



A study on Phytochemical analysis and anatomical comparison of two aquatic ferns in Salviniaceae

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Abstract: Ferns play an important role in the evolutionary studies with their unique phytochemicals and eccentric stellar regions. According to PPG, Polypodiales include maximum macro-ferns and this study focused on the anatomical and phytochemical properties of two aquatic sister Salviniaceae ferns, *Azolla filiculoides* and *Salvinia molesta*. The histological studies using safranin stain, GC-MS using the methanol solution and antimicrobial test by induction disk diffusion- agar method was used for the study. The hadrocentric anatomy, aquatic adaptations of *Azolla filiculoides* and *Salvinia molesta* were studied. The methanolic extract (ME) of these ferns showed Cyclohexasiloxan in large quantity, that has antibacterial and antiproliferative activity and ME of *Azolla* shows more antimicrobial activity than *Salvinia*. Also, the phytochemical contents are also high in *Azolla* sp than *Salvinia* sp. Anatomical and Antimicrobial comparison of sister ferns in Salviniaceae using methanol shows Cyclohexasiloxan in a notable sum and the ecological and phytochemical adaptations of *Azolla filiculoides* is comparatively advanced than *Salvinia molesta*.

Index Terms - *Azolla filiculoides*, *Salvinia molesta*, antibacterial, stellar evolution, Cyclo-hexasiloxan.

I. INTRODUCTION

The Pteridophytes including ferns and lycophytes, split into two monophyletic lineages, where ferns are more closely connected to plants that produce seeds [1]. In Plant kingdom, ferns are regarded as a crucial evolutionary connection between lower and higher plants. [2]. More than 12,000 species of ferns identified and on which the majority are found in tropical and subtropical areas. India is home to over 191 genera and over 1000 species [3]. Due to their peculiar life cycle on which the sporophyte and gametophyte generations are independent and their unique transitional position between higher vascular plants and lower cryptogams, ferns have long been a plant group of interest to botanists. The recent PPG classification (2016) on ferns is based on monophyletic groups, morphological analysis, homogeneity and hierarchical equivalency and it includes 2 classes, 14 orders, 51 families, 337 genera and 11, 916 species of living pteridophytes [4] [5]. According to their evolutionary history and biology, they produce a distinct collection of secondary metabolites [6]. Secondary metabolites, which plants produce under stressful conditions are metabolically inert phytochemicals with a range of therapeutic and biotechnological applications. The fact that the majority of Pteridophytes are resistant to microbial attack may be the reason for their 350-million-year lifespan and important role in evolutionary studies [7] [8]. Additionally, recent research has focused on the objective use and extraction of fern phytochemicals from large amounts of biomass [9]. Especially *in vitro* studies using the existing nutrient media can give multiple copies of plants that can be used in pharmaceutical and therapeutic industries [10]. An extensive study related with biodiversity hotspots are required to protect these niche-specific plants from overexploitation, monoculture, habitat destruction, species interventions and laborious *in vitro* propagations [11].

The ferns in the Salviniaceae family are floating annual herbs with austere aquatic adaptations in their leaves, roots, and growth. Based on their ecological and industrial applications, two common aquatic ferns from the Salviniaceae family viz. *Azolla filiculoides* Lam and *Salvinia molesta* Mitch—were chosen for this study. They are cultivated in some places and become weeds in others [12]. The sister ferns, *Azolla* and *Salvinia* of same family Salviniaceae, show internal differences with symbiotic association in all its seven species of *Azolla*, while any known co-symbiont was not observed in *Salvinia* ferns. A symbiont-host co-evolution may occur in the *Azolla* lineage, that differs it from *Salvinia* ferns [13]. Being blue-green algae as co-biont, they are associated with soil fertility [14] [15]. However, there are numerous unexplored therapeutic and biotechnological potentials for these enigmatic ferns. As they are frequently used as biofertilizers and feed, this study aims to discriminate the antimicrobial and Phyto-potential of these aquatic ferns.

II. METHODOLOGY

2.1. Collection of plant sample and anatomical study

Two aquatic Salviniaceae ferns, *Azolla filiculoides* Lam and *Salvinia molesta* Mitch were collected from the Attakatti, Valparai region (elevation 1193m from sea level) of Western Ghats, Tamil Nadu, India during the month of March. The phytochemical content of the plant was analyzed in Methanolic solvent as the high polar solvent like methanol can detect maximum secondary compounds [16]. The collected plant samples were taken to the laboratory, washed thoroughly, air-dried, and kept in an oven at 70°C for 24 hours. The dry weight of the plant was estimated. The dried plant specimen was ground to a fine powder. A known quantity (15g) of the sample was mixed with methanol (150 ml), and the phytochemicals were extracted by a Soxhlet apparatus. The crude extract was concentrated in a rotary evaporator, and stored in a freezer for further tests.

For anatomical studies, hand sections and staining with safranin/fast green was done and mounted in glass slides. The respective microphotographs were obtained by a light compound microscope. Antimicrobial studies were done in agar disk diffusion method with the antibiotics, Gentamicin as control against the plant pathogens.

2.2 Phytochemical analysis

2.2.1 Alkaloid

To 1ml of extract, 2ml of Mayer's reagent was added. The formation of a yellowish-white precipitate indicated the presence of alkaloids [17] [18].

2.2.2 Flavonoids

To ascertain the presence of flavonoids, an alkaline reagent test was performed. To 3ml of leaf extract, 4ml of 1N NaOH was added. The solution turned into an intense yellow color. On addition of dilute acid, the solution turned colorless that indicated the presence of flavonoids.

2.2.3 Glycosides

The presence of glycosides was ascertained by Legal's test. 1ml of each plant extract were treated with sodium nitroprusside in pyridine and sodium hydroxide separately. Formation of pink to red color indicated the presence of glycolysis.

2.2.4 Quinones

1ml of concentrated sulphuric acid was mixed with 0.5ml of the leaf extract mixed with isopropyl alcohol. The formation of red color showed the presence of quinones.

2.2.5 Saponins

The presence of saponins was ascertained by Foam test. 0.5 g of extract was shaken with 2 ml of boiling water and allowed to cool and mixed well. Bubble formation indicated the presence of saponins.

2.2.6 Steroids

Steroids were ascertained by the Salkowski test. In brief, to 2 ml of the leaf extract, an equal amount of chloroform was added followed by 2 drops of concentrated Sulphuric acid. The formation of a reddish-brown ring at the interface indicated the presence of steroids.

2.2.7 Tannins

To 1ml of leaf extract, 5% FeCl₃ was added. The formation of the brownish-green color showed the presence of tannins.

2.3 Histological studies

The fully grown and sterile plant parts are used for maceration by Schultze method using Conc. Nitric acid and KOH. The macerated cells were observed under a light microscope (Labomed 2019) at 10x, 60x and 100x magnification using safranin stain [19].

2.4 GC-MS analysis

The GCMS-QP2010 Ultra (Shimadzu) system was equipped with a BPX5 capillary column (30 m; 0.25 mm inner diameter; 0.25 mm film thickness; maximum temperature, 370°C) and a QP2010 Ultra (Shimadzu) MS. Ultra-high purity helium (99.99%) was employed as the carrier gas at a constant flow rate of 1.0 mL/min. The injection, transfer, and ion source all had a temperature of 280° C. The oven temperature was designed to raise at a rate of 3 °C per minute from 80 °C (hold for 2 minutes) to 280 °C. The crude samples were filtered after being diluted with the suitable solvent (1/100, v/v). In a syringe, particle-free diluted crude extracts (1 L) were delivered into an injector at a split ratio of 10:1. All of the data was gathered by collecting full-scan mass spectra in the 40–550 amu scan range. The crude extract elements' percentage composition was reported as a percentage by peak area. The GC retention time was used to identify and characterize chemical components in different crude extracts. The mass spectra were compared to the standards in the NIST 08 mass spectrum library using a computer program.

2.5 Antimicrobial activity

The antimicrobial activity was determined by Kirby Bauer agar well diffusion assay. In brief, the nutritional agar medium was prepared and sterilized by autoclaving at 121°C for 15 minutes at 15 lbs pressure, then aseptically put into sterile Petri plates and allowed to harden. Each Petri plate was swabbed on sterile buds with bacterial broth culture. Then, using a well borer, wells were created. Aseptically, the methanolic extract of plants in organic solvents was added to each well. This method was repeated for each Petri plate, after which the plates were incubated for 24 hours at 37 degrees Celsius. The plates were examined for the zone of inhibition after they had been incubated. This procedure was repeated for each Petri plate then the Petri plates were incubated at 37°C for 24 hrs. After incubation, the plates were observed for the zone of inhibition [20] [21].

III. RESULTS AND DISCUSSION

3.1 Morphological studies

The morphology of the ferns (Salviniaceae) was ascertained. *Azolla filiculoides* Lam is an aquatic fern consisting of a short, branched, floating stem, bearing 2 or 3 soft roots at each node that hang down to the water (Figure.1). The leaves are alternately arranged, each consisting of a thick aerial dorsal lobe containing chlorophyll and a slightly larger thin, colorless, floating ventral lobe. Under some conditions, an anthocyanin pigment gives the fern a reddish-brown color. Plant diameter ranges from 1-2 cm and root size is 1cm in length. The plant exhibits great morphological variation depending on the conditions of habitat and sunlight intensity. The optimum temperature for growth ranges from 20-30°C [22].

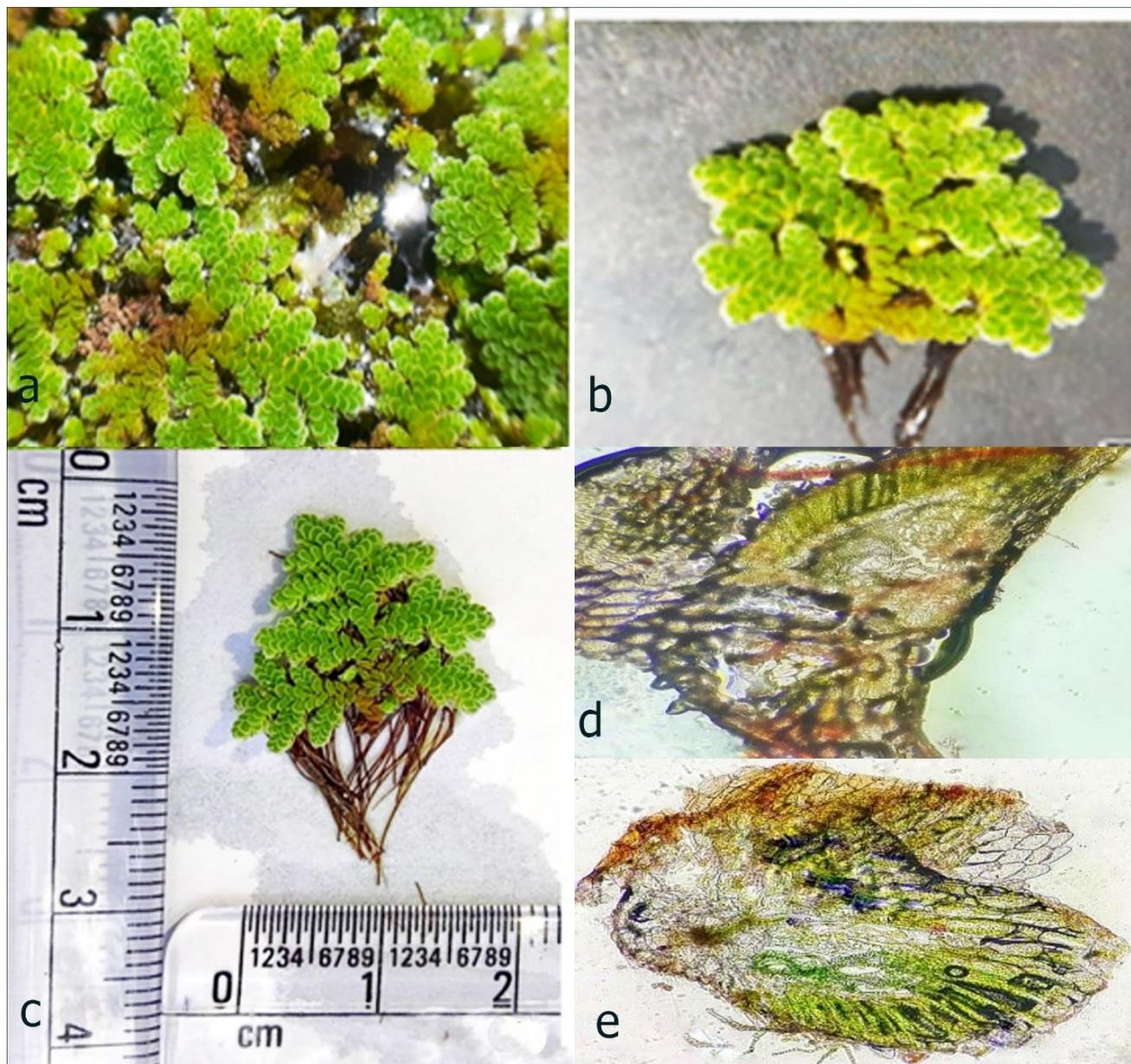


Fig. 1 a. Habitat of *Azolla*, b. a whole plant with fronds and roots, c. plant under measurement, d. V.S. of plant, e. T.S. of frond.

S. molesta Mitchell is an aquatic fern that floats freely on the water surface with a horizontal rhizome (below the water's surface). There are two different types of fronds (buoyant and submerged). Egg-shaped spore sacs containing sterile spores are produced by the mature plant (Figure.2). It does not have genuine roots, yet its submerged fronds serve as roots. It has three whorls of fronds (two floating and one submerged). The floating fronds are circular to oblong in shape and are oriented in the opposite direction to each other. At the distal end of each papilla, four hairs (each consisting of a single row of cells) are linked together at their terminals to form an inverted egg-beater. The cage-like structure of the end hairs is an effective air trap giving the plant buoyancy in the water. The papillae, end hairs and the upper surface of the plant are water-repellent. It grows extensively and branched. Most of the ferns are part of folk medicines and fodders [23].

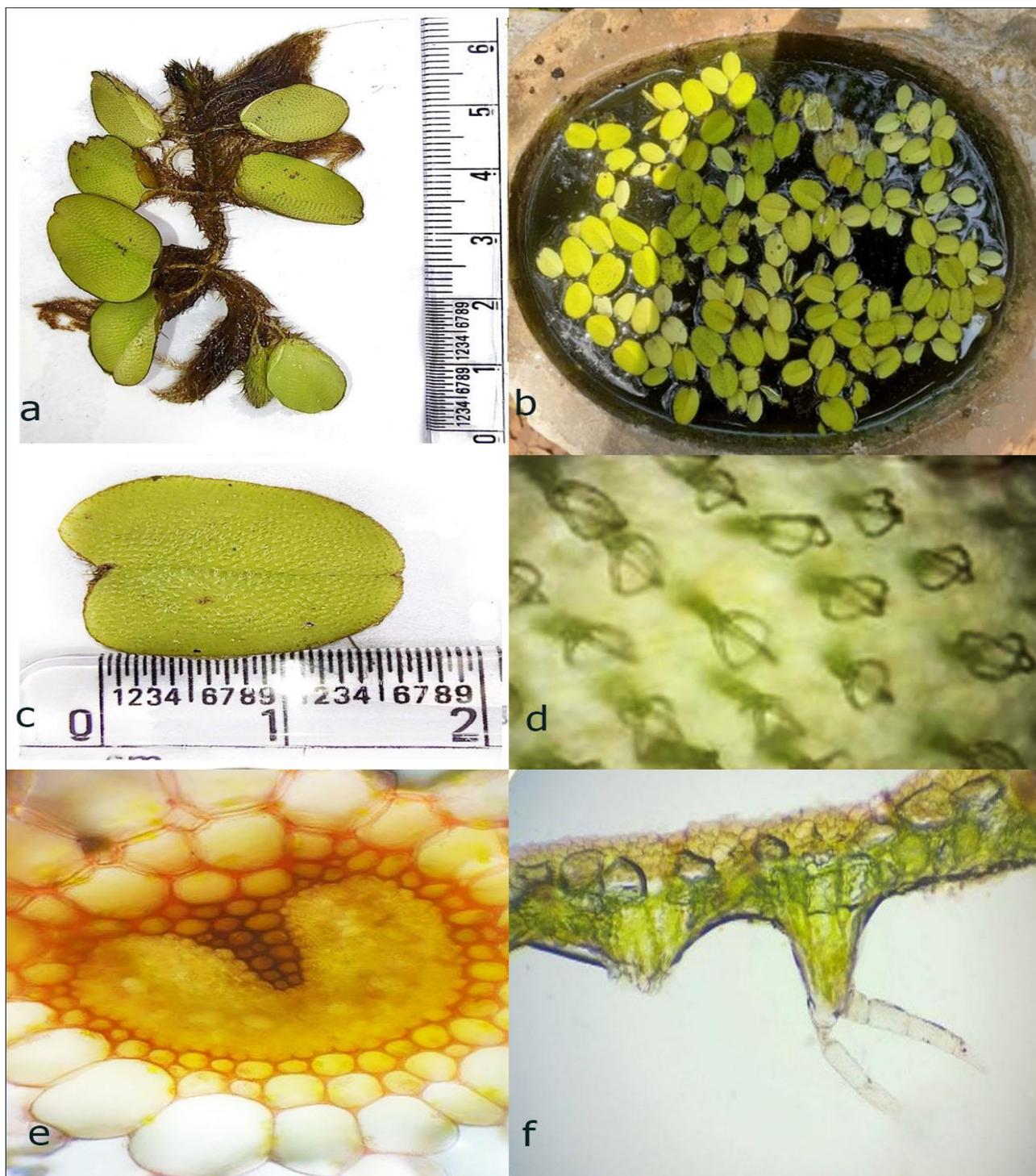


Fig. 2, a. Whole plant under measurement, b. *Salvinia* Habitat c. a leaf, d. leaf hairs showing 'egg-beater' shape, e. C.S of stele of stem f. V.S of leaf hair.

3.2 Identification of phytochemical compounds

Comparing to other solvents like ethanol, acetone, Petroleum ether and water, methanolic extract of aquatic ferns shows maximum phytochemicals in GC-MS analysis. The methanolic extracts of *Azolla* (MA) and *Salvinia* (MS) were taken for the qualitative estimation of alkaloids, carbohydrates, flavonoids, quinones, saponins, steroids, and terpenoids (Figure-3). The presence of alkaloids and quinones in both *A. filiculoides* and *S. molesta* were observed. Alkaloids in plants have toxicity against predators and diseases. Since ancient times, plant alkaloids of diverse classes have been used in many forms due to its sensitive reactions. Also, fern-based quinones and alkaloids exhibit anti-pathogenic properties. The remaining constituents like flavonoids, saponins, steroids and terpenes were absent or present in little. Plant alkaloids and quinines are secondary metabolites, highly particular in its habitat location and mostly therapeutic in nature. In earlier studies, presence of alkaloids was not reported from *Salvinia* sp. [24].

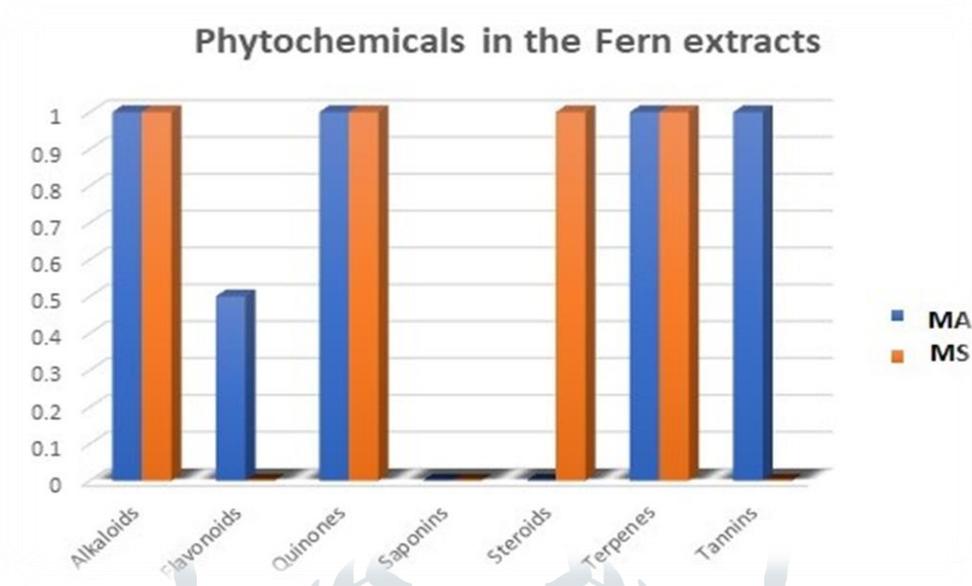


Figure. 3: Chart showing the Comparison of phytochemicals in two extract, MA- methano extract of *Azolla*, MS- methanol extract of *Salvinia*.

The methanolic extract of *A. filiculoides* and *S. molesta* (whole plant) gives 15 major phytochemicals. Cyclohexasiloxane, dodecamethyl- (CHDC) emerged as a major phytochemical both in *A. filiculoides* (23 %) and *S. molesta* (32%) in GC-MS analysis (Table-1,2). Interestingly, CHDC is useful in numerous industrial applications like cosmetics, electronics, health-care products, lubricants etc. Benzoic acid, 2,6-bis (trimethylsiloxy)-, trimethylsilyl ester forms the second important compound in *A. filiculoides* (16%) and *S. molesta* (18%) with anti-bacterial and anti-fungal properties.

Table 1. Identification of compounds from methanolic extract of *Azolla* plant

Peak	Retention time	Area	%	Height %	A/H	Compound
1	10.055	15.42	16.89	1.56	Benzoic Acid, 2,6-Bis (Trimethylsiloxy)-, Trimethylsilyl Ester	
2	13.988	21.68	23	1.61	Cyclohexasiloxane, dodecamethyl-	
3	17.609	2.9	3.74	1.32	(SS)- or (RR)-2,3-hexanediol	
4	26.579	3.79	4.11	1.57	Fluro[2,3-c] pyridine, 2,3-dihydro-2,7-dimethyl-	
5	38.85	3.33	3	1.89	1-Propene, 2-(3-methylphenyl)-1-phenyl-, (Z)	
6	38.935	4.48	6.71	1.14	3 -5-(3'-bu-benzyloxy tenyl)-4-	

Peak	Retention time	Area %	Height %	A/H	Compound
7	39.071	9.48	6.33	2.56	3-benzyloxy-5-(3'-butenyl)-4- [(t-butyl) dimethylsilyloxymethyl]]-2-methylpyridine
8	39.16	5.79	5.87	1.68	2-hexamethyl-6,7-diphenyl,9-hexahydro-
9	39.27	0.61	3.27	0.32	3-methylcholest-5-en-3-ol nitrite
10	39.29	5.76	5.74	1.71	D-Valyl-DL-Valine, N, N'-dimethyl-N'-(3- chloropropoxycarbonyl)-, pentyl ester
11	39.337	3.73	5.33	1.19	3-Benzyloxy-5-(3'-butenyl)-4-[(t-butyl) dimethylsilyloxymethyl]]-2-methylpyridine
12	39.37	2.38	3.55	1.14	Diethyl [2'-(1"- cyclohexenyl) ethylimino] malonate
13	39.81	2.47	3.36	1.25	Ethanone, 2-(4-chlorophenyl)-1-cyclohexyl-2-(1-piperidinyl)-
14	40.245	17.53	7	4.27	4,5-dianilino-1,3-imidazolidinedicarbaldehyde
15	40.3	0.65	2.09	0.53	Ethanone, 2-(4-chlorophenyl)-1-cyclohexyl-2-(1-piperidinyl)

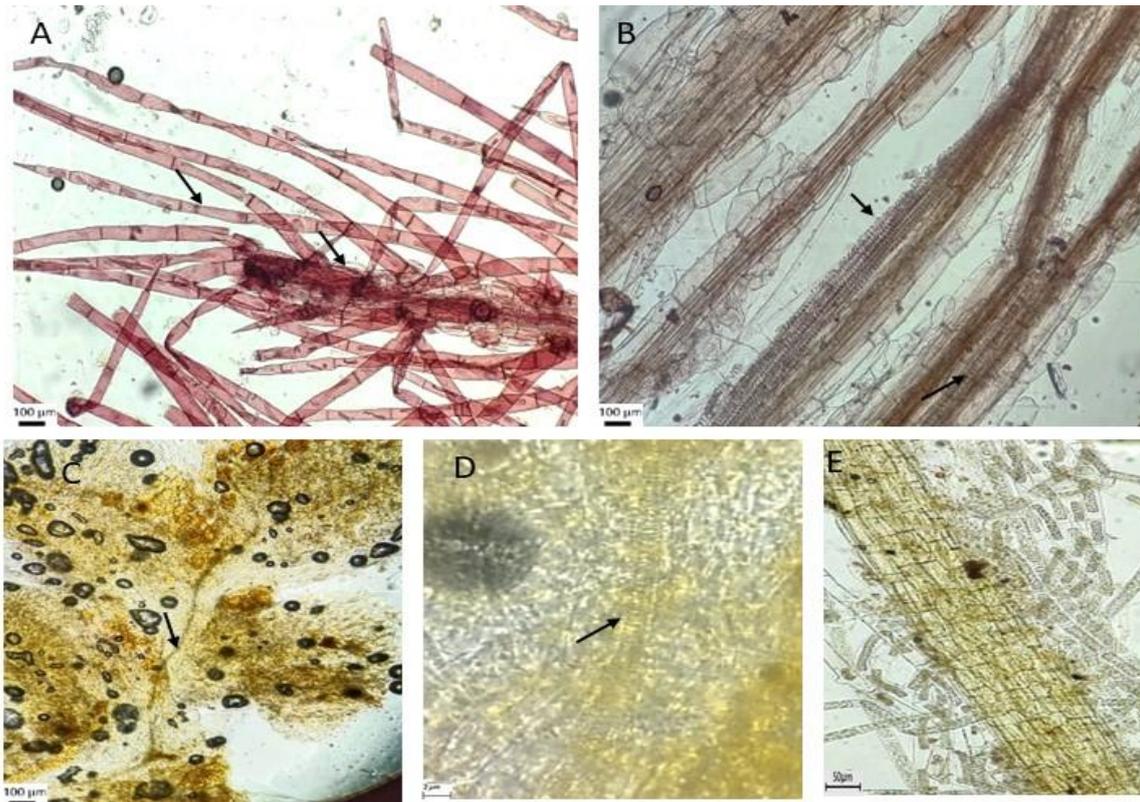
Table 2. Identification of compounds from methanolic extract of *Salvinia* plant

Peak	Retention time	Area %	Height %	A/H	Compound
1	10.048	21.12	18.69	1.74	Benzoic acid, 2,6-bis(trimethylsiloxy)-, trimethylsilyl ester
2	13.986	32.42	28.55	1.74	Cyclohexasiloxane, dodecamethyl-

3	17.603	5.63	7.41	1.17	2-cyclobuten-1-one, 4- [(1,1-dimethylethyl) dimethylsilyl] oxy]-2,3-dimethoxy-4-(3-phenyl-1-propynyl)-
4	37.062	3.7	3.36	1.69	4-(benzoylmethyl)-6-methyl-2h-1,4-benzoxazin-3-one
5	39.492	3.82	3.79	1.55	Dimethyl bis(3-iodopropyl) malonate
6	39.533	2.3	3.32	1.07	4 h-pyrazino carbazole, 5,6-dihydro-
7	39.58	1.24	3.38	0.56	Dimethyl bis(3-iodopropyl) malonate
8	39.722	3.27	3.61	1.39	3-methylcholest-5-en-3-ol nitrite
9	39.832	9.04	3.8	3.66	Octadecanoic acid, (1,1-dimethylethyl) dimethylsilyl ester
10	39.903	3.99	3.44	1.78	Dimethyl bis (3-iodopropyl) malonate
11	40.003	2.19	3.68	0.9	Ethanone, 2-(4-chlorophenyl)-1-cyclohexyl-2-(1-piperidinyl)-
12	40.065	2.4	6.2	0.6	3-methylcholest-5-en-3-β-ol nitrite
13	40.095	1.95	3.55	0.84	3 α -methylcholest-5-en-3-. β-ol nitrite
14	40.125	4.34	3.62	1.85	Dimethyl bis(3-iodopropyl) malonate
15	40.197	2.59	3.6	1.1	3 α -methylcholest-5-en-3-β-ol nitrite

3.3 Histological studies

The maceration studies using the Schultze method showed spirally coiled xylem tracheid (Figure.4). *A. filiculoides* have single tracheid in its whole anatomy and small branches bifurcates at nodes to the leaf lobes. The root has no specialized tracheid and elongated parenchymatous cells with thin walls and large vacuoles can be observed. In *Salvinia molesta*, the root carries multicellular pointed root hairs with blunt root tip. The root carries 1-2 celled spirally coiled xylem tracheid and in stem 4-5 bunches of xylem tracheid can be observed. The histological studies showed resemblances in spirally coiled tracheid cells with elongated large vacuole parenchyma cells in both plants. These structures may help them in the floating aquatic habitat of Polypodial plants.



A- Rhizodermal cells showing multicellular trichoblast, 1-2 spiral tracheids in *Salvinia* root, B- 4-5 spiral tracheids of *Salvinia* stem, C- single tracheid in *Azolla* plant, D- single tracheid in stem of *Azolla* (enlarged view), E-elongated parenchyma cells in *Azolla* root.

Figure. 4: Histological comparisons of *Azolla filiculoides* and *Salvinia molesta*.

3. 4 Antibacterial activity

The antibacterial activity of *A. filiculoides* and *S. molesta* were performed with gentamicin as a control. *Pseudomonas aeruginosa* and *Xanthomonas citri* are two common gram-negative bacterial pathogens affecting crop plants [25]. The methanolic extract of *Azolla* (MA) and *Salvinia* (MS) tested for antibacterial activity at various quantities (25 μ l, 50 μ l, 75 μ l and 100 μ l) against known concentration of *P. aeruginosa* and *X. citri* (Figure.5). Comparatively, *A. filiculoides* demonstrated better antibacterial activity against *P. aeruginosa* and *X. citri*. *A. filiculoides* showed inhibition with 22mm and 20mm diameter (100 μ l) against *P. aeruginosa* and *X. citri* separately (Table-3). *S. molesta* presented maximum inhibition (100 μ l) of 20mm and 17mm diameter against *P. aeruginosa* and *X. citri* respectively. Presumably, the antibacterial activity was pertaining to the presence of phytochemicals like alkaloids and quinones.

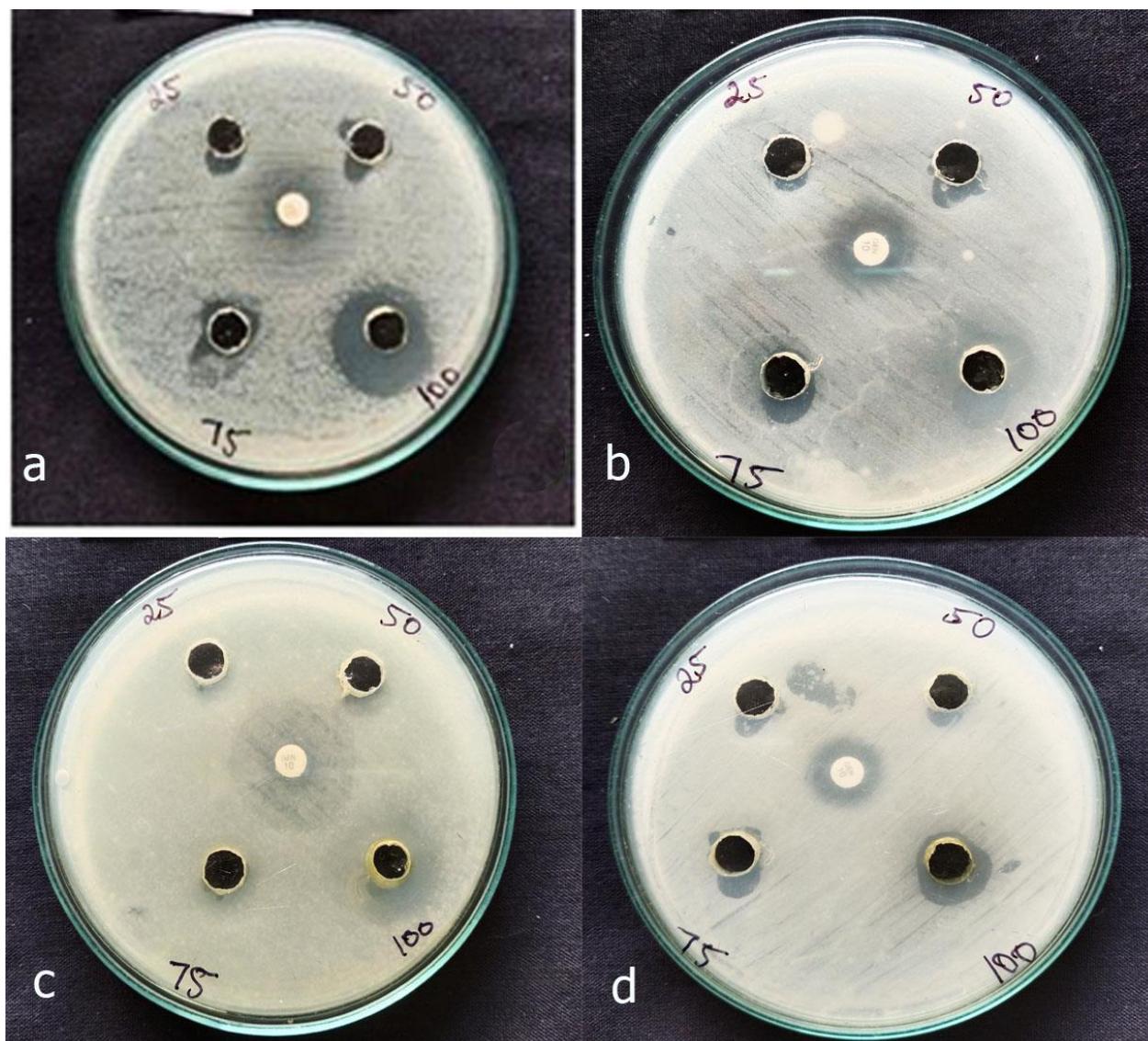


Figure. 5 Anti-microbial study on crude methanolic extract against *P. aeruginosa* and *X.citri*.

Table 3. Antibacterial activity of MA and MS

MA	MA extract and the Zone of inhibition (mm/ml)				
	25 μ l	50 μ l	75 μ l	100 μ l	Control
<i>P. aeruginosa</i>	13	15	18	22	24
<i>X. citri</i>	12	15	17	20	20

MS	MS extract and the Zone of inhibition (mm/ml)				
	25 µl	50 µl	75 µl	100 µl	Control
<i>P. aeruginosa</i>	13	15	18	20	24
<i>X. citri</i>	11	13	15	17	20

IV. RESULTS AND DISCUSSION

4.1 Results of Descriptive Statics of Study Variables

The habitat specific phytochemical and antibacterial properties of Salviniaceae were reported earlier [26] [27] and this study also revealed a significant level of antagonistic action on the microbes. The anatomical features of these ferns are only primitive level. The hadrocentric vascular bundles with phloem surrounded by xylem adaptations in vegetative parts help them to multiply faster. The *Salvinia* grows without roots and the leaf hairs are highly adapted to their buoyancy and reproduction. The ecological modifications of the *Azolla* helps them to grow faster and multiply in a good organic medium. Quinones, alkaloids and CHDC were found in both plant species. Commercially, these ferns are useful to support farmers' requirements in crop productivity, animal feed/husbandry/treatment of diseases, eco-friendly and sustainability. Salviniaceae ferns are widely used as biofertilizers and they indirectly act as a fuel for the plant growth. The enrichment of soil fertility by these ferns and also as fodder make them potential plant species. As the primitive Cryptogames, these ferns may be invasive to some places, but comparing to their soil reclamation they enrich nutrients to the soil. They made sociable niche to the diverse nitrogen enriching cyanobacteria and there by enhance the fertility of soil by reducing the noxious hydrocarbons [28], [29]. The Cyanophytes can also dwell in most of the hostile environment even the climate is also adverse. [30]. In this study, the anatomical comparison of ferns in evolutionary aspects has studied and also observed a direct correlation between their antibacterial efficacy with their biomass. Further, much studies on niche-based phytochemicals of ferns are essential for their preservation and use.

IV. ACKNOWLEDGMENT

The authors are grateful to the management of Bishop Heber College for the support to complete this work.

REFERENCES

- [1] Li FW, Brouwer P, Carretero-Paulet L. 2018. Fern genomes elucidate land plant evolution and cyanobacterial symbioses. *Nature Plants*. 4: 460–472. <https://doi.org/10.1038/s41477-018-0188-8>
- [2] Schuettelpelz E, Schneider H, Smith AR, Hovenkamp P, Prado J. 2016. A community-derived classification for extant lycophytes and ferns. *Journal of systematics and evolution*. 54 (6): 563–603. <https://doi.org/10.1111/jse.12229>
- [3] Priti Giri, Prem Uniyal. Edible Ferns in India and Their Medicinal Uses: A Review. *Proceedings of the National Academy of Sciences, India - Section B: Biological Sciences*. 2022; 92(4). [10.1007/s40011-021-01293-4](https://doi.org/10.1007/s40011-021-01293-4)
- [4] Michael G Simpson. *Plant Systematics* 2nd ed. Academic Press; 2010. 2(4): pp. 71-128. <https://www.sciencedirect.com/science/article/pii/B978012374380050004X>
- [5] Mickel John T, Yatskievych George, Walker Warren F, Wagner Warren H and Gifford Ernest M. *Fern*. *Encyclopedia Britannica*. 2023. <https://www.britannica.com/plant/fern>.
- [6] Vetter, J. Secondary Metabolites of Ferns. In: Fernández, H. (eds) *Current Advances in Fern Research*. Springer, Cham. 2018. https://doi.org/10.1007/978-3-319-75103-0_15
- [7] Jadhav D, Manda G, Vanita K. Phytochemical studies on three Epiphytic ferns From Mahabaleshwar and Panchgani hills. *Research journal of Life Science, Bioinformatics, Pharmaceutical and Chemical Sciences* .2019;5(3): 680-689. <https://doi.org/10.26479/2019.0503.55>
- [8] Tomaszewicz W, Cioć M, Dos Santos Szewczyk K, Grzyb M, Pietrzak W, Pawłowska B, Mikuła A. Enhancing In Vitro Production of the Tree Fern *Cyathea delgadii* and Modifying Secondary Metabolite Profiles by LED Lighting. *Cells*. 2022; 11(3):486. <https://doi.org/10.3390/cells11030486>
- [9] Bandyopadhyay A, Dey A. Medicinal pteridophytes: ethnopharmacological, phytochemical, and clinical attributes. *Beni-Suef Univ J Basic Appl Sci*. 2022; 11: 113. <https://doi.org/10.1186/s43088-022-00283-3>.
- [10] Deepa, K P. 2019. *In vitro* studies of *Ocimum sanctum* using various plant growth regulators. *ZENITH International Journal of Multidisciplinary Research*, 9(1): 252-257. <https://www.indianjournals.com/ijor.aspx?target=ijor:zijmr&volume=9&issue=1&article=028>.
- [11] Vasco, Alejandra and Moran, Robbin and Ambrose, Barbara. The evolution, morphology and development of fern leaves. *Frontiers in Plant Science*.2013. <https://www.frontiersin.org/articles/10.3389/fpls.2013.00345>
- [12] Ali S, Abbas Z, Rizwan M, Zaheer IE, Yavas I, Unay A, Abdel-Daim MM, Bin-Jumah M, Hasanuzzaman M and Kalderis D. Application of floating aquatic plants in phytoremediation of heavy metals polluted water: A review. *Sustainability*. 2020; 12: 1927. <https://doi.org/10.3390/su12051927>

- [13] Güngör E, Brouwer P, Dijkhuizen LW, Shaffar DC, Nierop KGJ, de Vos RCH, Sastre Toraño J, van der Meer IM, Schlupepmann H. Azolla ferns testify: seed plants and ferns share a common ancestor for leucoanthocyanidin reductase enzymes. *New Phytologist*. 2021; 229(2):1118-1132. doi:10.1111/nph.16896.
- [14] Deepa KP, Panneerselvam A and Thajuddin N. Seasonal variation of planktonic micro algal and cyanobacterial diversity in the temple pond of Tepakulam, Tiruchirappalli, Tamil Nadu. *ZENITH International Journal of Multidisciplinary Research*. 2019; 9(3): 23-28. <https://www.indianjournals.com/ijor.aspx?target=ijor:zijmr&volume=9&issue=3&article=003>
- [15] Yang YQ, Deng SF, Yang, YQ. Comparative analysis of the endophytic bacteria inhabiting the phyllosphere of aquatic fern *Azolla* species by high-throughput sequencing. *BMC Microbiology*. 2022; 22, 246. <https://doi.org/10.1186/s12866-022-02639-2>
- [16] Farook MA, Muthu Mohamed HS, Santhosh Kumar G, Subash S, Paranjothi M, Muhammed Naveez V, Naveen Kumar M, Muhammed Shariq K and Aadil Ahmed I. Phytochemical screening, Antibacterial and Antioxidant activity of *Azolla pinnata*. *International Journal of Research and Analytical Reviews*. 2019; 6 (1): <https://www.researchgate.net/publication/353038052>
- [17] Pugazh, Santhosh TG, Nithya & Lakshmi, Gokila Marino, Subramanian G, Bala vaishnavi Murugesan, Kamaraj. Assessment of phytochemicals, antioxidant, antibacterial activity, and profiling of functional molecules in novel freshwater fern *Salvinia cucullata* Roxb. *South African Journal of Botany*. 2022; 151. <https://doi.org/10.1016/j.sajb.2022.02.030>
- [18] Kokate CK, Purohit AP, Gokhale SB. *Pharmacology: Pathway to screen phytochemical nature of natural drugs*. Pune, India: Nirali Prakashan; 2008. p. A1-A6.
- [19] Shashank Kumar, Pramod Kumar, Anzari SA. A rapid and economic method for the maceration of wood fibres in *Boswellia serrata* Roxb. *Tropical Plant Research*. 2015; 2(2) 108-111. <https://www.tropicalplantresearch.com/archives/2015/vol2issue2/7.pdf>
- [20] Mabel Merlen Jacob, Magna Jom, Ameena Sherin, Binu Shahla. *Azolla pinnata*: Potential Phytoremediation, Antimicrobial, and Antioxidant applications. *Letters in Applied Nano BioScience*. 2020; 9 (1)4: 1673 – 1679. <https://doi.org/10.33263/LIANBS94.16731679>
- [21] Deepa, K. P., and N. Thajuddin. "Biofilm Inhibitory Potential of *Oscillatoria tenuis* Against *Candida albicans*". 2023. *Plant Science Today*, vol. 10, no. 3, July 2023, pp. 422-9, [doi:10.14719/pst.2434](https://doi.org/10.14719/pst.2434).
- [22] Upendra Kumar, Nayak AK. *Azolla* Germplasm at NRRI Conservation- Characterization and Utilization. ICAR- National Rice Research Institute [Internet]. 2019. https://icar-nrri.in/wp-content/uploads/2019/07/1.-NRRI_Research-Bulletin-No.-19.pdf
- [23] Coetzee JA and Hill MP. *Salvinia molesta* D. Mitch. (Salviniaceae): impact and control. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*. 2020; 15: 033. Doi:10.1079/pavsnr202015033
- [24] Hien Giang Pham, Manh Tuan Ha, Thao Quyen Cao, Ngoc Khanh Vu, Thi Thanh Le, Byung Sun Min. Chemical constituents from *Salvinia natans* (L.) (Salviniaceae) and their chemotaxonomic significance. *Biochemical Systematics and Ecology*. 2022; 102. <https://doi.org/10.1016/j.bse.2022.104410>.
- [25] Miller SA, Ferreira JP, LeJeune JT. Antimicrobial Use and Resistance in Plant Agriculture: A One Health Perspective. *Agriculture*. 2022; 12, 289. <https://doi.org/10.3390/agriculture12020289>
- [26] Castillo Loría K, Emiliani J, Herrero MS, Bergara, CD, Salvatierra LM, Perez LM. Effect of daily exposure to Pb-contaminated water into *Salvinia biloba* physiology and phytoremediation performance. *Aquatic Toxicology*. 2019; 210: 158–166. <https://www.sciencedirect.com/science/article/abs/pii/S0166445X18310877?via%3Dihub>
- [27] Anjana VJ and Lekshmanaswamy M. Biomass increase and preliminary phytochemical screening of *Azolla microphylla* Kaulf grown under four soil nutrient medium. *Journal of Advanced Scientific Research*. 2019; 10 (4) 2: 275-278. <http://www.sciensage.info/jasr>
- [28] Deepa, K. P., A. Panneerselvam, and N. Thajuddin. "Biodegradation studies of a microalgae, *Coelastrella* SP. NTAPD 01 isolated from hydrocarbon spills." *Research Journal of Biotechnology* 15.3. 2020: 114-119.
- [29] Deepa, K. P., A. Panneerselvam, and N. Thajuddin. "A study on the waning effect of oil spill isolated microalga *Coelastrella* sp. on a synthetic dye, Eriochrome black T." *Asian J. Microbiol. Biotechnol. Environ. Sci* 21 (2019): 205.
- [30] Deepa, K. P. A comprehensive analysis on carbon assimilation and longevity of algae in induced stress condition. *Journal of Emerging Technologies and Innovative Research*. 2019 6 (3), 340-342. <https://www.jetir.org/papers/JETIR1903654.pdf>