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Azolla pinnata R. Br. : An aquatic macrophyte as a potential therapeutic candidate

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Abstract

The aquatic plants are gaining popularity in nutrition studies because of their wide range of uses in animal and human food. Pteridophytes like *Azolla* (Azollaceae) float freely in water. It could be used as a natural plant-based antimicrobial and also a water purifier in a laboratory or for industrial wastewater management. It could have been used as animal/bird feed, human food, a water purifier, green manure or vermicompost, biogas, a biolarvicide, and to enhance soil microbial diversity. Other than significant amounts of β-carotene and vitamin B_{12}, *Azolla pinnata* R. Br. is a great source of protein and comprises all those basic amino acids and minerals such as iron, calcium, magnesium, potassium, and so on. India has a large number of marine macrophytes, which had not yet been thoroughly investigated phytochemically and pharmacologically. Phenols, saponins, flavonoids, tannins, proteins, and other phytochemicals are found in this aquatic weed. Because of its phytoconstituents, it has been broadly used in pharmacological circumstances such as anticancer, antioxidant, and anti-inflammatory, antimicrobial, analgesic, antipyretic, hepatoprotective, *etc*. The purpose of this article is to review the findings of other researchers' studies on the phytochemical and pharmacological characteristics of *A. pinnata*, in the hopes that they will be useful for future science investigation.

1. Introduction

Aquatic spermatophytes (flowering plants), pteridophytes (ferns), and bryophytes (mosses, hornworts, and liverworts) are all examples of aquatic macrophytes. These aquatic macrophytes are usually classified into four groups depending upon their growth forms, including Group I: emergent macrophytes, Group II: floating left macrophytes/plants, Group III: submerged macrophytes or plants mostly emerging entirely underneath the water surface, comprising mosses, charophytes, several pteridophytes and many angiosperms, and Group IV: free-floating macrophytes, or plants that are not rooted to the ground, are a diverse group in terms of ecosystems and forms (*e.g*., *Eichhornia crassipes*, *Salvinia* sp., *Azolla* sp., and Lemna sp.) (Hassan *et al*., 2014; Malik *et al*., 2020). *Azolla* spp. seem to be free-floating freshwater ferns with heterosporous spores. The genus *Azolla* contains six species that are found around the world in temperate, sub-tropical, and tropical climates. Within the genus, the six noticeable species are divided into two subgenera: Euazolla and Rhizosperma. *A. filiculoides*, *A. caroliniana*, *A. mexicana*, and *A. microphylla* are the four species that comprise the Euazolla subgenus. *A. nilotica* and *A. pinnata* are the two species that belong to the Rhizosperma subgenus.

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Figure 1: *Azolla pinnata* R. Br.

2. Description

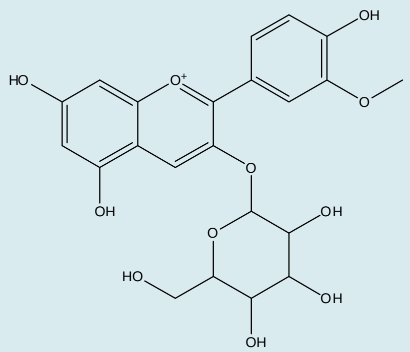
The only genus in the Azollaceae family, *Azolla pinnata* R. Br. (Figure 1), is an aquatic fern with the diameter varying from 1-2.5 cm, a short, branched, floating stem bearing roots which dangle in warm-temperate and tropical water ponds, ditches, and rice fields around the world. The fern *A. pinnata* is known by many names, such as mosquito fern, duckweed fern, fairy moss, green gold mine, and water velvet and it could be mostly found in African and Asian territory (Lal and Nayak, 2012; Sri Bhuvaneswari *et al.*, 2021). Each leaf has a thick aerial dorsal lobe with green chlorophyll and a relatively greater thin, colorless, floating ventral lobe. Anthocyanin pigment provides the fern a reddish-brown color in certain environments. The reddish carpet takes on a dark green hue from them. *Azolla* plants are triangular or polygonal in texture and sail on the water surface, individually or in mats (Lumpkin *et al*., 1980).

3. Phytoconstituents

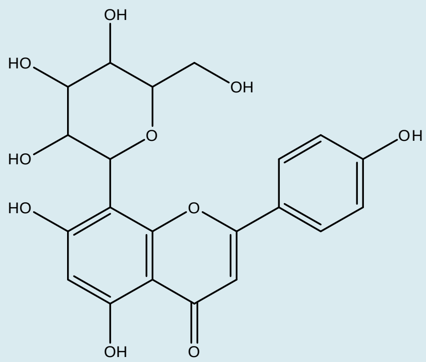
Many secondary metabolites are found in *A. pinnata*, including basic amino acids, vitamins, beta-carotene, minerals, saponin, and flavonoids (Thi Linh Nham *et al.*, 2020). It is often regarded as a high-quality protein source (Kumar *et al.*, 2017). *Azolla* is abundant in phenols, tannins, carbohydrates, flavonoids, hormones, proteins, and other phytochemicals, and has a wide variety of biological and pharmacological properties (Abraham *et al.*, 2012). Bioactivities are similar to antioxidant and anti-inflammatory (Duraisami *et al.*, 2021) behaviours ultimately depend on phenolic and flavonoid contents present in the weed plant (Selvaraj *et al.*,2013). Muraleedharan and his team studied phytochemical analysis on this plant, *A. pinnata* and found phenolic compounds, tannin, sugars, steroid, saponin, xanthoprotein, flavonoid, and protein in the different extracts (Muraleedharan *et al.*, 2011). The active constituent's peonidin 3-O-glucoside, vitexin, rutin, thiamine, choline, tamarixetin, hyperoside, astragalin, and quercetin were found in *A. pinnata* ethanolic extract (Table 1) (Ekanayake *et al.*, 2007). Farook and his team identified alkaloids, phenol, terpenoids, carbohydrate, flavonoids, saponin, anthocyanin, coumarin, and oxalate in their phytochemical study of four solvent extracts, *viz.*, benzene, methanol, water, and ethanol of *A. pinnata* (Farook *et al.*, 2019).

The author Paul Brouwer and his team investigated alkaline protein extraction for protein development from the aquatic weed, *A. pinnata* (Paul Brouwer *et al.*, 2019). *Azolla* is made up of 25-35% protein, 10-15% minerals, and 7-10% amino acids, which are a mixture of amino acids, bioactive compounds, and biopolymers (Kathirvelan *et al.*, 2015). As a result of its biocomposition, *Azolla* is an effective feed replacement for livestock and broilers (Sonam Mishra *et al.*, 2020). Other than significant amounts of α-carotene (vitamin A precursor) and vitamin B12, *Azolla* has a decent amount of protein and almost all essential amino acids and minerals such as iron, calcium, magnesium, potassium, phosphorus, manganese, and so on (Shrikant *et al.*, 2017).

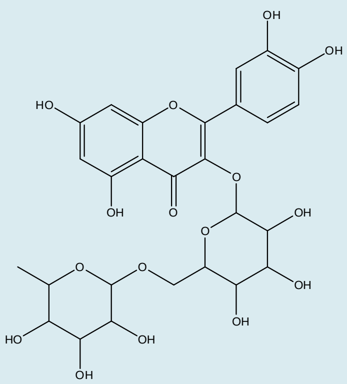
The growing efficiency and biochemical phenotype of *A. pinnata* and *A. caroliniana* grown in greenhouse conditions were studied by Taylan and Mustafa (2019). In comparison to *A. caroliniana*, *A. pinnata* had greater protein contents, lipid, cellulose, and ash levels. Palmitic, oleic, and lignoceric acids were identified to be the most important acids in *A. pinnata*. The LC-MS findings of *A. pinnata* extracts revealed primarily three essential chemical compounds, including 1-(O-alpha-D-glucopyranosyl)- -(O-alpha-D-glucopyranosyl)- (O-alpha (1,3R,25R) nicotinamide N-oxide, -hexacosanetriol, and pyridate (Table 1) (Rajiv *et al.*, 2020).



Peonidin 3-O-glucoside



Vitexin



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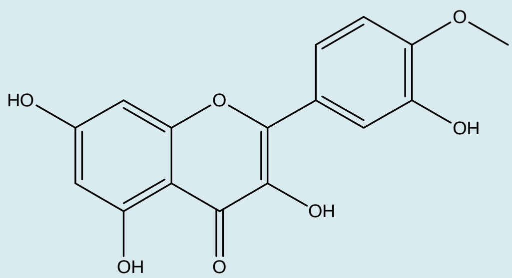

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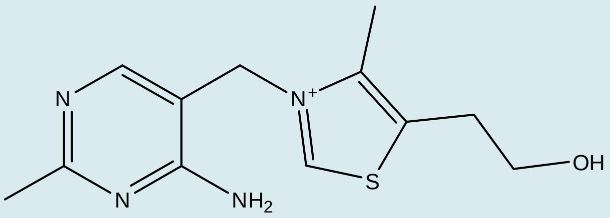
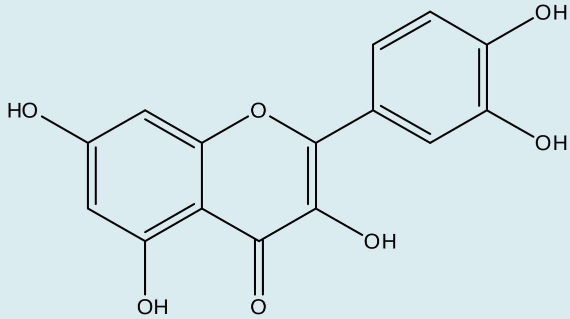
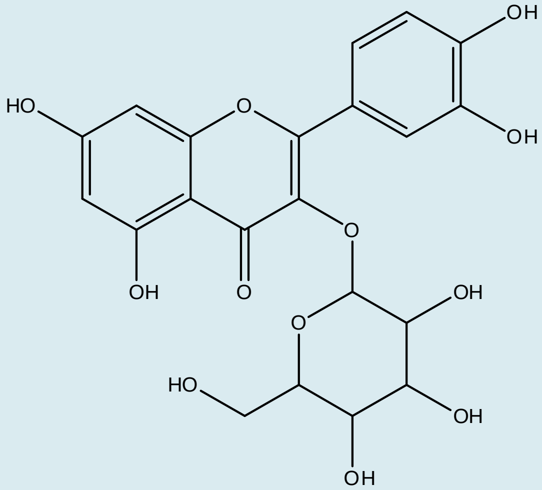
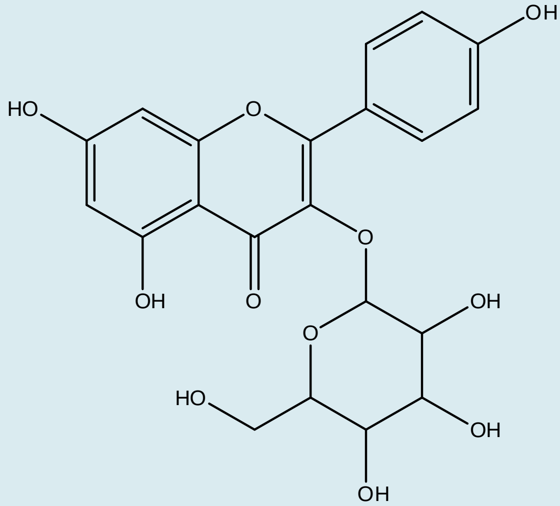
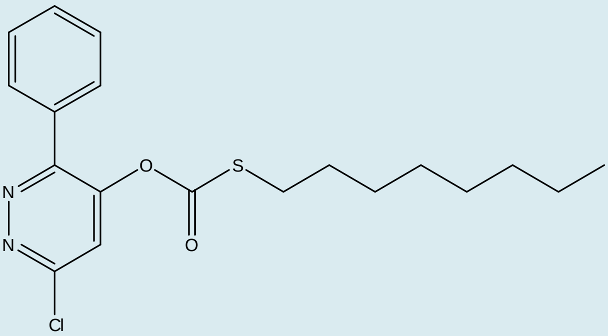
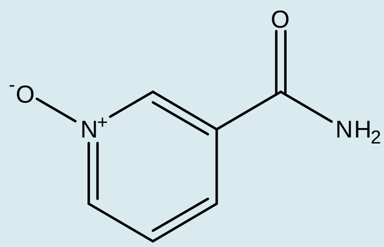
 **Thiamine**	 **Quercetin**
 **Hyperoside**	 **Astragalin**
 **Pyridate**	 **Nicotinamide N-oxide**

Table 1: Reported phytoconstituents from *Azolla pinnata* R. Br.

4. Biological and pharmacological significance of *A. pinnata*

4.1 In environmental studies

Azolla has a long history in agriculture, and its nitrogen-fixing properties have allowed it to be used as a biostimulant and green manure for rice crops. *Azolla* could be a valuable protein source for several animals, especially dairy cattle, chickens, pigs, and fish, since it has a high protein content (19-30%) than most green grass species and aquatic macrophytes and an essential amino acid content (lysine) that is beneficial for animal feed (Van Hove *et al.,* 2002; *Hasan et al*., 2009).

A. pinnata has been found to be effective in the mitigation of environmental pollutants. *A. pinnata* is an essential organic fertilizer in the cultivation of lowland tropical rice in Southeast Asia. The potential of *Azolla* to cure nitrogen and green manure is the key reason for its long-term success among farmers (Bhuvaneshwari *et al.*, 2012). *Azolla* prevents algae photosynthesis and, as a result, increases pH and NH_3 volatilization by lowering light intensity underwater. Since up to 50% of nitrogen fertilizer added to rice paddies is destroyed due to volatilization, *Azolla* may support to decrease the nitrogen fertilizer in rice farms (Lejeune *et al.,* 2000). The preservation of saline soils and the development of biofuels are two other advantages listed in the reported literature (Raja *et al.,* 2012).

4.2 Pytoremediation properties

A. pinnata has a high capacity for absorbing heavy metals, such as mercury (Hg) and cadmium (Cd) (70-94%), and could be used as a bioaccumulator to refine heavy metals in ash slurry and chloral-alkali effluent. Under a microcosm environment, *A. pinnata* has purify waters contaminated by two heavy metals, *viz*., Hg and Cd. The free-floating aquatic fern does have additional value for use in mitigation practices since it is easy and inexpensive to cultivate, it has increased nutrient efficiency combined with a high degree of nitrogen-fixation, this could thrive in a variety of conditions and concentrate nutrients (Kumar *et al.,* 2012), and it can be used in a wide range of applications as a biofertilizer, livestock feed, biofilter, bioweedicide, and heavy metal phytoremediation from floodwater.

Nitrogen and phosphorus, which induce water eutrophication, are removed by *Azolla* and it could even get rid of sulpha medications. *Azolla*, which contains the nitrogen-fixing symbiotic *Anabaena azollae*, is a well-established nitrogen-biofertilizer for rice that has been widely used in Asian countries for nitrogen-fertilization of rice production and as green manure helpful in processing metropolitan wastewater for irrigation, and the biomass generated could be used as biofertilizer or green manure after a slight acid treatment (Costa *et al.,* 2009). Through, microcosm environments, the degree of chromium (Cr) contamination in the Singrauli industrial area of India was evaluated, and the phytoremediation potential of a small water fern, *A. pinnata* was found to detoxify Cr-polluted waters (Muradov *et al.,* 2014).

Plant species have well-defined heavy metal-binding ligands called metallothioneins (MTs) and phytochelatins (PCs). Various *Azolla* species have diverse bioaccumulation potentials based on the heavy metal ions they produce. As an effect, the aggregation of Ni, Zn, Cu, and Cd in *A. pinnata* and *A. filiculoides* was investigated. Furthermore, the expression of genes encoding metallothionein and phytochelatin synthase was investigated at various metal concentrations. Cu and Cd accumulation were found to be greatest in *A. pinnata*. The heavy metal treatments greatly influenced MT2 and PCS1 gene expression patterns, supporting their functions in Azolla's phytoremediation capacity. According to the findings, *Azolla* is a best candidate for phytoremediation and the development of phytochelatin-heavy metal complexes and its sequestration in the vesicle is the key mechanism determining the vulnerability of *Azolla* to heavy metals (Majid *et al.,* 2019).

4.3 Organoleptic properties

A. pinnata is rich in nutritional content and protein, and it could be consumed by humans (Divya *et al.,* 2020), made yoghurt with *A. pinnata* extract in three separate amounts (1%, 2%, and 3%), and the lifespan was checked. The organoleptic characteristics of the *A. pinnata* integrated yoghurt were also investigated using the Nine-point Hedonic Scale of (Larmond *et al*., 1977). According to the findings in the separate trials, the organoleptic assessment of *A. pinnata* integrated yogurt in terms of color, flavor, taste, texture, and general acceptability was decreased significantly. The trials 1, 2, and 3 had a mean score of 7.6, 7.2, and 6.8, accordingly.

4.4 Weed and mosquito control

Azolla inhibits the production of certain aquatic weeds by creating a dense mat that harms weed seedlings of sunlight whereas mechanically blocking them from growing, which has been experimentally demonstrated and well accepted by farm workers. In the early twentieth century, it was proposed that *Azolla* could inhibit mosquito breeding, and thus the spread of paludism (therefore the name "mosquito fern") (Lumpkin *et al.,*1980; Van Hove *et al.,* 2000).

4.5 Antimicrobial activity

Evy Ratnasari and her team studied *A. pinnata* and discovered that it contains a range of ingredients and phytochemical compounds, including flavonoid, tannin, and saponin, which have antimicrobial action against *Salmonella typhi*. The zone of inhibition with a diameter of less than 10 mm were observed at varying concentrations of *Azolla* extract (20% -100% b/v). According to statistical analysis report, the treatment of *A. pinnata* has the capacity to suppress the growth of *S. typhi* (p<0.05). There was no major difference in the administration of *A. pinnata* towards *S. typhi* at varying concentrations of 60%, 80%, and 100% (Evy Ratnasari *et al.*, 2019 ; *Sreenivasagan et al.,* 2020).

Talreja and his team studied the *in vitro* antibacterial effects of ethyl acetate, methanol, and benzene plant extracts from different weed plant parts of *Achyranthes aspera*, *A. pinnata*, and *Cissus quadrangular* is towards pathogens like *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Escherichia coli*. According to their findings, the ethyl acetate extract of *Cissus quadrangular* had the maximum zone of inhibition towards *S. aureus* (25 ± 1.3 mm), accompanied by *Achyranthes aspera* (20.0 ± 0.5 mm) and *Azolla pinnata* (20 ± 0.9 mm), while the methanolic extract of the plants tested had outstanding results towards *P. aeruginosa*. Benzene extract has no activity against any of the experiment pathogens (Talreja *et al.,* 2017).

Vanmathi Selvi and her team investigated the antimicrobial efficacy of *A.pinnata* utilizing ethanol, chloroform, and water as solvents and screened towards human dental microbes, such as dental decaying pathogen strains 1, 2, 3, and 4, which were defined as *Streptococcus mutans* 1, 2, 3, and 4. The maximum growth inhibition diameter in ethanol extract was observed in *Streptococcus mutans* 2 and 3 with a diameter range of 24.3 ± 1.40 mm, 22.5 ± 1.56 mm, respectively. Meanwhile, water extract, displayed a maximum inhibitory activity towards *S. mutans* 1 and 4 with diameters of 20.5 ± 1.02 and 17.6 ± 0.93 mm, accordingly. The activity of the chloroform extract was regulated and limited to 12 ± 0.54 – 22.6 ± 0.56 mm, respectively. Their findings indicate that the ethanolic extract of *A. pinnata* was effective in treating periodontal disease (Vanmathi Selvi *et al.,* 2017).

The diverse extracts, namely; acetone, benzene, ethanol, methanol, and water of *A. pinnata* were assessed for their antimicrobial property against *Pseudomonas aeruginosa* and *Staphylococcus aureus* was reported by Farook *et al.,* (2019). The zone of inhibition was observed for *S. aureus* and *P. aeruginosa* to be 13 mm and 10 mm for acetone extract, 12 mm and 8 mm for benzene extract, 12 mm and 9 mm for ethanol extract, and 12 mm and 11 mm for methanol extract, accordingly. The aqueous extract, on the other hand, had no effect. According to their observations, *A. pinnata* and its metabolites had promising antibacterial activity.

Mahyuddin and his team discovered that methanolic extracts of *A. pinnata* were tested towards *Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* in their antibacterial experiments. Antimicrobial testing indicated that both extracts found to inhibit the growth of *B. subtilis* and *S. aureus*, but none prevented the growth of *E. coli* or *P. aeruginosa*. In their research, they discovered the maximum inhibitory activity with a diameter of 2.67 ± 1.53 mm and a minimum inhibitory concentration of 0.125 mg/ml towards *B. Subtilis*. It also suggests that the bacteriostatic characteristics of the extracts examined were responsible for the suppression of *B. subtilis* and *S. aureus* (Mahyuddin *et al.,* 2020).

The aim of the author, Thiripurasundari and her team analysis was to see if *A. pinnata* could be used as a class of antimicrobial substances and to look into the fern's antioxidant capacity. Phytochemical analysis of various solvents, including ethyl acetate, methanol, chloroform, acetone, and water extracts, showed the presence of diverse phytochemicals, particularly phenols and flavonoids in the extracts. Further, the antimicrobial effect was also tested using a variety of bacterial and fungal strains. Whereas, the antioxidant activity was assessed using the DPPH free radical scavenging assay and the FRAP assay, respectively. The *A. pinnata* methanolic extract had a high phenolic and flavonoid content, which showed in substantial antimicrobial and free radical scavenging and reducing capacity (Thiripurasundari *et al.,* 2018).

Mangesh Kumar and his team investigated the antibacterial effect of diverse extracts, including ethyl acetate, methanol, and benzene extracts of *A. aspera, A. pinnata, C. quadrangularis, T. cordifolia* on *S. aureus, P. aeruginosa,* and *E. coli* . Their findings revealed that plant extracts became effective in their activity at bacteria growth and displayed a zone of inhibition, however no growth of the tested microorganism was detected in benzene extracts. The ethyl acetate extract of *T. cordifolia* (30 ± 1.7 mm) showed the increased inhibition of growth towards *S. aureus*, accompanied by *C. quadrangularis* (25 ± 1.3 mm), *A. aspera* (20 ± 0.5 mm), and *A. pinnata* (20 ± 0.9 mm). The methanol extract of *T. cordifolia* showed the most antibacterial activity against *S. aureus* (25 ± 0.87 mm), accompanied by *A. aspera* (11 ± 0.61 mm), *C. quadrangularis* (10 ± 0.5 mm) and *A. pinnata* (10 ± 0.14 mm). Whereas, the antibacterial effect of methanol and ethyl acetate extracts of these selected plants towards *P. aeruginosa* and *E. coli* was also showed a good activity (Mangesh Kumar *et al.,* 2017).

The therapeutic efficacy of organic and aqueous extracts on bacteria and yeasts was studied by *Pereira* and Histeam. Organic (dichloromethane: methanol) and water extracts from six *Azolla* species were screened against bacterial potential pathogens and non-pathogenic strains, as well as *Candida albicans* and *Candida glabrata* pathogenic fungi. According to the findings, organic extracts of *A. caroliniana, A. rubra,* and *A. filiculoides* inhibited the growth of *B. subtilis,* while those of *A. caroliniana* and *A. microphylla* inhibited the growth of *S. aureus.* For *A. caroliniana, A. microphylla*, and *A. rubra*, the minimum inhibitory concentrations were greater than 4 mg/ml, and for *A. filiculoides,* they were greater than 3.25 mg/ml. With a minimum inhibitory concentration greater than 12.5 mg/ml, the water extracts of *A. filiculoides, A. caroliniana, A. microphylla, A. rubra*, and *A. pinnata* produced a limited inhibitory activity (1 mm) in *C. albicans*. Furthermore, the author revealed that the organic and water extracts of certain *Azolla* species could be used to treat gram-positive bacteria and *Candida albicans* infections, respectively (Pereira *et al.*, 2015).

4.6 Antioxidant activity

Under various concentrations of NaCl, the function and regulation of antioxidant components in two variants of *Azolla* (*A. pinnata* and *A. filiculoides*) were compared. In *A. pinnata*, overall superoxide dismutase (SOD) and ascorbate peroxidase (APX) was up regulated, while in *A. filiculoides,* all antioxidant activity was decreased. *A. pinnata* plants introduced to 30 mM NaCl contained less Na^+ ions and had less electrolyte leakage compared to *A. filiculoides* plants. Our findings show that antioxidant enzymes react differently to salt tolerance in *Azolla* plants. *A. pinnata* has been classified as salt resistant, as contrasted to *A. filiculoides*, which is salt sensitive (Masood and his team *et al.,* 2006).

According to Noor Nawaz and his team, the effectiveness of extracts of *A. pinnata* and *A. rubra* to act as antioxidants was evaluated by DPPH free radical scavenging assay, and the results indicated pronounced dose-dependent radical scavenging behaviour. *A. pinnata* had a greater scavenging ability (IC_{50} value 7.32 µg/ml) than *A. rubra* (IC_{50} value 14.47 µg/ml) as compared to regular ascorbic acid, which had a better IC_{50} value of 1.39 µg/ml. The existence of high phenolic and flavonoid content in *A. pinnata* could justify its greater antioxidant efficacy (Noor Nawaz *et al.,* 2014).

The potential of non-enzymatic antioxidants like vitamin C and E, enzymatic antioxidants like superoxide dismutase and catalase, and lipid peroxidation were assessed by Radhakrishnan *et al.* (2014) in *Macrobrachium rosenbergii* post larvae provided with formulated diet that included *Spirulina platensis, Chlorella vulgaris,* and *A.pinnata*. Their study report revealed that the levels of vitamins C and E in the hepatopancreas and muscle tissue was improved significantly ($p<0.05$) with *S. platensis, C. vulgaris*, and *A.pinnata* incorporated diet fed groups. While compared to the control, the formulated materials in the 50% incorporated feed fed groups performed higher in non-enzymatic antioxidant function. In the enzymatic antioxidant analysis, the 50% fish meal substitute diet fed groups displayed no substantial changes in SOD, CAT, or LPX as compared to the control. The authors concluded that the formulated feed enhanced the vitamins C and E, and decreased the level of enzymatic antioxidants and moreover, these ingredients could be used as an alternative protein source.

Mabel Merlen and his team investigated the antimicrobial activity of benzene and ethanol extracts on *Bacillus* and *Staphylococcus* pathogens and found that benzene inhibits *Bacillus* and *Staphylococcus* organisms to a lesser extent than ethanolic extracts, *viz*., 20 µl and 30 µl, accordingly. When compared to standard ascorbic acid, the antioxidant effectiveness of benzene and ethanol extracts was discovered to be 10 and 20 µl, approximately, by using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay procedure. The greater amount of total phenolics and flavonoids in the plant extract might cause higher antioxidant effectiveness. The percentage antioxidant effect was recorded to be 74, 58.47, and 63% for 10 µl, respectively, and for 20 µl, 75, 60.81, and 65%, correspondingly (Mabel Merlen *et al.*, 2020).

The green synthesis of selenium nanoparticles with *A. pinnata* extract act as a catalyst in oxidation and effective antioxidant and antimicrobial agent against *E. coli, E. faecium, C. albicans*, and *S. aureus* (Gopalan Rajagopal *et al.*, 2021). The crystallite size of the *A. pinnata* stabilized nanoparticle was found to be 36.45 nm with spherical in shape. The nanoparticle's radical scavenging potency was found at highest concentration of 500 µg/ml. However, the zone of inhibition on antimicrobial activity against *E. coli* (17 ± 0.67 mm), followed by *E. faecium* (15 ± 0.13 mm), *C. albicans* (15 ± 0.32 mm), and *S. aureus* (14 ± 0.93 mm). Hence, it was proved that the synthesized nanoparticles act as a catalyst in oxidation and effective antioxidant and antimicrobial agents.

Eltabakh and his team produced the sodium alginate (SA) maltodextrins (MD) based functional films incorporated with phenolic extract of *A. pinnata* leaves fern (AF). AF at different concentrations, *viz*., 0.8, 1.2, and 1.6% w/w inside the films were characterized by scanning electron microscope, thermal disposal by differential scanning chromatography, crystallization by X-ray diffraction, potential interaction by infrared spectroscopy, and along with its mechanical properties. However, the antioxidant and antimicrobial properties were enhanced by the presence of its phytoconstituents, *viz*., ferulic acid, rutin, thiamine, tamarixetin, astragalin, quercetin, chlorogenic acid, and epicatechin of the extract. Thus, the authors concluded that the resulted films could be utilized as composite material for diverse food applications (Eltabakh *et al*., 2021).

4.7 Hepatoprotective activity

The protective efficacy of *A. pinnate* ethanolic extract on lead-induced hepatotoxicity in rats was explored by (Elrasoul *et al.*, 2020). Lead acetate raised serum levels and changed the morphology of the hepatic tissue. Moreover, it reduced the interleukin and glutathione levels in the blood, as well as the catalase and superoxide dismutase activity in hepatic tissue. The treatment of an ethanol extract that is rich in the photochemical such as, tamarixetin, rutin, and quercetin alleviated the effects of lead on liver function and structure. As a result of its antioxidant and anti-inflammatory properties, *A. pinnate* extract is a preventive and therapeutic agent for lead-induced hepatotoxicity.

A. pinnata extract modulated the toxic effects of ranitidine and normalized hematological parameters after 30 days treatment. The intoxication of the rats with ranitidine elevated significantly ($p<$ 0.05) RBCs count and WBCs count. Similarly, *A. pinnata* modulated the toxic effects of ranitidine on liver and kidney functions biomarkers and the intoxication of the rats were elevated significantly ($p<0.05$), the activities of serum ALT and AST and serum levels of urea and creatinine. However, treating with *A. pinnata* had no significant effect on hematological parameters and liver and kidney functions biomarkers as compared with the control rats ($p<0.05$). *A. pinnata* extract relapsed the effects of ranitidine on serum levels of inflammatory and anti-inflammatory cytokines. *A. pinnata* extract ameliorated the deleterious effects of ranitidine on oxidative and antioxidant statues in hepatic tissues of rats (Abd Elrasoul *et al.*, 2012).

4.8 Analgesic and antipyretic activity

Jerine Peter and his team investigated the various extracts of *A. pinnata*, including an ethanol, methanol, and water, which were serially diluted at concentrations of A (1:32), B (1:16), C (1:8), D (1:4), and E (1:2) and subjected to pharmacological testing such as the hot plate test, analgesic, and antipyretic testing, and assorted assays such as the DPPH assay, peroxidase and catalase activity. According to the findings, the percentage inhibition was higher at 1:2 ratios of ethanol extract than methanol or water extracts, and the percentage was equal to 0.9 µ/mg. While, at 250 mg/kg, the *A. pinnata* administration improved the reaction time, extended the response time, and increased the pain-relieving movement analgesic activity on the hot plate method (Jerine Peter *et al.*, 2021).

4.9 Larvicidal effect

Rajiv and his team found that *A. pinnata* plant extracts were effective for *Aedes aegypti* and *Aedes albopictus* mosquitoes in four separate tests. In the adulticidal experiment, there was a substantial rise in death as the test concentration rises, and with *A. pinnata* extracts observed the LC50 and LC95 values of 2572.45 and 6100.74 ppm, correspondingly, on *Aedes aegypti* and 2329.34 and 5315.86 ppm, respectively, on *Aedes albopictus*. The ovicidal result demonstrates that all concentrations tested at 1500 ppm, 1000 ppm, 500 ppm, 250 ppm, and 125 ppm resulted in 100% egg mortality in both species. During the oviposition preventive experiment, both *A. aegypti* and *A. albopictus* samples did not lay eggs in the plastic cups packed with *A. pinnata* extract, whereas, they laid eggs in the plastic cups filled with water. This means that *A. pinnata* contains bioactive compounds that are responsible for adulticidal and ovicidal behavior. The possibility for identifying natural products against dengue fever has been demonstrated by the overall assessment of these active compounds from *A. pinnata* extracts (Rajiv *et al.*, 2020).

The larvicidal activity of *A. pinnata* extracts employing methanol and acetone solvents on *A. albopictus* late 3rd instar larvae were tested by (Rajiv Ravi *et al.*, 2018). The findings of methanol solvent exhibited the greatest larvicidal effet towards late 3rd instar to early 4th instar *A. albopictus* larvae with LC_{50} and LC_{95} values of 867 ppm and 1293 ppm at 24 h and 647 ppm and 972 ppm at 48 h, correspondingly. While, at 24 h, acetone solvent substances had

LC_{50} and LC_{95} values of 1072 ppm and 1302 ppm and at 48 h, had 904 ppm and 1126 ppm, correspondingly. The authors found that the plant bioactive molecules found in *A. pinnata* are effective and could be established as an environmentally friendly, “go-green” strategy to mosquito larvicidal control (Adamu Yunusa *et al.,* 2019).

Husna Zulkrnin and her team published a report on the larvicidal effectiveness of *A. pinnata* in both raw and powdered form on larvae in the late third stage (6 days, 5 mm body length) of *A. aegypti*. The powdered concentration of *A. pinnata* used in the larvicidal study ranges from 500 to 2000 ppm; in the meantime, fresh *A. pinnata* varies from 500 to 9,000,000 ppm. The maximum death was observed at 1853 ppm for powdered *A. pinnata*, relative to fresh *A. pinnata* 2,521, 535 ppm, whereas, the LC_{50} observed at 1262 ppm and 1853 ppm for both powdered and fresh *A. pinnata*, accordingly. Eventually, in a 24 h bioassay examination, the ANOVA result revealed a substantial difference in *A. aegypti* larval mortality ($F = 30.439$, $df = 1$, $p \leq 0.001$) and concentration ($F = 20.002$, $df = 1$, $p \leq 0.001$) relative to powdered and fresh *A. pinnata*. The author concluded that the powdered *A. pinnata* is an effective larvicidal substance against *A. aegypti* (Husna Zulkrnin *et al.,* 2018).

4.10 Herbicidal stress

The 2,4-D hyper aggregation induced cellular responses of *A. pinnata* to stabilise the herbicidal stress were described by (Arnab Kumar *et al.,* 2020). The objective of their study was to assess the Azolla’s 2, 4-D tolerance effectiveness and, as a result, its potential use in biological xenobiotic pollution prevention was observed. This species might well be developed as a better phytoremediation due to the wide spectrum of 2, 4-D biosorption following changes in a few physiological and cellular operations. With its better anti-oxidation pathways, *Azolla* has considerable remembrance as a bio-resource to reduce herbicidal stress such as xenobiotic toxicity.

4.11 Green biosynthesis of nanoparticles

Hassan and his team used hydroalcohol extract of *A. pinnata* whole plant to perform a green biosynthesis of silver nanoparticles and examined their characteristics parameters (Hassan *et al.,* 2014).

Asha and her team used *Azolla* plant extract to create green biosynthesis of ZnO nanoparticles using a traditional chemical technique and a microwave-assisted environmentally friendly method. Greenly synthesised ZnO nanoparticles observed to have the better antibacterial and antioxidant activity while compared to chemically synthesised ZnO nanoparticles. Standard characterization analyses such as UV visible, FT-IR, and X-RD were used to validate the formation of nanoparticles (Asha *et al.*,2015; Abubucker Peer Mohideen, (2021).

5. Conclusion

Unfortunately, the potential use of *Azolla* in medicine or pharmacology, as well as in pathogenic targets, has yet to be studied due to its lack of available bioactivity data. As a result, the intent of the current review report was to summarize the pharmacological functions of *A. pinnata* as described by various researchers around the world.

So far, we have realized that *Azolla* species are commonly used as a source of protein and other basic nutrients for poultry. *Azolla* has the ability to be a potential and cost-effective feed for a variety of animal species. Combining *Azolla* with agricultural by-products such as wheat and rice bran could increase animal intestinal absorption and feed quality, as well as their productivity. According to the relevant literature, *Azolla* is a cost-effective and sustainable feed supplement for various animal species, containing large quantities of protein, amino acids, vitamins, and minerals that greatly decrease feeding costs.

Based on the aforementioned reports, the current review report on *A. pinnata* is important in terms of its medicinal value. According to the survey, India has a large number of marine macrophytes that have yet to be studied phytochemically and pharmacologically. Moreover, these species could also produce essential secondary metabolites such as flavonoids, phenolics, tannins, saponins, and , *etc*. The literature report collected from the biological and pharmacological results observed from various researchers submitted worldwide with exciting information along with phytoconstituents obtained from this aquatic plant. On the other hand, ongoing further research work on this aquatic plant and its secondary metabolites is required to make this into clinical significance *via* its structural modification, mechanism, *etc*.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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