestris* are broadly studied against different cell lines both in-vitro and in-vivo. Secondary metabolites of plants can contribute a lot to their inhibitory effect. Secondary metabolites from *Oroxylum indicum*, especially Baicalein, Baicalein-7-O-glucoside, Chrysin, and Baicalin, contribute to its antiproliferative effects. Anti-cancer properties of *Desmodium gangeticum* are mainly due to the presence of Salicin, a secondary metabolite. Two alkaloids solamargine and solasonine from *S. xanthocarpum* imparts anti-proliferative potential to it. *Steriospermum suaveolens, Uraria picta, Solanum xanthocarpum, Solanum indicum*, and *Premna integrifolia* are comparatively less examined for their anticancer properties. Dashamoolam plants can act as effective sources for novel drugs in the field of cancer research.

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A SHORT REVIEW ON POTENTIALS OF PHYTOCHEMICALS IN THE PIONEERING BIOMEDICAL RESEARCH AND DEVELOPMENT

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Abstract:

There are various kinds of plants on earth such as Terrestrial, Coastal and Marine plants, which are incomparable mediums to get precious organic chemicals. These chemicals are applicable in various domains of Science, Technology, Research and Development. Present review paper highlights the role of various phytochemical along with recent case study in biomedical application. The present review paper also deals with the key challenges and future prospects associated withthe use of plant biomolecules. Thus emphasiszed the sparkling and active appliances of plant phytochemicals.

Keywords: Phytochemicals, applications, health, energy and environment.

Introduction:

The plants are magnum opus platforms for research and development because of the presence their exceptional, remarkable and diverse biomolecules (Ancín-Azpilicueta *et al.*, 2020; Dawane and Pathak, 2020; Defossez *et al.*, 2021). These plants are rich sources of organic chemicals (Krishnaswamy and Sundaresan, 2012; Yadav *et al.*, 2021). Different class of plants

such as terrestrial plants (Ruess and Müller-Navarra, 2019), marine plants (Goud *et al.,* 2009; Mollo *et al.,* 2015) and coastal plants (Ksouri *et al.,* 2012), all possess very dissimilar and diverse profiles of their plant metabolites (Mollo *et al.,* 2014). The various secondary metabolites and their various concentrations that are directly and/or indirectly influenced by their genetic and/or non-genetic factors present in the side of plants, which are responsible for a variety of applications of plants (Gottlieb, 1990; Wagner and Bladt, 1996; Tripodi *et al.,* 2018). Plant organic chemicals possess a varied nature in their biochemical arrangements (Dawane and Fulekar, 2018), unknown chemical configurations (Dawane and Fulekar, 2016) and natural capriciousness (quantitative and qualitative) allow them to differ noticeably and difficult to assess (Waksmundzka-Hajnos *et al.,* 2018) and diverse in applications (Uversky, 2021). The precise environmental influences (like geography and place to place dissimilarities, physiology of the plant, its habitation, different soil structure and salinity stages, time of reaping, levels of exact contamination and seasonal dissimilarities) and several other factors (Waksmundzka-Hajnos *et al.,* 2018) found to be extremely influencing factors for phyto-constituents types and quantity existence in the plants. This noteworthy phytochemical profile in the plants has been a great source of research and development since a long time.

Use of phytochemicals in various fields of sciences: importance and selected case studies:

Plant biomolecules are having very diverse applications which includes Biomedical, Pharmaceutical and Health sector, Material sciences, Nanotechnology and Nano engineering as well as Agriculture, Energy and Environment etc.

Plant produces both primary metabolites (central metabolites) and secondary metabolites. Primary metabolites are present in the various parts of the plants. It’s particularly related to the growth, development and reproduction of the plants. Various chemical compounds including proteins, amino acids, polysaccharides, fatty acids, etc. are the primary metabolites and it is present in all types of plants weather it is terrestrial, marine or any other plant (Waksmundzka-Hajnos *et al.,* 2018). Secondary metabolites are not responsible for the growth of the plants but they are essential for other important functions and mechanisms involved in the plant. These secondary metabolites are responsible for the bioactivities (Waksmundzka-Hajnos *et al.,* 2018). Nucleosides, antibiotics, terpenoids, peptides etc. chemical compounds are known as secondary metabolites.

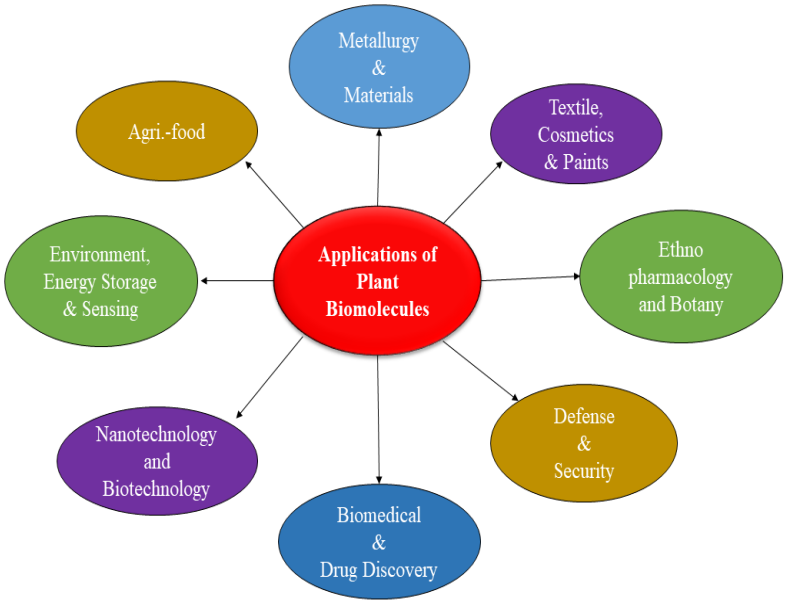

Figure 1: Various applications of plant biomolecules in diverse domains of research and development

Various bioactive compounds of the plants represent versatile biological activities and thus having a noteworthy role in the various biotechnological aspects (Salehi *et al.*, 2019). Protein and amino acids are interrelated and their specific polymerisation produces the protein entities. Both are present in all living things, prominently in the plants (Prasad, 2004). Enzymes, hormones, vitamins, and pigments are the modified form of the protein and amino acids. The polysaccharides are also the most important component in the plants (Deffosez *et al.*, 2021). They are also responsible for the biological activity of the plants (Wagner and Bladt, 1996). This short review deals with the case studies that are having the studies of identification of primary and secondary metabolites present in the plants and their special biomedical applications. Some selected recent case studies are as follows -

Javed *et al.* (2020) worked on extraction of tannins from *Salix alba L.* and their antibacterial activity. They used methanolic extract for the extraction of tannin from *Salix alba* L. They also did phytochemical analysis of various part of the *Salix alba* L. plant. They reported that various types of phytochemical including Tannins, Quinones and Phenols were presented in both leaves and bark. While Steroids and Resins were present in leaves and Flavonoids were present in bark of the *Salix alba* L. plant. They also reported that in leaves and bark of the *Salix alba* L. plants contain 5 and 10% tannins respectively. Total 6 types of pathogenic bacteria were

considered in antibacterial evaluation. They reported that tannins extracted from bark of the plant more effective to kill the bacteria than leaves extract.

Khalil *et al.* (2020) examined *Conocarpus erectus* plant extracts and their biomedical applications. *Conocarpus erectus* plant exists in tropical and subtropical region. Methanol extract of different part of the *Conocarpus erectus* plant were used for biomedical applications. They also did phenolic acid profiling by (RP-HPLC–UV–ESI-MS*). E. coli, S. Aureus* and *K. Pneumoniae* were used as microbes in the present study. HepG2 cell line also used for anticancer activity in the study. Various types of phenolic, flavonoid and tannin substances were identified in the *Conocarpus erectus* plant by HPLC results. Results revealed that methanol extract of leaves, stems, fruits and bark of the *Conocarpus erectus* plant displayed good biomedical activities.

Alam *et al.* (2020) worked on biomedical applications of *Ipomoea mauritiana Jacq.* Plant. They used methanol extract of whole for this study. Preliminary phytochemical studies of *Ipomoea mauritiana Jacq.* also done in the present investigation. Total 10 pathognenic bacteria were used for antimicrobial activity and DDPH activity used for antioxidant study in the present work. They reported that Terpenoids, Saponins, Flavonoids, Steroids and Alkaloids were present in the plant due to presence of these phytochemicals in the *Ipomoea mauritiana Jacq*, Thisplant also indicates excellent antimicrobial and antioxidant properties.

ZakariaNabti *et al.* (2020) antibacterial activity of essential oil (EOs) extracted from *Origanumglandulosum*Desf. In this study EOs extracted from hydro distillation method and chemical composition analysed by GC/MS. The antibacterial activity done on the eight different *E. Coli* strain. GC/MS revealed that various components including thymol, carvacrol, γ-terpinene and p-cymene were present in the EOs. Due to presence of these chemical components in EOs it exhibit excellent antibacterial acivity.

de Oliveira Filho *et al.* (2020) worked on antibacterial activity of Chitosan Film with *Citrus limonia* Essential Oil. They used hydrodistillation method for Essential Oil extraction from *Citrus limonia*. After essential oil extraction chitosan based film was prepared using very simple method. GC-MS results reported that total 20 various chemicals were present in the ECO including Myrcene, Limonene, 1,8-Cineol, Neroletc. They reported as prepared Film exhibit good antibacterial activity *S. aureus*, but not against *E. coli*.

Reddy *et al.* (2020) worked on biomedical application of *Tectaria coadunate* extract. They used ethanolic extract for phytochemical investigation using UHPLC-PDA-ESIMS/MS. The various phytochemical constituents including phenolic acids, flavonoids and anthocyanins

compounds were identified by UHPLC-PDA-ESIMS/MS. They have reported whole crud extract of *Tectaria coadunate* exhibit good Anticancer, Antibacterial and Antioxidant Properties.

Ouattara *et al.* (2020) worked on Antifungal Activity of Aqueous Leaf Extract of *Trema guineensis*. They used 3 fungal strain, *C. albicans, A. fumigatus* and *C. neoformans* for antifungal activity. Chemical test methods have been used for phytochemical analysis in the present study. They reported presence of saponins, alkaloids, flavonoids, steroids, quinones in the leaf extract of *Trema guineensis*. Due to presence of these phytochemicals *Trema guineensis* exhibit good antifungal activity against fungal strain,which is under investigation.

Adarsh *et al.* (2020) et al worked on biomedical applications of *Cinnamon zeylanicum*. They used ethanol extract for the phytochemical screening of bark. They reported Alkaloids, Carbohydrates, Steroids, Terpenoids, Reducing sugar, Glycosides, Phenolic and tannins chemical compounds in the ethanolic extract of bark. Due to presence of these compounds in the bark of *Cinnamon zeylanicum* exhibit excellent antimicrobial activity against *E. coli, Salmonella Typi* and *Enterococcus faecalis*.

Trong Le *et al.* (2020) examined biomedical applications of *Leoheodomatiophorus*. For this they used essential oil present in the leaves of *Leoheodomatiophorus*. Essential oils were extracted using hydro distillation process from leaves of the plants. GC-MS results represented total 52 different types of chemical compound have been identified in the leaves. They reported these essential oils exhibit excellent antibacterial, antimycotic, antitrichomonal and antiviral properties.

Okla *et al.* (2021) revealed antimicrobial activity of *Avicennia marina* (Forssk.) Vierh. They used different parts including roots, stem, leaves, fruits, and seeds. Ethanol and water extracts of these parts have been used for the phytochemical and antimicrobial activity of the plants. GC-MS studies reported that various special compounds including 1,2-Benzenedicarboxylic acid, Cis-cinnamic acid, hexadecanoic acid, and 1- deoxy-D-altritol have been present in the different parts of the *Avicennia marina*. Four different bacteria were used for antimicrobial activity and different parts of this plant shown good antimicrobial activity against pathogens under investigation.

The key challenges and future prospectus:

The cutting edged applications and future directions of R and D have focused their consideration on selective plant biomolecules as a noteworthy source of unique components. These unique metabolites are found to be more structurally important, multifaceted, with inimitable usefulness and distinctive properties. This present short review emphasized on update

going forward towards the potential applications of various plant biomolecules for biomedical applications. Though many plants including old ethnobotanical or ancient uses as well as nice plant species or new natural plant hybrids are elucidated for their remarkable properties, the investigation effort on them is still inadequate as well as the stepwise scientific evaluation has not been executed properly. Thus a significantly cost effective and well organised unconventional authentic method in herbal technological point of view is a need of hour along with an eye on their efficacy and biological action are present in rising request.

Figure 2: Key Challenges involved with the plant biomolecules related research and technologies

In future viewpoints, the accountable molecular mechanism and safety worries of these compounds are very significant for upcoming challenges in interdisciplinary fields related to plant biomolecules applications. Therefore, further studies are gaining a direction that can reduce the time, money and affords along with the increment in safety and authenticity issues regarding the plant biomolecules, their extracts, and formulation and multifarious motifs.

The exploration of precise mechanism related to the molecular basis for the beneficial activity should be undertaken that can highlight the accurate and eject use. Recently, advanced chromatographic techniques and spectroscopic techniques and *in silico* tools have been in trend to deal with the authenticity issues and safety concerns. Thus a combination of all above techniques and interdisciplinary approaches will be a significantly important strategy deals with the technological aspects of science along with utilization of plant phytomolecules.

Conclusion:

The wide utilization of plant biomolecules is the need of the hour. The current technological shifts are truly impressed by the appliances of plant biomolecules specifically in the fields of biomedical domains. The cutting edge researches in drug discovery and drug targeting are exercising this great potential of plants and plant phytochemicals. There is a strong need to work on the authentic use of these plant organic compounds by the means on various analytical techniques to achieve maximum output and sustainable development goals for the humanity.

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USE OF SPLINE REGRESSION TECHNIQUE INFORECASTING AGRICULTURE DATA

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Forecasting of agriculture data in the present-day scenario is a very important and also challenging task. The agriculture data are subjected to vagaries of nature and thus shows changed pattern in different time periods. A long run growth path is seldom unidirectional. Very often the growth path changes its direction with ups and downs. When the data are plotted over a long period of time, some significant changes (jumps or breaks) in the direction of growth path may be seen. In such cases fitting a single regression line for the entire period to forecast the future period values may give misleading results. A spline regression model avoids the inappropriate "Jump" (i.e., break) in joining the two regression lines. In spline regression technique, the regression line for the dependent variable of interest may suddenly change its slope without causing an abrupt "Jump" in the line itself. This is accomplished by allowing a kink in the line (a change in the slope) without allowing a break in the line. This amounts to forcing the two separate regression lines to touch at their joining point known as spline knot while their slopes are allowed to be different at that point.

ARIMA models are commonly used for forecasting purpose. But the drawback of ARIMA model is that it can give reliable forecast for a short future period as the uncertainty increases as prediction is made for periods which are quite far in future times (Sarika *et al.*, 2011). The ordinary regression model has the demerit that the predictions obtained from the regression model are valid only when the relationship between the independent variables and the dependent variable does not change significantly. Spline regression is thought to get rid of these demerits as the technique fits different curves for different section of data range without losing the continuity of the curve. Hence, spline regression model can be used to obtain forecast for comparatively longer future period.

Fitting of spline regression model is explained below in an elaborative manner. To illustrate the fitting of spline regression model, the data on area and yield of rabi cereals in Odisha are used.

The forecasts are obtained for area and yield of rabi cereals of Odisha for the period from 2020-21 to 2025-26, by fitting appropriate spline model. The forecast values of area and yield gives the forecast values of production of rabi cereals.

Spline regression models are fitted to data on area and yield of rabi cereals for the state of Odisha. The data are considered for the period 1970-71 to 2019-20 out of which the data for the period 1970-71 to 2015-16 are used as training data set i.e.for building the model and remaining data are kept as testing data set i.e., used for cross validation. The data are obtained from Odisha Agricultural Statistics published by Directorate of Agriculture and Food Production, Odisha.

The entire procedure includes the following steps:

1. selection of suitable regression models
2. partitioning of the whole study period
3. fitting of the selected models by using spline regression technique
4. selecting the best fit model
5. cross validation of the selected best fit model
6. using the selected model for future forecasting

1. **Selection of suitable regression models**

The study of scatter plot of the data on areaand yield of cereals in the state of Odisha for rabi season helps us to find the regression models that could fit well to the data. The regression models found to be suitable to the data are linear, compound, logarithmic and power model.

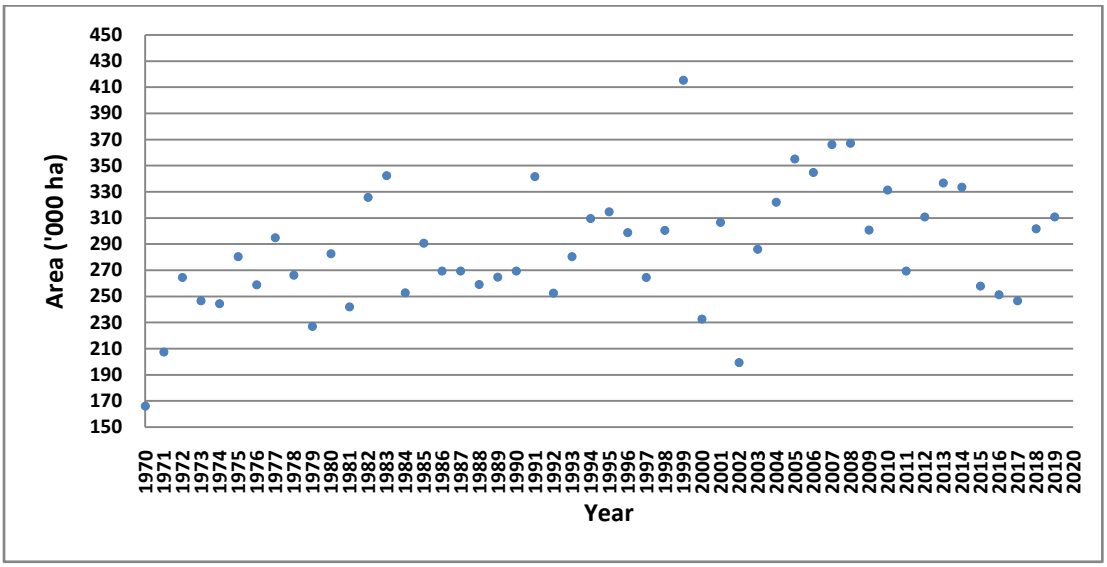

Figure 1: Scatter plot of area under rabi cereals

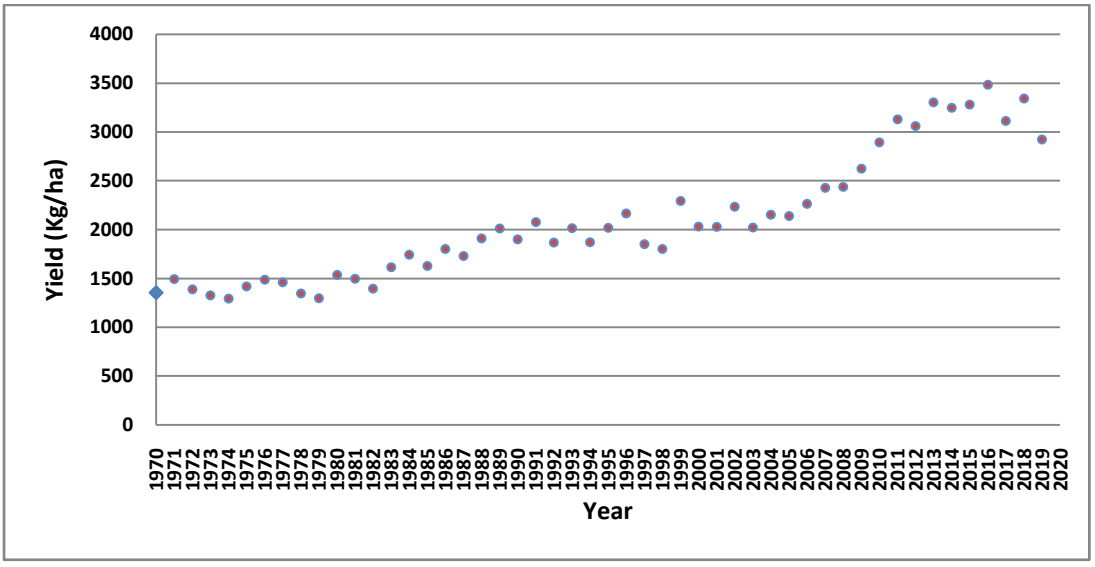

Figure 2: Scatter plot of yield of rabi cereals

2. Partitioning of the whole study period

The scatter plot gives an idea for partitioning of the whole study period into different periods such that the data within a period follows a definite pattern and abruptly changes in the consecutive periods.

The scatter plot of area under rabi cereals as shown in the Figure 1 shows that the area undergoes three different phases in the entire period from 1970-71 to 2019-20 with first knot at the year 1983-84 and second knot at the year 1998-99 which corresponds to the time, t = 14 and 29 respectively. Thus the entire period of study is divided into three sub-periods: sub-period I (1970-71 to 1983-84), sub-period II (1984-85 to 1998-99) and sub-period III (1999-00 to 2019-20).

The scatter plot of yield of rabi cereals as shown in the Figure 2 shows that the yield undergoes three different phases in the entire period from 1970-71 to 2019-20 with first knot at the year 1979-80 and second knot at the year 1998-99 which corresponds to the time, t = 10 and 29 respectively. Thus, the entire period of study can be divided into three sub-periods: sub-period I (1970-71 to 1979-80), sub-period II (1980-81 to 1998-99) and sub-period III (1999-00 to 2019-20).

The partitioning of data into periods can be further ascertained by calculating CV of each period and testing whether the difference in CV of consecutive periods is significant or not.

Let S_i be the observed standard deviation of the i^{th} period, $\bar{x}_i$ be the observed mean of thei^{th}period, and let $m_i = n_i - 1$. Where, $n_i \rightarrow$ no. of years in i^{th} period,

Let CV^* be the estimate of population CV,

$CV^* = \frac{\sum_{i=1}^{k} m_i \frac{\sigma_i}{\bar{x}_i}}{M}$ and $M = \sum_{i=1}^{k} m_i$

Where, k→ no. of periods

Test statistics, $\chi^2 = (CV^*)^{-2}(0.5 + (CV^*)^2)^{-1} [\sum_{i=1}^{k} m_i (\frac{\sigma_i}{\bar{x}_i})^2 - M(CV^*)^2]$

The χ^2 value distributed as a central χ^2 variables with k-1 degrees of freedom, from which the p-value can be computed. The χ^2 value measures how far each sample CV is from the estimate of the population CV^*(Feltz and miller, 1996).

The partitioning of data into segments can be further ascertained by calculating χ^2 value for CV of each segment and testing whether the difference in CV of consecutive segments are significant or not.

Table 1 shows the partitioning of data on area and yield of rabi cereals based on the testing of Coefficient of Variation. The difference in CV of consecutive periods is found significant for area and yield of rabi cereals with p-value less than 0.05. The whole period of area and yield of rabi cereals is divided into three periods.

Table 1: Partitioning of data on area and yield of kharif and rabi cereals based on the testing of Coefficient of Variation

Season	Variable	Periods	Standard deviation	Mean	C.V. (%)	Chi-square (χ^2) value	
						I – II	II – III
Rabi	**Area**	**I**	45.33	260.74	17.39	5.093* (0.024)	4.93* (0.026)
		II	25.95	282.61	9.18		
		III	51.56	307.04	16.79		
	Yield	**I**	74.68	1389.05	5.38	4.897* (0.027)	4.42* (0.035)
		II	207.88	1814.33	11.46		
		III	514.18	2688.57	19.12		

The figures in the parentheses represents the p-value

*** p-value ≤ 0.001 ** 0.001 < p-value ≤ 0.01 * 0.01 < p-value ≤ 0.05

3. Fitting of the selected models by using spline regression technique:

Prior to fitting of models, the data are checked for presence of outliers. The Inter-Quartile Range of the data series, denoted as IQR is used for checking of outliers.

IQR = Q_3 - Q_1, where Q_1 and Q_3 are the first and third quartiles respectively.

The observations that are less than Q_1–3×IQR or more than Q_3+ 3×IQR are referred to as extreme outliers (Bhattacharya and Roychowdhary, 2010). The extreme outliers, if found in the data, are eliminated from the data set before analysis.

A brief description of different regression models used in the study is given below. In all the models $Y_t$is the value of the variable at time t, β_0 and β_1 are the parameters of the model used in the study and ε_t is the random error component.

(i) Linear model: Linear model is of the form $Y_t = \beta_0 + \beta_1.t + \varepsilon_t$

(ii) Logarithmic model: Logarithmic model is of the form, $Y_t = \beta_0 + \beta_1 . \ln(t) + \varepsilon_t$

(iii)Compound model: Compound model is a non linear model of the form,

$Y_t = \beta_0 . \beta_1^t . \exp(\varepsilon_t)$

The form of the compound model after logarithmic transformation is: $\ln(Y_t)= \ln(\beta_0) + \ln(\beta_1).t + \varepsilon_t$

(iv) Power model: Power model is of the form:$Y_t = \beta_0 . t^{\beta_1} \cdot \exp(\varepsilon_t)$.

The form of power model after logarithmic transformation is:

$\ln(Y_t) = \ln(\beta_0)+ \beta_1 . \ln(t) + \varepsilon_t$

The above-mentioned regression models fitted with spline regression technique withtwo knots placed at time period $k_1$and k_2 in the following manner:

Linear spline model:

$$Y_t= \beta_0 + \beta_1 . t . I_{(1\le t\le k_1)} + \{\beta_1 . t + A_1 (t - k_1)\} . I_{(k_1+1\le t\le k_2)} + \{\beta_1 . t + A_1 t + A_2 (t - k_2)\} . I_{(k_2+1\le t\le n)} + \varepsilon_t$$

Logarithmic spline model:

$$Y_t= \beta_0 + \beta_1 . \ln(t) . I_{(1\le t\le k_1)} + \{ \beta_1 . \ln(t) + A_1 . \ln(t - k_1)\} . I_{(k_1+1\le t\le k_2))} + \{ \beta_1 . \ln(t) + A_1 . \ln(t) + A_2 . \ln(t - k_2)\} . I_{(k_2+1\le t\le n)} + \varepsilon_t$$

Compound spline model:

$$Y_t= \beta_0 . \beta_1^t . I_{(1\le t\le k_1)} . \{ \beta_1^t . A_1^{(t-k_1)}\} . I_{(k_1+1\le t\le k_2)} . \{ \beta_1^t . A_1^t . A_2^{(t-k_2)}\} . I_{(k_2+1\le t\le n)} + \exp(\varepsilon_t)$$

The compound spline model can be transformed to linear form by a natural log transformation and written as,

$$\ln(Y_t) = \ln \beta_0 + t . \ln(\beta_1) . I_{(1\le t\le k_1)} + \{ t . \ln(\beta_1)+ (t - k_1) . \ln(A_1)\} I_{(k_1+1\le t\le k_2)} + \{ t . \ln(\beta_1)+ t . \ln(A_1)+ (t - k_2) . \ln(A_2)\} I_{(k_2+1\le t\le n)} + \varepsilon_t$$

Power spline model:

$$Y_t= \beta_0 . t^{\beta_1} . I_{(1\le t\le k_1)} \{ t^{\beta_1} . (t - k_1)^{A_1}\} . I_{(k_1+1\le t\le k_2)} . \{ t^{\beta_1} . t^{A_1} (t - k_2)^{A_2}\} . I_{(k_2+1\le t\le n)} \exp(\varepsilon_t)$$

The power spline model is transformed to linear form by natural log transformation as,

$\ln(Y_t)= \ln \beta_0 + \beta_1 . \ln(t) . I_{(1\le t\le k_1)} + \{ \beta_1.\ln(t) + A_1 \ln(t - k_1)\} . I_{(k_1+1\le t\le k_2)} + \{ \beta_1.\ln(t) + A_1.\ln(t) + A_2 \ln(t - k_2)\} . I_{(k_2+1\le t\le n)} + \varepsilon_t,$

Where, $I_{(P)}$is the indicator function which is 1 if P holds and 0 otherwise.

The models are fitted by OLS technique. R software is used for the purpose of fitting the spline models

Table 2: Estimated parametric coefficients, of spline regression models fitted to training data set on area under rabi cereals in Odisha

Model →	**Linear Spline**	**Compound Spline**	**Logarithmic Spline**	**Power Spline**
b_0	232.911*** (≤0.001)	228.149*** (≤0.001)	190.557*** (≤0.001)	189.806*** (≤0.001)
b_1	2.79 (0.06)	1.012* (0.02)	35.59** (0.011)	1.169** (0.001)
a_{11}	-0.372 (0.793)	-0.004 (0.428)	-4.23 (0.645)	-0.031 (0.326)
a_{12}	-1.473 (0.571)	-0.003 (0.75)	3.24 (0.715)	0.013 (0.66)

Figures inside the parentheses indicates the p-value

*** p-value ≤ 0.001 ** 0.001 < p-value ≤ 0.01 * 0.01 < p-value ≤ 0.05

Table 3: Estimated parametric coefficients of spline regression models fitted to training data set on yield of rabi cereals in Odisha

Model →	**Linear Spline**	**Compound Spline**	**Logarithmic Spline**	**Power Spline**
b_0	1199.9*** (≤0.001)	1261.428*** (≤0.001)	1328.4*** (≤0.001)	1355.601*** (≤0.001)
b_1	37.18*** (≤0.001)	1.02*** (≤0.001)	45.87 (0.62)	1.019 (0.610)
a_{11}	-15.133 (0.054)	-0.012 (0.159)	149.28* (0.02)	0.101*** (≤0.001)
a_{12}	60.238*** (≤0.001)	0.016*** (≤0.001)	288.56*** (≤0.001)	0.102*** (≤0.001)

Figures inside the parentheses indicates the p-value

*** p-value ≤ 0.001 ** 0.001 < p-value ≤ 0.01 * 0.01 < p-value ≤ 0.05

4. Selection of best fit model

The model to be considered for selection should have overall significanceand must satisfy the assumptions regarding the errors.The model fit statistics, viz., R^2, adjusted R^2, Root Mean square Error (RMSE), Mean absolute Percent Error (MAPE) and Akaike's Information Criteria corrected (AICc) are computed for the purpose of model selection. In spline regression models, the significance of the coefficients is tested by using t-test. F test is used to test the overall significance of the model.

The above models are fitted under the assumptions that errors are independently distributed, follow normal distribution and have constant variance i.e. homoscedastic.

The following statistical tests are considered for testing the assumptions regarding errors in the model:

(i) Durbin-Watson test for testing independence of residuals.

(ii) Shapiro-Wilk's test for testing normality of residuals.

(iii) Breusch-Pagan test for testing homoscedasticity of the errors

Durbin-Watson test:

This test considers the first order autocorrelation among the residuals (Montgomery *et al*., 2001).

Null hypothesis is taken as, H_0: the errors are independent.

And the alternative hypothesis as, H_1: the errors are not independent.

Durbin-Watson test statistic (D-W statistic), $d = \frac{\sum_{t=2}^{n}(e_t - e_{t-1})^2}{\sum_{t=1}^{n} e_t^{\,2}}$,

Where, $e_t = y_t - \hat{y}_t$, y_t and $\hat{y}_t$ are respectively the actual and estimated values of the response variable at time t and *n* is the no. of observations.As the p-value ofthe test statistic d is greater than 0.05, the independency of errors can be assumed.

Shapiro-Wilk's test: This test is used for testing normality of the residuals.

Null hypothesis here is H_0: the errors follow normal distribution.

Alternative hypothesis, H_1: The errors do not follow normal distribution.

To carry out the test, the data pertaining to errors are arranged in ascending order so that $e_{(1)} \leq e_{(2)} \leq \ldots \leq e_{(n)}$

The Shapiro-Wilk's (S-W) test statistic as given by, $W = \frac{s^2}{b}$

Where, $s^2 = \sum_{k=1}^{m} a(k)\{e_{(n+1-k)} - e_{(k)}\}^2$; $b = \sum_{t=1}^{n}(e_t - \bar{e})^2$ (Lee *et al*., 2014)

If *n* is even, then $m = \frac{n}{2}$. If n is odd, then $m = \frac{n-1}{2}$

The parameter k takes the values 1, 2, …, m.

n is the number of observations, $e_{(k)}$ is the k^{th} order statistic in the set of residuals,

e_t is the residual at time 't' and $\bar{e}$ is the mean of e_t.

The values of coefficients are (k) for different values of *k* and particular values of *n* are obtained from the table of Shapiro-Wilk

For a given value of n, the value of pthat is closest to 'W' can be obtained from Shapiro-Wilk's table. If the p value exceeds 0.05, then the null hypothesis cannot be rejected. If it lies below 0.05 but above 0.01, then the null hypothesis is rejected at 5% level. If the *p* value is below 0.01, then the null hypothesis is rejected at 1% level

Breusch-Pagan test:

The homoscedasticity of errors obtained from the regression model can be tested by using Breusch-Pagan test (Zaman, 2000).

Null hypothesis, H_0: Errors have constant variance i.e. homoscedastic.

Alternative hypothesis, H_0: Errors have non-constant variance i.e. heteroscedastic

Breusch-Pagan test statistic is given as, $BP = n \times R^2$

Where, n is the no. of observations, R^2 is the coefficient of determination of the regression of squared residuals (obtained from the original regression) on the independent variable (which is time, in the present study).

BP statistic follows chi-square distribution with 'k' degrees of freedom.

If the BP statistic has a p-value below 0.05, then the null hypothesis is rejected and heteroscedasticity is assumed to be present in the residuals and the regression model used can be considered to be inappropriate fit.

Among the fitted models, model having overall significance and model satisfying the diagnostics tests, model having highest R^2, highest adjusted R^2 , lowest MAPE, AIC and AICc is considered to be the best fit model for that dependent variable.

$R^2 = \frac{SSM}{SSE}$, where, SSM is the sum of square due to model; SSE is the sum of square due to error.

The expressions for SSM and SSE are, respectively,

$SSM = \sum_{t=1}^{n}(\hat{y}_t - \bar{y})^2$, $SSE = \sum_{t=1}^{n}(y_t - \hat{y}_t)^2$,

Where y_t and $\hat{y}_t$ are respectively the actual and estimated values of the response variable at time t, and $\bar{y}$ is the mean of y_t.

Adjusted R^2 is defined as, Adjusted $R^2 = 1 - (1-R^2) \times \frac{(n-1)}{(n-p)}$

Where, p is the no. of coefficients involved in the model.

Adjusted R^2 penalizes the model for adding some independent variables which are not necessary to fit the data and thus adjusted R^2 will not necessarily increase with the increase in the number of independent variables included in the model. To check the significance of R^2 and adjusted R^2, F-value is calculated. If the F-statistic has a p-value below 0.05, the test is significant.

F-statistic = $\frac{R^2/(p-1)}{(1-R^2)/(n-p)}$,

Where, 'p' is the no. of coefficients involved in the model and 'n' is the no. of observations.
To check the significance of adjusted R^2, in use adjusted R^2 values in place of the R^2 values in the above mentioned f-statistic formula.

Mean Absolute Percent Error, $MAPE = (\sum_{i=1}^{n} \frac{|P_i - O_i|}{O_i} \times 100)/n$, where P_i and O_i are respectively the predicted and observed values for the i^{th} year, i= 1, 2,…, n.

Akaike's Information Criteria (AIC) estimates the relative amount of information lost by a given model. The less information a model loses, the higher the quality of that model.

AIC = n x ln($\hat{\sigma}^2$)+ 2 x k (Burnham & Anderson, 2002).

$\hat{\sigma}^2 = \frac{\sum \varepsilon_t^2}{n}$; Where, ε_t is the residual at time t

k is the number of parameters involved in the model.

R software has been used for the regression analysis including Durbin Watson-test, Shapiro-Wilk's test and Breusch Pagan test.

The study of table 4 shows that all the fitted models show overall significance by having highly significant F-value and significant R^2 and adjusted R^2 values. Both the linear spline model and compound spline model does not satisfy the assumption of homoscedasticity of errors as the BP-statistic used for testing the assumption of homoscedasticity is found to be significant. The power spline model does not satisfy the assumption of normality of errors as the SW-statistic

used for testing the assumption is found to be significant. The logarithmic spline is found to satisfy all the three assumptions of errors and also have moderately high value of R^2 and adjusted R^2 with low value of RMSE, MAPE and AIC as compared to linear, compound and power spline model. So, logarithmic model is selected for cross-validation purpose.

Table 4: Model diagnostics measures and model fit statistics of spline regression models fitted to training data set on area under rabi cereals in Odisha

Model →	**Linear Spline**	**Compound Spline**	**Logarithmic Spline**	**Power Spline**
Model Diagnostics Criteria				
DW Statistic	1.92 (0.452)	1.86 (0.342)	2.05 (0.764)	2.059 (0.781)
SW Statistic	0.988 (0.924)	0.923 (0.004)	0.981 (0.66)	0.85*** (≤0.001)
BP Statistic	11.263* (0.01)	10.08* (0.017)	5.107 (0.164)	3.55 (0.314)
Model Fit Statistics				
F Value	5.64** (0.002)	5.975** (0.002)	7.25*** (≤0.001)	8.831*** (≤0.001)
R^2	0.287** (0.002)	0.299** (0.002)	0.341*** (≤0.001)	0.386*** (≤0.001)
Adjusted R^2	0.236** (0.009)	0.249** (0.007)	0.294** (0.002)	0.343*** (≤0.001)
MAPE	11.23	11.292	10.417	10.411
AIC	347.705	348.58	344.081	344.753

Figures inside the parentheses indicates the p-value

*** p-value ≤ 0.001 ** 0.001 < p-value ≤ 0.01 * 0.01 < p-value ≤ 0.05

The study of table 5 shows the results obtained by fitting of selected spline regression models of the type linear spline, logarithmic spline, compound spline and power spline model. All the fitted models show overall significance by having highly significant F-value and significant R^2 and adjusted R^2 values. The linear spline model does not satisfy the assumption of normality of errors as the SW-statistic used for testing the assumption of normality is found to be significant. The logarithmic spline model does not satisfy the assumptions of normality and

independence of errors as the respective test statistic i.e., SW-statistic and DW-statistic used for testing the assumptions are found to be significant. The power spline model also does not satisfy the assumptions of normality, homoscedasticity and independence of errors as the respective test statistic i.e., SW-statistic, BP-statistic and DW-statistic used for testing the assumptions are found to be significant. The compound spline model is found to satisfy all the three assumptions of errors and also have moderately high value of R^2 and adjusted R^2 with low value of RMSE, MAPE and AIC as compared to linear spline, logarithmic spline and power spline model. So, compound spline model is selected for cross-validation purpose.

Table 5: Model diagnostics measures and model fit statistics of spline regression models fitted to training data set on yield of rabi cereals in Odisha

Model →	**Linear Spline**	**Compound Spline**	**Logarithmic Spline**	**Power Spline**
Model Diagnostics Criteria				
DW Statistic	1.151*** (≤0.001)	1.883 (0.08)	0.608*** (≤0.001)	0.891*** (≤0.001)
SW Statistic	0.975 (0.418)	0.949 (0.07)	0.986 (0.88)	0.894*** (≤0.001)
BP Statistic	5.08 (0.165)	2.08 (0.55)	23.55*** (≤0.001)	14.747*** (0.002)
Model Fit Statistics				
F Value	166.3*** (≤0.001)	160*** (≤0.001)	79.63*** (≤0.001)	108.5*** (≤0.001)
R^2	0.922*** (≤0.001)	0.919*** (≤0.001)	0.85*** (≤0.001)	0.885*** (≤0.001)
Adjusted R^2	0.916*** (≤0.001)	0.913*** (≤0.001)	0.839*** (≤0.001)	0.877*** (≤0.001)
MAPE	6.642	6.135	7.96	7.564
AIC	472.372	466.71	502.524	499.518

Figures inside the parentheses indicates the p-value

*** p-value ≤ 0.001 ** 0.001 < p-value ≤ 0.01 * 0.01 < p-value ≤ 0.05

5. Cross validation of the selected model

After exploring the best fit model from each group, cross validation is done for the selected models by obtaining the forecast values for the time period 2016-17 to 2019-20 as the

observations were left out for the validation purpose. From the actual and forecast values of the dependent variable, the absolute percentage error (APE) value is obtained for each observation in the left out period. The APE for the i^{th} year of validation period is obtained as, $APE_i = \frac{|P_i - O_i|}{O_i} \times 100$, where P_i and O_i are respectively the predicted and observed values for the i^{th} year, i= 1, 2, …, 9. Low value of APE ensures the appropriateness of the selected model for forecasting.

Cross validation of the selected models for area and yield of rabi cereals in Odisha for the year from 2016-17 to 2019-20 is shown in Table 6. The absolute percentage error for the selected logarithmic model for area under rabi cereals is found to be below 25% for all the years included in the testing data and the value of MAPE obtained is 12.33%. The absolute percentage error for the selected compound model for yield of rabi cereals is found to be below 15% for all the years included in the testing data and the value of MAPE obtained is 7.75%. These values of MAPE obtained in both the cases are sufficiently low to accept logarithmic spline model for area and compound spline model for yield of rabi cereals as the best fit model. So, these models can be used respectively for forecasting of area and yield of rabi cereals in Odisha for the future years from 2020-21 to 2025-26. The forecast values of area and yield can be used for forecasting the production of rabi cereals in Odisha for the future years from 2020-21 to 2025-26.

Table 6: Cross validation of the selected logarithmic spline and compound spline model for area and yield of rabi cereals respectively in Odisha

	Area			**Yield**		
Year	**Actual value**	**Predicted Value**	**APE**	**Actual value**	**Predicted Value**	**APE**
2016-17	251.37	289.35	15.01	3483.75	3064.11	12.04
2017-18	246.68	306.27	24.32	3114.89	3159.94	1.45
2018-19	301.91	321.15	6.37	3344.97	3258.78	2.58
2019-20	310.79	322.01	3.61	2923.77	3360.70	14.94
Mean Absolute Percentage Error			**12.33**			**7.75**

6. Forecasting of future period values

After successful cross validation of the selected model, it is used for the purpose of forecasting. The forecast values of area and yield and hence production for rabi cereals in Odisha for the future years from 2020-21 to 2025-26 are presented in table 7.

$$\text{Forecast values of production} = \frac{\text{Forecast values for area} \times \text{Forecast values for yield}}{1000}$$

The forecast values shows that the future values of area under rabi cereals is expected to increase. The future values for yield of rabi cereals is expected to increase. The future values of production for rabi cereals is expected to increase due to increase in both area and yield.

Table 7: Forecast values of area and yield and hence production of rabi cereals in Odisha for the year from 2020-21 to 2025-26.

Year	Rabi		
	Area ('000 ha)	Yield (kg/ha)	Production ('000 MT)
2020-21	322.84	3465.81	1118.90
2021-22	323.65	3574.21	1156.79
2022-23	324.43	3685.99	1195.85
2023-24	325.19	3801.28	1236.14
2024-25	325.93	3920.17	1277.70
2025-26	326.65	4042.78	1320.57

Forecasting of the area, yield and hence production of rabi cereals in Odisha is done through best fit spline regression models show that future forecast values of both area and yield of rabi cereals is found to be increasing and hence there is increase in the future forecast values of production for rabi cereals. The spline regression model assumes that the pattern of data in the future period will be same as that of the last partition period.

Thus it could be seen that spline regression technique effectively captures the variation data over a long period of time and can be used efficiently for forecasting purpose.

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Advances in Plant Science Volume III

ISBN: 978-93-91768-51-5

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ISBN 978-93-91768-51-5
9 789391 768515