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*Original Article*

# Identifying the potential activity of *Azolla pinnata* through *in vitro* assay and sem

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## Abstract

*Azolla pinnata* belongs to the family Salviniaceae. It has shown various beneficial effects in animals of lower phylogenetic order in curing hepatotoxicity. The main objective of the current study is, that one could add additional value to species holding great medicinal qualities. The different extractants tested were ethanol, methanol and water. The dilutions were varied among 1:32, 1:16, 1:8, 1:4, 1:2 and these were subjected to different pharmacological tests like Hot plate test, analgesic, and antipyretic tests. The different concentrations of *Azolla pinnata* were subjected to various assays like DPPH assay, peroxidase activity, and catalase activity. The surface morphology of *Azolla pinnata* was analyzed using Scanning Electron Microscope to potentially detect morphological features of its ultrastructure. Spectroscopy was performed to analyze the compositions of various components present in the plant. It was concluded that the ethanolic extract had very significant potential, higher than the rest of the extracts.

**Keywords**: *Azolla*, *In vitro* antioxidants, pharmacological activity, SEM

## 1. Introduction

*Azolla pinnata* is a member of the Salviniaceae family. In addition to the use of *Azolla pinnata* in fertilizers, it has shown great beneficial effects in various *in vitro* tests. Because of its nitrogen-fixing ability, this aquatic pteridophyte is well-known for its agricultural use. It has antioxidant activity and provides hepatoprotection, which can be attributed largely to the secondary metabolites. It grows more rapidly than any other algae. With the elimination of excess phosphorus, the water quality is also improved. *Azolla pinnata* grows very fast and in a very short time it produces a maximum biomass. An endosymbiont living in *Azolla's* leaf depressions is related to the improvement of all phases of the plant. It fixes nitrogen at a high rate and is easy to handle: *Azolla pinnata* can be used more often than not as organic manure, particularly in paddy fields (Mithraja, @ Antonisamy, Mahesh, Paul, &Jeeva, 2011). In addition, *Azolla pinnata* is also useful in dentistry and genuine current investigations into the refinement of *Azolla* focus on its complex segments and on confirmation of their inhibitory activities against cariogenic microorganisms (De, Sarkar, & Adak, 2017).

As a potential remedy, *Azolla pinnata* extricates the problem to a more prominent degree by normalizing a body's physiology and maintaining its cell integrity. *Azolla* is used as an oceanic plant for animals. It has a simplicity of development, profitability and nutritive value (A M MHalim, Shanab, & Abdel-Tawwab, 1998; Querubin, Alcantara, & Princesa, 1986). *Azolla* use in feeds for fish, swine and poultry have also been tried, described and recommended. *Azolla*, a family of amphibian plants widely endemic in the world's tropical and mild areas, harbors the cyanobacterium, and Anabaena *Azolla*e especially prefers the pits framed in its

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leaves. As it has the ability to fix nitrogen, *Azolla* species frequently grow bad water on nitrogen (Miranda, Liu, Rochfort, & Mouradov, 2018). *Azolla pinnata*, a sea-growing plant, has shown that it can be used as a biomaterial to help nano zero-valent particles to expel lethal metals from concentrated fluid. It stifled particles from oxidation and agglomeration of the nano zero-valent particles. The findings from TEM images showed that the balanced non-zero valent particles outside of the modified *Azolla* were shaped circularly by the compound reduction of ferrous iron (Martinez *et al*., 2017). The Langmuir isotherm depicts the take-up of lead and mercury onto the nano bio-composite, while energy is correlated with a false-second-request state. When temperature was elevated, the take-up efficiency rose (Yano, Thomas, Swenson, Lu, &Wharry, 2018). The adjusted marine plant showed high reusability after the seventh cycle due to its high expulsion efficiency (Trayner *et al*., 2015). The dead *Azolla* tended to adsorb major metals and colorants successfully. The level of gelatin methylation in the cell divider had been communicated as the overall substance between the amounts of methoxyl bunches in the chain just as the circulation of the carboxyl gatherings the chain. Likewise, the kind of living biomass has tended to remove metals viably through the dissemination of the cell divider to the inside. It has also been seen that extracts of *Azolla pinnata* such as ethanolic, methanolic and aqueous concentrate have potential advantages in lower phylogenetic strata. Work on these concentrates *in vitro* gives us deep insight. Lower phylogenetic queries show living beings are primarily focused on. It was also stated in late examinations that *Azolla* can also have hepatoprotective or mitigating effects, and that its further pharmacological activities are yet to be exploited (Radhakrishnan, SaravanaBhavan, Seenivasan, Shanthi, & Muralisankar, 2014).

## 2. Materials and Methods

### 2.1 *Azolla* preparation

The *Azolla pinnata* was collected from the Vellore district, Tamilnadu, India, and was taxonomically identified by Prof. Jayaraman, Director of Institute of Herbal Botany, Plant Anatomy Research Centre (PARC), Chennai, India. A sample of 15 grams of *Azolla pinnata* leaves was dried for about 20 minutes, ground to even sized particles, laid on a sheet of polymeric material, water added equal to pit depth, and cow-dung with protein rich panchagavya was well mixed with a solvent. The *Azolla pinnata* repeated about seven times the feed content after prominent two-day processing and this was used in research. This preparation mode made it more cost-effective to synthesize nutritional animal feed.

### 2.2 Animals

Rats have been collected from the VIT animal house, each of approximately 300g weight. The animal experiment procedures were approved by the institutional animal ethical committee, VIT, Vellore, per the guidelines of Indian Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The animals used in this experiment are controlled according to the guidelines provided by the Committee for Animal Experimental Control and Supervision (CPCSEA), Ministry of Culture, Government of India, Chennai.

### 2.3 Extracts and drugs

*Azolla pinnata* ethanolic, methanolic and aqueous extracts were prepared. Weighed and dissolved 1 g of *Azolla pinnata* in 10 ml of distilled water to prepare an aqueous extract of *Azolla pinnata* and it was held for at least 24 hours in a shaker. *Azolla pinnata* ethanol extract was prepared by dissolving 1 g in 10 ml of ethanol and holding it for 48hr in a shaker. *Azolla pinnata* methanol extract was prepared by dissolving 1 g in 10 ml of methanol and storing it for 24hr in shaker. Then these extracts were each distilled and placed in an oven for 10 minutes (Querubin *et al*., 1986). Indomethacin (3 mg/kg/b.wt) was also used in this experiment for comparison.

### 2.4 *In vitro* analysis

All three extracts were diluted by serial dilution to concentrations A (1:32), B (1:16), C (1:8), D (1:4) and E (1:2). The serial diluted samples were analyzed to observe the DPPH scavenging, catalase activity and peroxidase activity for each extract. The DPPH assay was done by the standard procedure (Blois, 1958). The sample was analyzed to measure the level of catalase activity by a standard procedure (Suanarunsawat et al, 2014). The peroxidase level of the sample was measured using a standard procedure (Reddy et al, 1985, p.).

### 2.5 Experimental designs

Thirty *Wistar albino* rats of female sex were used in the study at 4 weeks of age. The rats were divided into 5 Groups as follows

Group1 – Normal control

Group 2- Indomethacin (3 mg/kg/b.wt)

Group3- Aqueous extract of *Azolla pinnata* (250 mg/kg/b.wt)

Group4- Methanolic extract of *Azolla pinnata* (250 mg/kg/b.wt)

Group5- Ethanolic extract of *Azolla pinnata* (250 mg/kg/b.wt)

### 2.6 Pharmacological analysis

Hot Plate Method – After the administration of the drug, the rat was kept in the hotplate at 40 degrees for 3 minutes. This method is to monitor how much temperature can be normally tolerated by the rat. Normally when the rat starts to lick its paws or tries to leap out, the pain threshold is considered to have exceeded. Stopwatch has been used to monitor the length of time. The rats were tested for paw leaching or jumping behaviors, and the rats that responded after 5 seconds were generally used for evaluation. Six rats per dose were tested 30 minutes after *Azolla pinnata* (250mg / kg b.wt, i.p) or Indomethacin (3mg/kg b.wt, i.p). The animals that were kept as control were given an equal dose of saline (Chindo, Schröder, Koeberle, Werz, & Becker, 2016; Gupta *et al*., 2005).

Analgesic – After half an hour of intraperitoneal injection of CH3COOH (10 ml/kg body weight), the drug was administered. For muscle contractions, rats were observed when 0.6 percent CH3COOH solution (10 ml/kg b.wt.) was given intraperitoneally. The animals were placed in cages when the CH3COOH administration was completed and the number of "stretching" per rat was recorded for half an hour. A critical reduction in the quantity of squirming was viewed as a positive pain-relieving response by any intervention when compared with control animals. Extracts of *Azolla pinnata* (250 mg/kg b.wt. *i.p.*) and Indomethacin (10 mg/kg b.wt. i.p.), which was suspended with 0.5% of carboxymethyl cellulose of phosphate supported saline, were managed intraperitoneally thirty minutes ahead of the $CH_3COOH$ infusion (Bhutia *et al.* 2010; Ferreira-Gomes, Adães, Mendonça, & Castro-Lopes, 2012).

Antipyretic test – Rats were exposed to 10 ml/kg subcutaneous infusion of 20 percent watery baker yeast suspension and 18 hrs reported rectum temperature. After 18 hours of perusing, extracts of *Azolla pinnata* (250mg/kg b.wt) and Indomethacin (3mg/kg b.wt) were administered intraperitoneally. At the time when temperature expansion was reported, it was estimated to be up to 5 hours per hour following the management of test drugs (Asha & Pushpangadan, 1999; Muhammad, Saeed, & Khan, 2012; Mukherjee *et al*., 1996).

Ulcerogenic test - For sixteen hours the animals were kept under fasting and administered the drugs orally after fasting. *Azolla pinnata* extracts were administered at a dose level of 250mg / kg b.wt and indomethacin was administered separately at a dose level of 3mg / kg b.wt to test ulcerative activity. Animals were sacrificed after 3h when the drug administration is over, the stomach was completely removed and was cut along the lesser curvature and the gastric mucosa was washed with normal saline and scored according to the scale, 0: no lesion, 0.5: hyperemia, 1: one or two lesions, 2: severe lesions, 3: very severe lesions, 4: mucosa full of lesions (Gürbüz, Özkan, Yesilada, & Kutsal, 2005; Sabina, Nasreen, Vedi, & Rasool, 2013).

## 2.7 Scanning Electron Microscope analysis

The electrons communicate in the sample with atoms, creating various signs that contain data on the specimen's surface topography and organizational structure. In a raster test model, the electron shaft is filtered and the bar situation is accompanied by the defined signal to generate an image (Hayes, Cnuts, & Rots, 2019). SEM can achieve resolution better than 1 nm. Specimens were studied in high vacuum at standard SEM, low vacuum or wet conditions at traditional SEM, and a wide variety of cryogenic or warm temperatures. The tests used a wavelength at 289 nm (Kasuga *et al*., 2016).

Ultraviolet–Visible spectroscopy (UV–Vis or UV / Vis) refers to spectroscopy retention or reflectance spectroscopy in the UV spectral field using light in the visible and neighboring ranges. In this range, absorption or reflection directly affects the apparent shade of the synthetic compounds included (Martelo-Vidal & Vázquez, 2016). Atoms are undergoing electronic changes in this region of the electro magnetic spectrum. Absorption spectroscopy is analogous to fluorescence spectroscopy, where fluorescence transitions from the energized state to the surface, while assimilation measurements proceed from the earliest stage to the energized state (Tanabe & Kurawaki, 2018).

## 2.8 Statistical analysis

All observations have been recorded at least six times and the mean ± standard deviation (SD) is reported. ANOVA was used for statistical analysis, with n=6 using the Instat graph pad software.

## 3. Results

### 3.1 Effect of *Azolla pinnata* on DPPH assay

From DPPH assay (Figure 1) of all the 3 extracts it can be concluded that at 1:2 ratio of ethanolic extract shows maximum effect higher than those of methanolic or aqueous extracts. The percent inhibition is more at 1:2 ratio of ethanolic extract than that of methanolic or aqueous. This assay shows that ethanolic extract has got maximum potential over methanolic and aqueous extracts in a DPPH scavenging assay.

### 3.2 Effect of *Azolla pinnata* on peroxidase activity

The peroxidase activity (Figure 2) which was recorded at every 30 sec for 3 min displayed that ethanolic

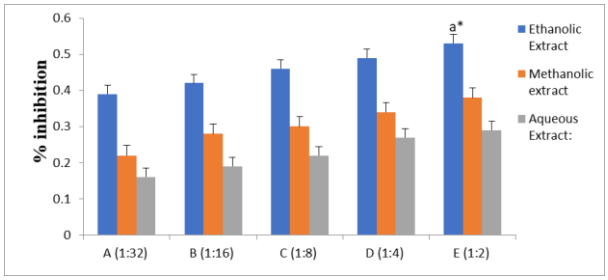


Figure 1. DPPH assay

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-A vs groups B, C, D, E; b- Group-B vs Group-C, D, E; c-Group-C vs Group-D, E; d-Group-D vs Group-E. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

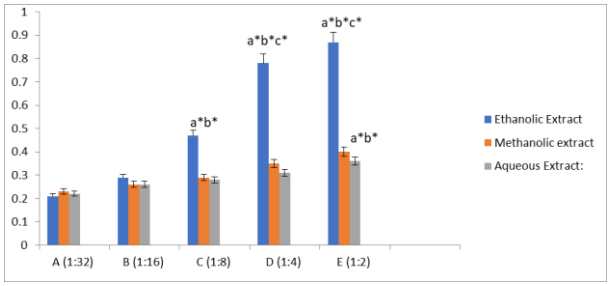


Figure 2. Peroxidase activity

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-A vs groups B, C, D, E; b- Group-B vs Group-C, D, E; c-Group-C vs Group-D, E; d-Group-D vs Group-E. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

extract at 1:2 ratio displayed maximum response to peroxidase activity: the rate was equal to 0.9 U/mg extract. In this peroxidase activity ethanolic extract shows maximum effect larger than the other extracts, at 1:2 dilution.

### 3.3 Effect of *Azolla pinnata* on catalase activity

The catalase activity (Figure 3) shows that ethanolic extract at 1:2 dilution had the highest activity of the extracts tested.

### 3.4 Effect of *Azolla pinnata* on hot plate method

Hot plate tests (Figure 4) displayed that rats administered with methanolic extract of *Azolla pinnata* (250 mg/kg/b.wt) reacted late at 40 degrees whereas, rats administered with ethanolic extract took 8-9 sec to respond whereas rats administered with Indomethacin responded in the least amount of time. In the hot plate method, the *Azolla pinnata* treatment increased the reaction time and showed more significant analgesic activity at 250mg/kg. b.wt. In Hot plate method methanolic extract shows maximum effect larger than the other extracts. Methanolic extract administration had the rats react at maximum time duration.

### 3.5 Effect of *Azolla pinnata* on analgesic test

Methanolic extract again displayed significance over the other extracts (Figure 5). In the acetic acid initiated squirming tests, *Azolla pinnata* treatment like Indomethacin, delivered a critical decrease in the quantity of stomach choking influences in rats. This sort of decrease is portion related and observed to be most extreme with (250 mg/kg/b.wt). In the hot plate strategy, the *Azolla pinnata* treatment expanded the response time and indicated progressively critical pain-relieving movement at 250 mg/kg. b.wt. Analgesic activity is represented in Figure 5.

### 3.6 Effect of *Azolla pinnata* on antipyretic test

The true potential of ethanolic extract was demonstrated at 22$^{nd}$ hour of antipyretic test. Ethanolic extract showed more potential than any other extract (Figure 6). It is observed that rats treated with *Azolla pinnata* extracts, especially ethanolic extracts, displayed an increase in body temperature in comparison to other extracts.

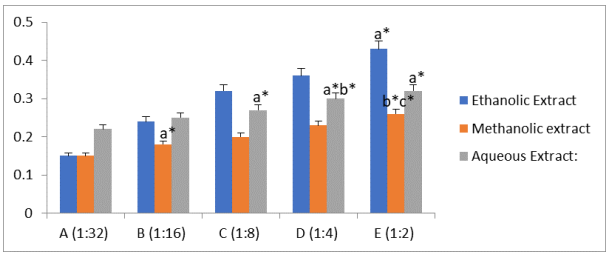


Figure 3. Catalase activity

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-A vs groups B, C, D, E; b- Group-B vs Group-C, D, E; c-Group-C vs Group-D, E; d-Group-D vs Group-E. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

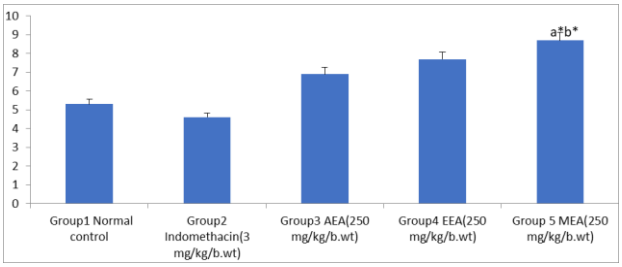


Figure 4. Hot plate method

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-I vs groups II, III, IV, V; b- Group-II vs Group-III, IV, V; c-Group-III vs Group-IV, V; d-Group-IV vs Group-V. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

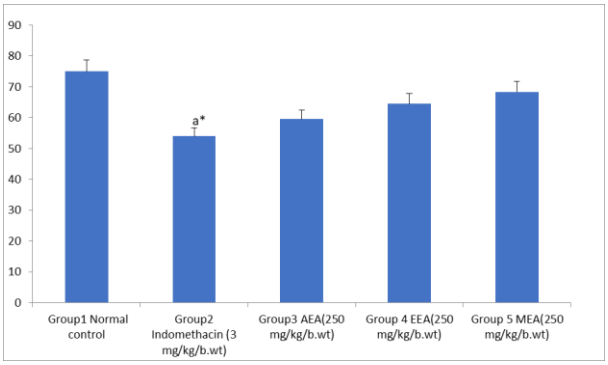


Figure 5. Analgesic property

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-I vs groups II, III, IV, V; b- Group-II vs Group-III, IV, V; c-Group-III vs Group-IV, V; d-Group-IV vs Group-V. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

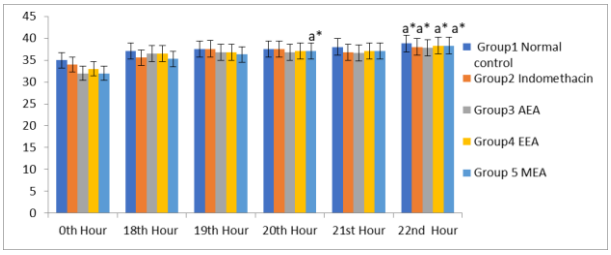


Figure 6. Antipyretic activity

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-I vs groups II, III, IV, V; b- Group-II vs Group-III, IV, V; c-Group-III vs Group-IV, V; d-Group-IV vs Group-V. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

### 3.7 Effect of *Azolla pinnata* on ulcerogenic test

In ulcerogenic test it was observed that Indomethacin played a major role in affecting the gastric mucosa of the rat, the ethanolic, methanolic and aqueous extract of *Azolla* showed little effect, the normal control did not show any effect at all. In antipyretic test the ethanolic extract of *Azolla pinnata* displayed maximum effect at 22$^{nd}$ hour; the temperature was about 36 degrees Celsius. Figure 7

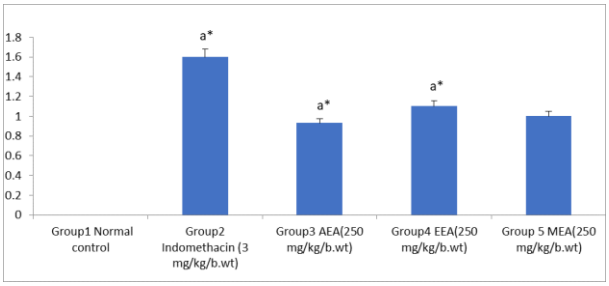


Figure 7. Ulcerogenic test

Each bar shows the mean ± SD of six rats. Comparisons were made as follows: a-Group-I vs groups II, III, IV, V; b- Group-II vs Group-III, IV, V; c-Group-III vs Group-IV, V; d-Group-IV vs Group-V. The symbols represent statistical significance at *p < 0.05. Statistical analysis was calculated by one-way ANOVA followed by the Student Newman–Keul's test.

presents the findings. Indomethacin displayed maximum potential in ulcerogenic test in comparison to any extract. However, among the extracts of *Azolla pinnata*, ethanolic extract had more effect than the others. No gastric lesions were found in the rats administered with ethanolic, aqueous or methanolic extracts of *Azolla pinnata* at a fixed dosage of (250 mg/kg/b.wt) whereas Indomethacin administered rats showed lesions.

### 3.8 Effect of *Azolla pinnata* on SEM Analysis

The Figure 8 and 9 illustrates the Scanning electron microscope (SEM) images of the top surface of *Azolla pinnata*. SEM is a kind of electron beam based magnifying lens that produces images of a specimen by examining the surface with a focused ray of electrons. The white spot represents bacterial strain on the top surface. A decrease in the inter particle distance was noticed with concentration.

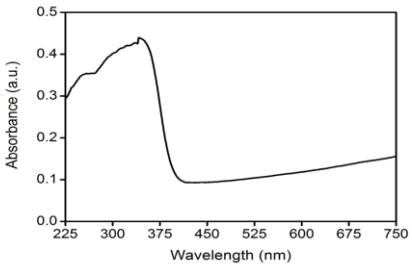


Figure 9. Ultraviolet–visible diffuse reflectance spectroscopy

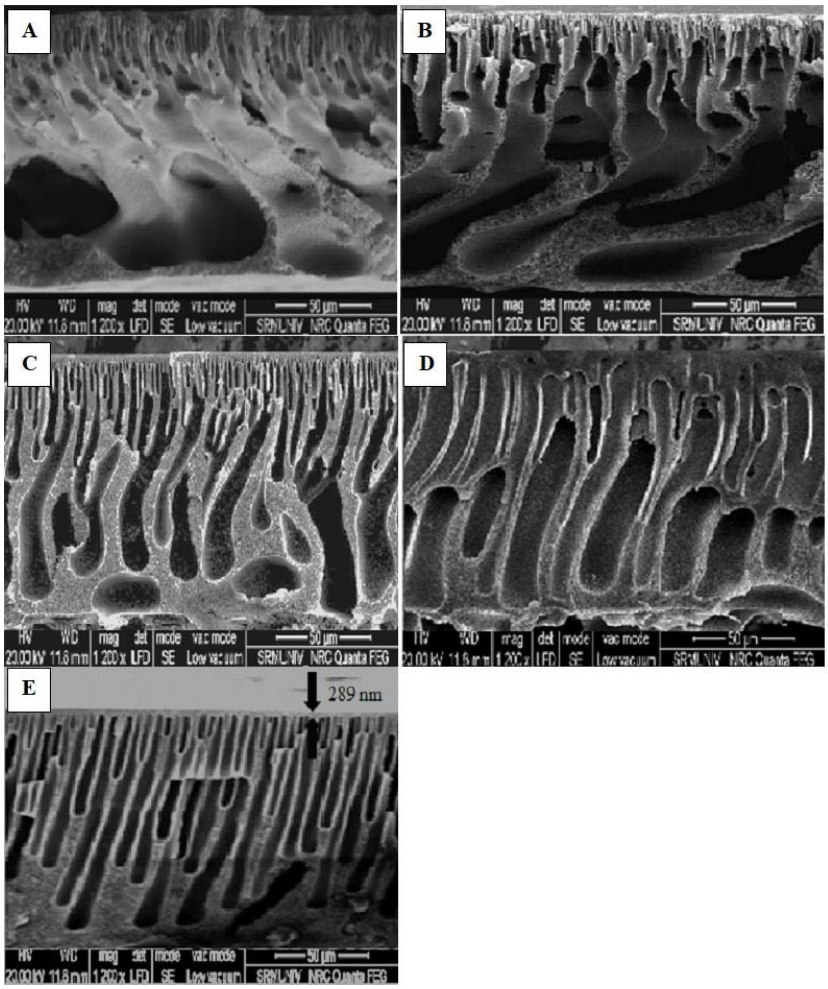


Figure 8. SEM analysis

## 4. Discussion

In key areas of health, *Azolla pinnata* assumes an imperative task. Current tests show *Azolla pinnata* handiness in ecological poison remediation and regulation. For the most part, *Azolla pinnata* can be used more often than not in two separate ways to clean up natural poisons. The primary technique is adsorption, which for a settled length of time allowed the *Azolla pinnata* to be prepared in powder and shake with the wastewater. The pollution will hold fast on the outside of the *Azolla pinnata* powder to the normal utilitarian gatherings. In adsorption, *Azolla pinnata* was accounted for in the remediation of methyl violet 2B, color malachite green, rhodamine B corrosive red 88 and corrosive blue wastewater (Querubin *et al*., 1986). There are several mitigation drugs available in the business sectors that are used for various common problems in treatment. By and large, these medications have pain-relieving and antipyretic effects that are frequently associated with gastric damage. In this manner, an endeavor is made in the present examination to assess the pain-relieving and antipyretic impact of *Azolla pinnata* and to investigate whether it goes with any noteworthy gastric harm (Mithraja *et al*., 2011).

The second remediation technique is essentially phytoremediation, where *Azolla pinnata* is suspended in the sewage outside. Due to its high resistance to ecological contamination, *Azolla pinnata* was primarily considered. Phytoremediation of mechanical wastewater containing metals, e.g. zinc, lead, mercury, cadmium, copper, or arsenic; just like natural colors, e.g. methyl violet 2B in literature. *Azolla pinnata* also has many other *in vitro* advantages as it is useful in curbing hepatotoxicity, and the lower phylogenetic species are typically aimed at finding the true potential of *Azolla* in different ways. To see the true potential of *Azolla pinnata* it was leached with various extractants (A M M Halim *et al*., 1998).

In general, these extracts amplify the properties of *Azolla pinnata*, making *Azolla pinnata* more efficient. Through performing various assays such as DPPH, the activity of catalase and peroxidase, it appears that the extract is suitable for use. The pharmacological activity also underlines its advantages, that indomethacin is suitable for ulcerogenic screening. It is shown that the extracts of *Azolla pinnata* have a more prominent pharmacological effect than the one of the anti-inflammatory agent Indomethacin. The extracts of *Azolla pinnata* are further observed to increase the rectal temperature compared to Indomethacin. The rat reaction times increases when methanolic extract (250 mg / kg / b.wt) was administered but no such effects were shown by the other extracts. The hot plate reaction experiment is commonly used to monitor the drug's analgesic function on the central nervous system. As a rule, the hot plate test estimates the reaction to a non-fiery, intense non-ciceptive info and is one of the typically used models for non-ciceptive concentrate focal movement. Narcotics specialists use supra-spinal and spinal receptors to apply their pain-relieving effects (Nemirovsky *et al*., 2001). In the hot plate study, after its organization, *Azolla pinnata* (250 mg / kg b.wt.) showed a critical pain relief effect at 30 min. The results suggest that in both the hot plate response test and the acidic corrosive squirming reaction, *Azolla pinnata* demonstrated a notable pain relieving impact. *Azolla pinnata's* pain-relieving effect in both models confirms that it has worked through both the fringe and focal system. The tender has reached maximum absorbance from spectroscopy at a wavelength of 352 nm, which endorses that the plant particles are in the nano range and *Azolla pinnata* reveals its major advantages at this correspondence level. It is very clear from the results obtained from SEM that the pore size began to be oriented towards the narrow deep section. *Azolla pinnata* beneficial properties would be the major factor in its pore size (Kasuga *et al*., 2016). It was also observed that various extracts of *Azolla pinnata*, such as ethanolic, methanolic and aqueous extract, have potential benefits in lower organism phylogenetic order. The true potential of *Azolla pinnata* has been demonstrated by various assays such as DPPH, catalase and peroxidase activity. Analyzing these extracts in vitro gives us a profound insight. The model organisms of lower phylogenetic order are mainly targeted. In recent studies it has also been cited that *Azolla pinnata* can also have hepatoprotective, anti-inflammatory effects, its other pharmacological activities are yet to be mined (P. R. Subudhi& K. Singh, 1978; Querubin *et al*., 1986).

## 5. Conclusions

It can be concluded that the ethanolic extract of *Azolla pinnata* showed maximal DPPH scavenging, peroxidase, and catalase activities among the extracts, confirming that ethanolic extract of *Azolla pinnata* is more potent than methanolic or aqueous extracts. Hot plate test rats with methanolic extract of *Azolla pinnata* (250 mg/kg/b.wt) reacted late in comparison to the other extract treatments, which means that methanolic extract best suits the pharmacological activity. Indomethacin played a major role in ulcerogenic test, and out of all the extracts ethanolic extract also played a major role; but in comparison to Indomethacin it was much less potent. Ethanolic extract also played a major role in 22$^{nd}$ hour of antipyretic tests. This indicates that the ethanolic extract of *Azolla pinnata* is more beneficial than the other extracts tested.

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## References

A M M Halim, A., Shanab, S., & Abdel-Tawwab, M. (1998). Evaluation of Azollapinnata meal as an ingredient in diets for Tilapia zillii fry.

Asha, V. V., &Pushpangadan, P. (1999). Antipyretic activity of Cardiospermumhalicacabum. *Indian Journal of Experimental Biology, 37*, 411–414.

Bhutia, Y. D., Vijayaraghavan, R., Pathak, U. (2010). Analgesic and anti-inflammatory activity of amifostine, DRDE-07, and their analogs, in mice. *Indian Journal of Pharmacology, 42*(1), 17.

Chindo, B. A., Schröder, H., Koeberle, A., Werz, O., & Becker, A. (2016). Analgesic potential of standardized methanol stem bark extract of Ficusplatyphylla in mice: Mechanisms of action. *Journal of Ethnopharmacology, 184*, 101–106.

De, A. K., Sarkar, B., & Adak, M. K. (2017). Physiological explanation of herbicide tolerance in Azollapinnata R.Br. *Annals of Agrarian Science, 15*(3), 402–409.

Ferreira-Gomes, J., Adães, S., Mendonça, M., & Castro-Lopes, J. M. (2012). Analgesic effects of lidocaine, morphine and diclofenac on movement-induced nociception, as assessed by the Knee-Bend and CatWalk tests in a rat model of osteoarthritis. Pharmacology *Biochemistry and Behavior, 101*(4), 617–624.

Gupta, M., Mazumder, U. K., Kumar, R. S., Gomathi, P., Rajeshwar, Y., Kakoti, B. B., &Selven, V. T. (2005). Anti-inflammatory, analgesic and antipyretic effects of methanol extract from Bauhinia racemosa stem bark in animal models. *Journal of Ethnopharmacology, 98*(3), 267–273.

Gürbüz, I., Özkan, A. M., Yesilada, E., &Kutsal, O. (2005). Anti-ulcerogenic activity of some plants used in folk medicine of Pinarbasi (Kayseri, Turkey). *Journal of Ethnopharmacology, 101*(1), 313–318.

Hayes, E., Cnuts, D., & Rots, V. (2019). Integrating SEM-EDS in a sequential residue analysis protocol: Benefits and challenges. *Journal of Archaeological Science*: *Reports, 23*, 116–126.

Kasuga, K., Muralidhar, M., Diko, P., Jirsa, M., Inoue, K., & Murakami, M. (2016). SEM and SEM by EDX analysis of air-processed SmBa2Cu3Oy. Physics Procedia, Proceedings of the 28th International Symposium on Superconductivity (ISS 2015) November 16-18, 2015, Tokyo, Japan, 81, 41–44.

Martelo-Vidal, M. J., &Vázquez, M. (2016). 3 - Advances in ultraviolet and visible light spectroscopy for food authenticity testing. In G. Downey (Ed.), *Advances in food authenticity testing*, Woodhead publishing series in food science, technology and nutrition (pp. 35–70). Sawston, England: Woodhead Publishing. Retrieved from http://www.sciencedirect.com/science/article/pii/B9780081002209000035

Martinez, M., Fleurier, G., Chmelík, F., Knapek, M., Viguier, B., & Hug, E. (2017). TEM analysis of the deformation microstructure of polycrystalline cobalt plastically strained in tension. *Materials Characterization, 134*, 76–83.

Miranda, A. F., Liu, Z., Rochfort, S., &Mouradov, A. (2018). Lipid production in aquatic plant Azolla at vegetative and reproductive stages and in response to abiotic stress. *Plant Physiology and Biochemistry, 124*, 117–125.

Mithraja, M. J., Antonisamy, J. M., Mahesh, M., Paul, Z. M., & Jeeva, S. (2011). Phytochemical studies on Azollapinnata R. Br., Marsileaminuta L. and Salviniamolesta Mitch. *Asian Pacific Journal of Tropical Biomedicine, 1*(Supplemental 1), S26–S29.

Muhammad, N., Saeed, M., & Khan, H. (2012). Antipyretic, analgesic and anti-inflammatory activity of Viola betonicifolia whole plant. *BMC Complementary and Alternative Medicine, 12*(1), 1.

Mukherjee, P. K., Das, J., Saha, K., Giri, S. N., Pal, M., & Saha, B. P. (1996). Antipyretic activity of Nelumbonucifera rhizome extract. *Indian Journal of Experimental Biology, 34*(3), 275–276.

P. R. Subudhi, B., & K. Singh, P. (1978). Nutritive value of the water fern Azollapinnata for chicks. *Poultry Science, 57*, 378–380.

Querubin, L. D., Alcantara, P. F., & Princesa, A. O. (1986). Chemical composition of three azolla species (A. caroliniana, A. microphylla and A. pinnata) and feeding value of azolla meal (A. microphylla) in broiler ration, 2 [Philippines]. Philippine Agriculturist (Philippines). Retrieved from http://agris.fao.org/agris-search/search.do?recordID=PH8810158

Radhakrishnan, S., SaravanaBhavan, P., Seenivasan, C., Shanthi, R., &Muralisankar, T. (2014). Replacement of fishmeal with Spirulinaplatensis, Chlorella vulgaris and Azollapinnata on non-enzymatic and enzymatic antioxidant activities of Macrobrachium rosenbergii. *The Journal of Basic and Applied Zoology, 67*(2), 25–33.

Sabina, E. P., Nasreen, A., Vedi, M., & Rasool, M. (2013). Analgesic, antipyretic and ulcerogenic effects of piperine: an active ingredient of pepper. *Journal of Pharmaceutical Sciences and Research, 5*(10), 203–206.

Tanabe, I., & Kurawaki, Y. (2018). Far-ultraviolet spectral changes of titanium dioxide with gold nanoparticles by ultraviolet and visible light. *Spectrochimica Acta Part A*: *Molecular and Biomolecular Spectroscopy, Festschrift in honor of Prof. Yukihiro Ozaki, 197*, 103–106.

Trayner, S., Hao, A., Downes, R., Park, J. G., Su, Y.-F., & Liang, R. (2015). High-resolution TEM analysis of flatten carbon nanotube packing in nanocomposites. *Synthetic Metals, 204*, 103–109.

Yano, K. H., Thomas, S., Swenson, M. J., Lu, Y., & Wharry, J. P. (2018). TEM in situ cube-corner indentation analysis using ViBe motion detection algorithm. *Journal of Nuclear Materials, 502*, 201–212