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ANATOMICAL FEATURES AND ANTIMICROBIAL ACTIVITY OF DUCKWEED

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Abstract

Duckweeds are common floating flowering plants belonging to Lemnaceae. Five main genera known as duckweed are found in tropical water bodies mostly under eutrophic condition. Despite of their minute size, duckweeds exhibit distinction as per morpho-anatomical characterisation and production of secondary metabolites, that show antimicrobial potentialities. Connective stalk of duckweeds is an organ of attachment between the mother and daughter fronds, whose anatomy has been studied here in details. The present study focuses on the critical anatomical differences between *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid. using scanning electron microscope. The antimicrobial potency especially against Gram-negative bacteria were studied using duckweeds biomass collected from Indian aquatic environment, which were cultured in laboratory conditions. Activity against fish pathogen like *Aeromonas* sp. were investigated also.

Introduction

Lemna minor L. commonly called as lesser duckweeds, are seen to form thick mats on the surface of nutrient rich brackish and fresh water habitats. The plant bears a single oval to elliptical frond which measures about 6-8 mm and a single long root which can measure up to 2 cm. The root length of *Lemna minor* L. can be found directly proportional to the nutrient content in the water in which it grows. Duckweeds belonging to the monocot order Alismatales, family Lemnaceae. They are represented by five genera namely *Lemna*, *Spirodela*, *Wolffia*, *Wollfiella* and *Landoltia* with 37 species (Bog 2010). Unlike advanced flowering plants, they do not show morphological differentiation into root, stem and leaves. Rather a leaf like structure lacking stem, called fronds, represents the vegetative body of duckweeds.Such morphological reduction may be compared with "neoteny" as are found in the animal kingdom (Wang *et al*. 2014). Duckweed reproduction is primarily vegetative. Daughter fronds bud off from reproductive pockets called meristematic primordial on the proximal part of mature frond (Cao *et al*. 2015). A single frond can produce as many as ten generations of daughter plants over a period of 10 days to several weeks before dying. Several factors may limit the growth of unorganized duckweed colonies. The daughter fronds remain connected to the mother fronds through organic connection within their reproductive pockets, called Connective stalk (CT). It originates from the meristematic region of the abaxial fronds and remain attached to the daughter fronds until separation (Lemon and Posluzny, 2000). This structure is comparable with the umbilical cord of placental mammals.

The dry weight of duckweeds is known to contain 35 to 45 % crude protein (Mbagwu and Adeniji 1988). Essential amino acids like lysine and methionine are found in high concentration in comparison to other plant proteins. In this regard, they resemble animal protein. Duckweeds are also known to possess a high concentration of trace elements and pigments like carotene and xanthophylls, in this regard, duckweed aquaculture can generate a valuable supplement for poultry and other animal feeds (Casani and Caton 1983).

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Flowers are rare, if produced are covered within a flowering spathe (Landolt 1998). Reproduction mainly occurs through vegetative means and daughter fronds remains connected with mother fronds by means of connective stalk (CT). *Spirodela polyrrhiza* (L.) Schleid. commonly called greater duckweeds or giant duckweeds, is a free floating aquatic macrophyte that can pile up very quickly in eutrophic water bodies within a short period of time. It shows much reduced morphology with a conspicuous green frond to which several small roots remain attached (Kim 2007). Some reports are available on the antimicrobial potency of duckweeds but mostly they are concerned with effects on Gram-positive rods (Duangjarus *et al.* 2022), however the action of duckweed extract on Gram-negative rods are very few, especially on fish pathogens like *Aeromonas* sp. Plants are known to produce turions under unfavourable conditions. They propagate mainly through vegetative means and the daughter fronds remain attached to the mother body by means of connective stalk. The present paper focuses on the anatomical distinction of the fronds between *Spirodela polyrrhiza* (L.) Schleid. and *Lemna minor* L , which were collected from eutrophic water bodies of Malda district and were studied under Scanning Electron Microscopy.

Materials and Methods

The duckweed species namely *L. minor* L. and *S. polyrrhiza* (L.)Schleid. were collected from different stagnant water bodies named as Duckweed Collection point within the English bazar municipality located between 25^{0}01” (N) to 25^{0}14” (N) latitude and 88007” (E) to 88^{0}85” (E) longitude. The collected fronds were placed in Murashige and Skoog (MS) Basal; pH 5.8 Relative Temperature was maintained at 28±2 degree Celsius for 21 days of culture. Fronds were collected from mid log phase (Day 9^{th}) of the liquid culture and were subjected to SEM study and aqueous methanolic extract was used for assessment of antimicrobial potentiality.

To study the leaf surface topology of *L. minor* L. and *S. polyrrhiza* (L.) Schleid., the fronds are dehydrated following the chemical fixation procedure of Pathan *et al.* (2015). At first the fronds were fixed using 2.5% Gluteraldehyde for overnight. It was followed by dehydration in different grades of alcohol. (50, 70, 80 and 95%). The dehydrated biomass were then subjected to SEM. using JEOL (JSM-IT 100).Biomass from mid-log phase (15-18 days after inoculation) used for methanolic extract. Antimicrobial assay following disc diffusion method was performed against *Aeromonas hydrophila* and *Escherichia coli*. Ampicillin (30 mcg/ disc) was taken as reference antibiotic.

Results and Discussion

Figure 1a and b shows the axenic growth of *L. minor* L. and *S. polyrrhiza* (L.) Schleid in the mid log phase. Stomatal dimensions of the fronds of both the duckweed species were compared along with critical observations of their guard cell and subsidiary cell. The attachment and the dimension of the connective stalk (CT) were also taken into consideration. The thickness of veinlets of both the duckweed species under SEM are more or less similar, but the vein islet dimensions were found more in case of *L. minor* L. as compared to *S. polyrrhiza* (L.) Schleid (Fig. 2A and B). The dorsal frond surface was found to be covered with trichomes (peltate trichomes and pointed trichomes) for both the duckweeds under study. However, the diameter of the globular head of peltate trichomes was found to be greater in *S. polyrrhiza* (L.) Schleid.as compared to that of *L. minor* L (Fig. 2C and D). Pointed trichomes were absent over the frond surface of *L. minor* L. and were exclusively present on the frond surface of *S. polyrrhiza* (L.) Schleid (Fig. 2 E). However, the stomatalaperature were more or less elongated to oval (Fig. 2F and H). All critical anatomical comparisons are presented in Table 1. The connective stalk (CT) of both the duckweeds were compared. The daughter fronds remains physically attached to the mother fronds with CT at various stages of development (Kim and Kim 2000). The CT has proven to be very efficient for separating offspring from the mother frond (Kwak and Kim 2008). Such structural complexity effectively contributes to the better adaptation of smaller plants to superficial aquatic environments during relatively short growing seasons, while also enabling rapid growth (Witzum 1974).

Table 1. Anatomical comparison between *Lemna minor* and *Spirodela polyrrhiza.*

Characters	*Lemna minor*	*Spirodela polyrrhiza*
Veinlet thickness	0.769 to 0.814 μm.	0.475 to 0.762 μm.
Vein islet dimension.	Length : 8.066 μm to 16.082 Width: 13.915 μm to 15.267	Length: 10 to 10.001 μm Width: 10 to 11.003 μm
Trichome type	Peltate trichomes	Both peltate and pointed trichomes
Trichome dimensions	Diameter of the head of the peltate trichome 3.88 to 4.23μm. Pointed trichomes are absent.	Diameter ranges from 21.846 to 23.866 μm. Length of each trichome 15.854 to 21.779 μm.
Stomatal type	Cyclocytic stomata	Cyclocytic stomata
Dimension of stomatal aperture.	Length: 3.606 μm to 13.652 μm Width: 8.240 μm to 20.452μm	Length: 3.986 to 3.893 μm Width: 5.319 μm to 16.454

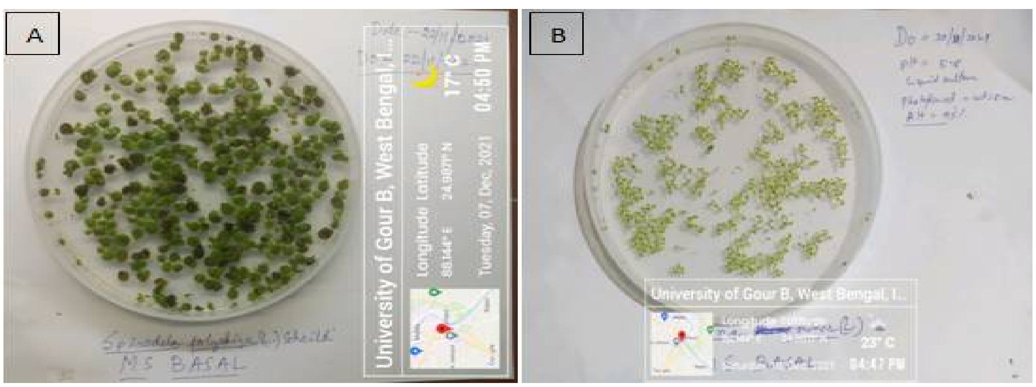

Fig. 1. *In vitro* liquid culture in MS Basal medium of the two duckweeds in the mid log phase (Day 9). (A) Mid log phase of *Spirodela polyrrhiza* (L.) Schleid. (B) Mid log phase of *Lemna minor* L.

Antimicrobial efficacy with reference to ampicillin (30 microgram/ disc) showed superiority of *Spirodela* over *Lemna*. Zone of inhibition for same dose were 14.6 mm and 13.9 cm respectively as compared to 16.2 mm for ampicillin against *A. hydrophila*. The dimension of inhibition zone were recorded to be 14.1, 13.5 and 15.8 mm, respectively against *E. coli*. The efficacy of crude extract of duckweeds as studies here for controlling the growth of *Pseudomonas* sp. was also tested. Both the greater and lesser duckweed species performed almost with similar potentiality rendering the inhibition zone 12.8 mm and 13.1 mm respectively. For synthetic antibiotics, Ampicillin performed better than Vancomycin scoring the dimension 28.5 mm and 25.3 mm, respectively.

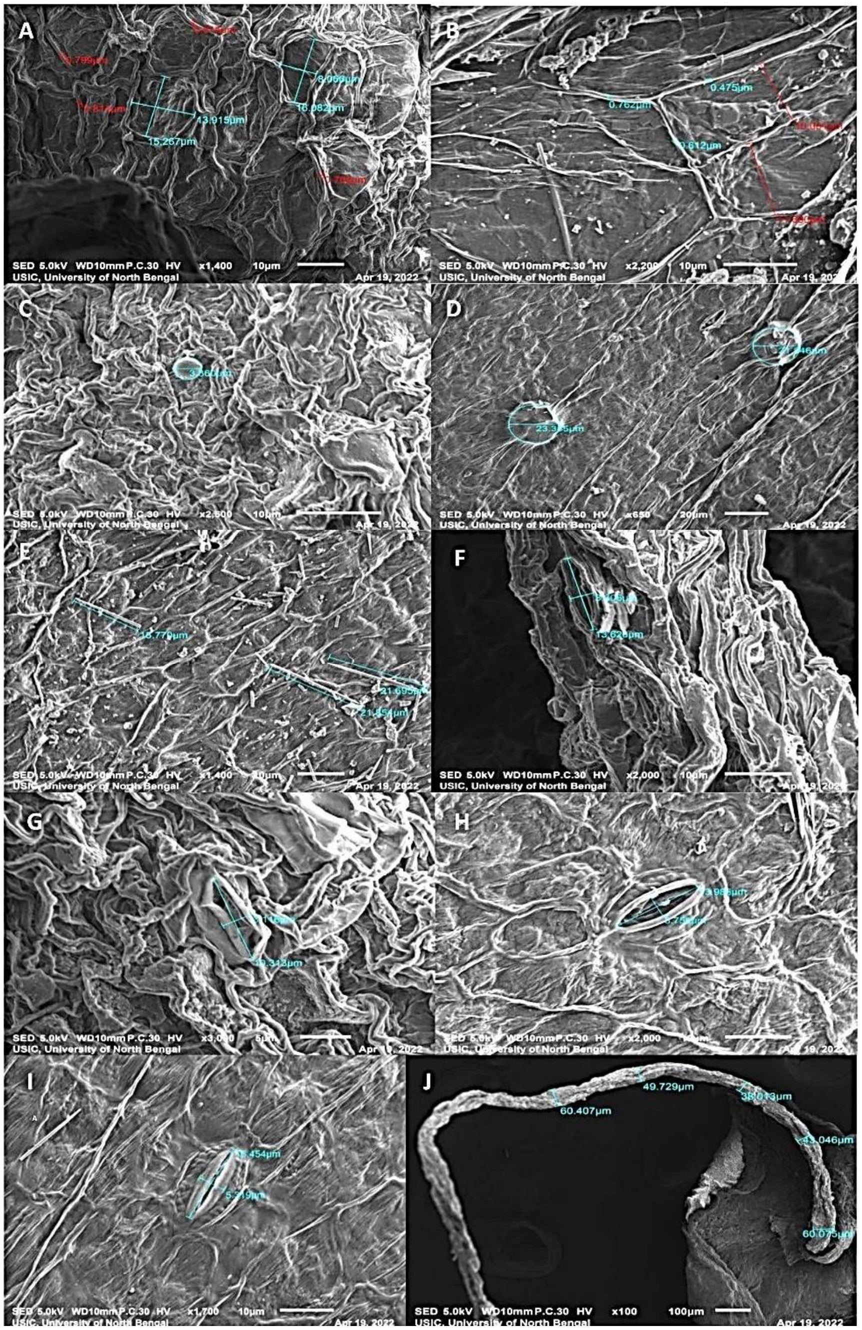

Fig. 2. Scanning electron micrographs of *Lemna minor* L. and *Spirodela polyrrhiza* (L.) Schleid. (A) Veinlet and vein islet of *Lemna minor* L. (B). Veinlet and vein islet of *Spirodela polyrrhiza* (L.)Schleid. (C) Peltate trichomes of *Lemna minor* L. (D) Peltate trichomes of *Spirodela polyrrhiza* (L.) Schleid. (E) Pointed trichomes of *Spirodela polyrrhiza* (L.) Schleid. (F) Stomata of young fronds of *Lemna minor* L. (G) Stomata of mature fronds of *Lemna minor* L. (H) Stomata of *Spirodela polyrrhiza* (L.) Schleid.(I) Stomata of *Spirodela polyrrhiza* (L.)Schleid. (J) CT of *Lemna minor* L. (k & l) CT of *Spirodela polyrrhiza* (L.) Schleid.

Figure 3C represents the action of duckweed extract on *Pseudomonas* sp. Spots**-** a, b, c, d and e represent inhibition zones by the action of distilled water (control), *Lemna***-** extract (100 mcg/disc), *Spirodela***-**extract (100 mcg/disc), Ampicillin (30 mcg/ disc), and Vancomycin (30 mcg/ disc) respectively.

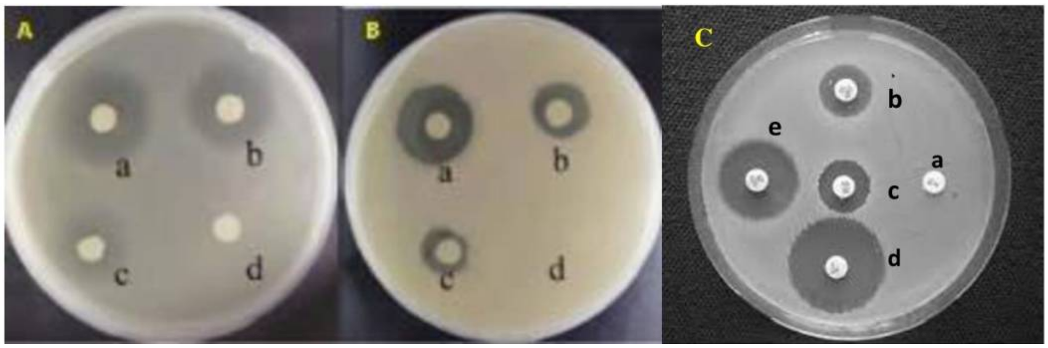

Fig. 3A and B. Antimicrobial action against *Aeromonas hydrophila* and *E. coli* respectively. Spots- a, b, c and d represent the zone of inhibitions with Ampicillin (30 mcg/ disc), *Spirodela*- extract (100 mcg/ disc), *Lemna*- extract (100 mcg/ disc) and control (without extract).

Reports on antimicrobial activity, particularly in case of the members of Lemnaceae are limited; whatever studies were conducted, mostly carried out on *Shigella flexneri, Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus* (Ziegler 2016). The effective concentration used to record any visible zone of inhibition were reported to be 12, 40, 60 and 170 mcg/ml. Although a few preliminary observations were available regarding the applicability of crude extract against the fish pathogens in Malaysian water bodies especially against the *Aeromonas* sp. (Lipeng *et al.* 2018), detailed assessment has been lacking. The present study exhibited that *Aeromonas hydrophila* and *Escherichia coli* can be controlled effectively using greater and lesser duckweeds from Indian aquatic environments. Such report may be considered as the pioneering one in this field.

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