

Cultivation, Phytochemical analysis and antimicrobial activity of *Azolla pinnata*

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Abstract— *Azolla* is a free-floating aquatic fern also known as duck weed. *Azolla* is a good source of protein and minerals. In the present investigation the cultivation of *Azolla pinnata* was carried out, the phytochemical analysis and antimicrobial activity of ethanolic extract of aerial parts of *A. pinnata* was also investigated. In the qualitative phytochemical tests the ethanolic extract showed the presence of alkaloids, terpenoids, phenolic compounds, proteins and carbohydrates. The other phytochemicals including tannins, flavonoids, glycosides and saponins were absent. The ethanolic extract showed antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The result of the current study suggested that the extract of *A. pinnata* possesses phytochemical compounds with significant antimicrobial activity.

Index Terms- *Azolla pinnata*, phytochemical analysis, ethanol, antimicrobial activity.

I. INTRODUCTION

Azolla is an aquatic fern or small leafed floating plant, seen in quiet and slow-moving water bodies. It produces maximum biomass in a relatively shorter period of time (Jacob et. al. 2020). It is commonly known as mosquito fern, duckweed fern, or water velvet. It has a symbiotic relationship with the nitrogen fixing cyanobacterium *Anabaena azollae*, which resides in special cavities of the dorsal leaf lobe. The endosymbiont provides adequate nitrogen to its host. The fern, in turn, provides a protected environment for the alga and also supplies it with a fixed carbon source (Bocchi et. al. 2010; Jacob et. al. 2020).

It is typically found all across India in places with standing water, such as ponds, streams, canals, and other areas where water is present for longer periods of time under sunlight or shade of tree (Masoodi and

Khan, 2012; Katole et. al., 2017). Indian species are triangular in shape, measuring 1.5 to 3.0 cm long and 1 to 2 cm wide. *Azolla* is rich source of proteins, amino acids, vitamins (A, B12, beta-carotene), growth promoter intermediates, and minerals such as calcium, phosphorus, potassium, iron, copper, and magnesium (Herath et. al., 2023).

Phytochemicals are natural bioactive compounds synthesized in plants that appear to have important physiological impacts in the human body. They cover a broad range of chemical substances such as, alkaloids, flavonoids, polyphenols, saponins, steroids, vitamins, among others. Depending on their role in plant metabolism they are divided into two types viz primary and secondary metabolites (Rex et. al., 2018). Sugars, amino acids, proteins, chlorophyll etc. are examples of primary metabolites whereas, the secondary metabolites includes flavonoids, alkaloids, terpenoids, saponins, tannins and phenolic compounds. The therapeutic properties of plants are due to phytochemicals (Savithamma et al., 2011).

Azolla has several applications such as green manure, N biofertilizer, cattle, chicken, and fish feed supplements because of its high protein content. *Azolla* is one of nature's protein-rich wonder plants. It can grow on wastewater improving its quality. *Azolla* biomass can be utilized for energy production. *Azolla* biomass has been used for biodiesel, biogas, and biohydrogen production.

II. MATERIALS AND METHODS

Collection and cultivation of *azolla*

The *Azolla pinnata* samples were procured from the *Krishi Vigyan Kendra College, Parbhani, Maharashtra* and its identification and authentication was carried out. *Azolla* was grown in plastic trays of size 32cm length and 18cm breadth. Initially the sieved soil was added in the trays and the depth of soil layer was kept about 2cm. Sufficient water was added and cowdung as fertilizer was also added. The culture of green *azolla* was inoculated and the water level was maintained regularly 15 to 21 days until harvesting.

Preparation of extract: The harvested *A. pinnata* was thoroughly washed with distilled water to remove all the impurities, dust particles, shadow dried at room temperature and grinded to coarse powder using a mechanical mixer. The powder was subjected to extraction by maceration using ethanol solvent. To 5gm of the powder, 100ml solvent ethanol was added and stirred in orbital shaker (Mangesh Kumar et al., 2016). The mixture was filtered on the 2nd day and the solvent was evaporated at room temperature for 18-24 hours to obtain a solid mass, and stored in refrigerator (4°C) for further use (Farook et. al. 2019).

Phytochemical analysis:

The ethanolic extract of aerial parts of *A. pinnata* were tested for presence and absence of phytochemicals (in qualitative forms), like alkaloids, tannins, terpenoids, flavanoids, saponins, phenolic compounds, glycosides, proteins and carbohydrates using standard procedures and reagents.

1. Test for Alkaloids

Wagner's test

For the detection of alkaloids in the plant extracts few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube. A reddish-Brown precipitate confirms the test as positive (Raaman, 2006).

2. Detection of Tannins

Braymer's test

For the detection of presence or absence of tannins in the plant extracts, to the 1mL of plant extract 3mL distilled water was added. To this 3 drops 10% Ferric chloride solution was added. Blue-green colour formation indicates positive test (Uma et. al., 2017)

3. Test for Terpenoids

Liebermann-Burchard test: 2 ml of the extract, 2 ml of the chloroform and 2 ml of the acetic acid, 1 ml of the

conc.H₂SO₄ are added. Appearance of blue green colour/reddish ring indicates the presence of Terpenoids.

4. Test for Flavonoids

Ferric chloride test:

This test was carried out by adding few drops 10% ferric chloride solution to the plant extracts. A green precipitate shows positive test (Audu, 2007).

5. Test for Glycosides

Aqueous NaOH test

To the ethanolic plant extract 1mL of water was added mixed it properly and few drops of aqueous NaOH solution was added. The formation of a yellow colour indicates positive test (Jagessar 2017).

6. Test for Phenolic compounds

Ferric chloride test:

The test for the detection of phenolic compounds was performed by adding few drops of 5% FeCl₃ solution to the plant extracts. The appearance of Dark green/bluish black colour indicates positive test (Tiwari et. al., 2011).

7. Test for Proteins and Amino acids

Millon's test:

This test is performed by adding few drops of Millon's reagent to 2mL plant extract.

A white precipitate formation indicates positive test.

8. Test for Carbohydrates

Molish test

2 ml of extract, 2 ml of Molish reagent and 2 ml of Conc.H₂SO₄ are added. Presence of reddish ring indicates the presence of Carbohydrate

9. Test for Saponins

Foam test:

For the detection of saponins in plant extracts, the 50 mg of plant extract is diluted with distilled water and made up to 20 ml. The suspension is shaken for 15 minutes. The formation of two cm layer of foam shows the presence of saponins (Devmurari, 2010).

Antimicrobial Activity: The well Diffusion Method was used to evaluate the presence of antibacterial activity of ethanolic extracts of *A. pinnata*. The microorganisms *Pseudomonas aeruginosa* and *Bacillus subtilis* were used. 0.1ml of selected pure bacterial culture of *Pseudomonas aeruginosa* and *Bacillus subtilis* was added in each nutrient agar plate individually and spread uniformly by using a glass spreader. Agar well of 5 mm in diameter was prepared in each plate with the help of a sterilized stainless cork

borer. The plates were labeled appropriately. In each well 50 µL of selected plant extract was added using a micro-pipette. All the plates were incubated at 37°C for 24 hours. Each plate was observed for the presence or absence of growth inhibition zone (Anosike et al., 2012).

III. RESULTS AND DISCUSSION

After 14-21 days of incubation the azolla plants formed a green colour mat due to its rapid multiplication. The green mat of azolla was harvested.



Fig.1 *Azolla pinnata*

The qualitative screening is very important to determine the phytochemical compounds present in herbal plants. This procedure is a simple preliminary pre-requisite before going for detailed phytochemical investigation (Kavitha, 2017).

Phytochemical analysis of ethanolic extract of aerial parts of *A. pinnata*

The preliminary phytochemical screening of ethanolic extract of aerial parts of *A. pinnata* revealed the presence of alkaloids, terpenoids, phenolic compounds, proteins and carbohydrates. These results are in accordance with the results observed by Farook et. al., 2019. The other phytochemicals including tannins, flavonoids, glycosides and saponins were absent as shown in Table 1.

Table 1: Phytochemical analysis of ethanolic extract of aerial parts of *A. pinnata*

Sr.	Phytochemical	Test	Observation
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n o.			
1	Alkaloids	Wagners test	+
2	Tannins	Braymer's test	-
3	Terpenoids	Lieberman-Burchard test	+
4	Flavonoids	Ferric chloride test	-
5	Glycosides	Aqueous NaOH test	-
6	Phenols	Ferric chloride test	+
7	Proteins	Millon's test	+
8	Carbohydrates	Barfoed's test	+
9	Saponins	Foam test	-

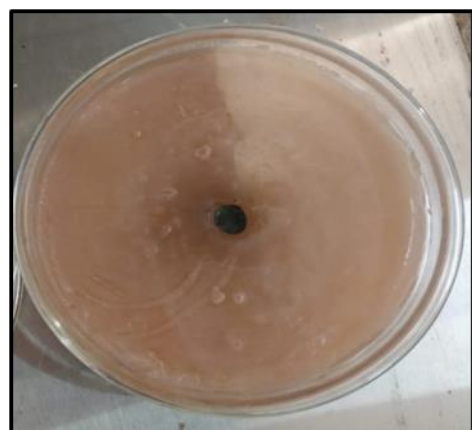
+ Present - Absent

In the present investigation ethanolic extract of *A. pinnata*, showed antibacterial activity against the disease-causing organisms *Pseudomonas aeruginosa* and *Bacillus subtilis* as shown in table 2.

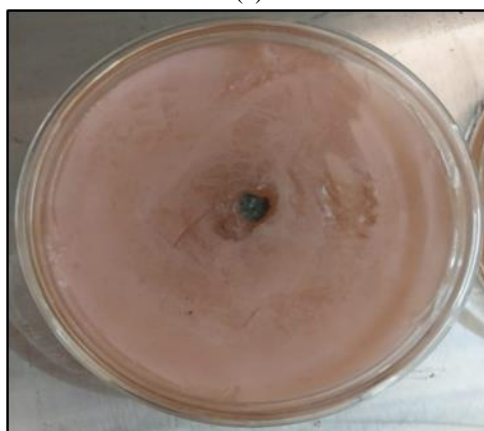
The bioactive compounds in the ethanolic solvent extracts of *A. pinnata* have antimicrobial potency and perhaps may have contributed to its antimicrobial activity.

Table 2 Antibacterial activity of ethanolic extract of *Azolla pinnata*

Test organism	Zone of inhibition in mm
<i>Pseudomonas aeruginosa</i>	08
<i>Bacillus subtilis</i>	05



(a)



(b)

Fig. 2: Antibacterial activity of ethanolic extract of *Azolla pinnata*

(a) *Pseudomonas aeruginosa* (b) *Bacillus subtilis*

CONCLUSION

Plants are the basic source of medicines for the modern life sciences. The cheap cost, low incidences of adverse reactions when compared to modern pharmaceuticals are encouraging public and health care institutes to turn to plant medicines. With this preliminary research studies, it was found that the species *A. pinnata* found to be a good source of various bioactive compounds and also have antibacterial activities.

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REFERENCES

- [1] Anosike, C. A., Ogili, O. B., Nwankwo, O. N., & Eze, E. A. (2012). Phytochemical screening and antimicrobial activity of the petroleum ether, methanol and ethanol extracts of *Ceiba pentandra* stem bark. *Journal of Medicinal Plants Research*, 6(46), 5743-5747.
- [2] Audu, S.A., Mohammad, I. and H.A. Kaita (2007) Phytochemical screening of the leaves of *Lophira lanceolata* (Ochanaceae). *Life Sci J*. 4(4):75-79.
- [3] Bocchi, S.; Malgioglio, A. *Azolla-Anabaena* as a Biofertilizer for Rice Paddy Fields in the Po Valley, a Temperate Rice Area in Northern Italy. *International Journal of Agronomy* 2010, 1-5.
- [4] Devmurari, V. P. (2010). Phytochemical screening study and antibacterial evaluation of *Symplocos racemosa* Roxb *Arch. Appl. Sci. Res.* 2(1): 354-359.
- [5] Herath, Bimal Manuranga & Yapa, Neelamanie & Karunaratna, Samantha. (2023). *Azolla* as the multifunctional fern in organic agriculture: Prospects and challenges: A Review Article. 19. 63-82.
- [6] Jacob M.M., Jom M. , Sherin A. and Shahla B. (2020). *Azolla pinnata*: Potential Phytoremediation, Antimicrobial, and Antioxidant Applications. *Letters in Applied NanoBioscience*, Volume 9, Issue 4, 1673 - 1679
- [7] Jagessar, R.C. (2017). Phytochemical screening and chromatographic profile of the ethanolic and aqueous extract of *Passiflora edulis* and *Vicia faba* L. (Fabaceae). *J Pharmaco and Phyto.* 6(6):1714-1721.
- [8] Katole S.B., Lende S.R. and S.S. Patil (2017). A Review on Potential Livestock Feed: *Azolla*. *Livestock Research International*, Vol. 05(01), Pages 01-09.
- [9] Masoodi A and Khan FA (2012). A new record to the invasive Alien Flora of India: *Azolla cristata*. *National Academy Science Letters*, 35: 493-495
- [10] Raaman, N. (2006). *Phytochemical Techniques*. New India Publishing Agency, New Delhi, 19-24.

- [11] Rex, J.R.S., Muthukumar, N.M.S.A. and P. M. Selvakumar (2018). Phytochemicals as a potential source for anti-microbial, anti-oxidant and wound healing - a review. *MOJ Biorg Org Chem.* 2(2):61-70.
- [12] Savithamma, N., Linga Rao M., and D. Suhulatha (2011). Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research.* 8: 579-84.
- [13] Tiwari, P., Kumar, B., Kaur, M., Kaur, G. and H. Kaur (2011). Phytochemical screening and extraction: A Review. *Internationale Pharmaceutica Scientia.* 1(1):98-106.
- [14] Uma, K.S., Parthiban, P. and S. Kalpana (2017). Pharmacognostical and preliminary phytochemical screening of Aavaarai Vidhai Chooranam. *Asi J of Pharma and Clin Res.* 10(10):111-116.