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Antibacterial aromatic compound and steroid glycoside isolated from duckweed *Lemna paucicostata* Hegelm

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Abstract

Lemna paucicostata Hegelm is traditionally used as healthy feed for fish, broilers and as a source for vitamin A. In the present study, we report the isolation of an aromatic compound and a steroid glycoside from *Lemna pauciscostata*. The methanolic extract of dried and powdered *Lemna paucicostata* was fractionated using solid phase extraction (SPE). The compound was collected in a series of fractions. While first fraction contributed (80% pure) to an aromatic compound, 3rd fraction contained a steroid glycoside. Preliminary analysis of the compounds was performed with nuclear magnetic resonance and mass spectrometry. Aromatic compound was analyzed by ^{1}H NMR and MS spectra, steroid glycoside were analyzed by ^{1}H NMR and IR spectra. Aromatic compound showed significant inhibition of growth (10 mm) against Gram-negative bacteria, *Pseudomonas aeruginosa*.

Key words: *Lemna paucicostata*, Lemnaceae, *Pseudomonas aueriginosa*, aromatic compound, steroid glycoside

1. Introduction

Natural products have inspired chemists and physicians for millennia. Their rich structural diversity and complexity has prompted synthetic chemists to produce them in the laboratory, often with therapeutic applications in mind. Many drugs used today are natural products or natural-product derivatives. As the resistance for antimicrobial compounds is increasing, scientists are searching for new compounds coupled with improvements in approaches for natural-product isolation, characterization and their chemical synthesis in the laboratory for further modifications (Clardy and Christopher, 2004). Previously search for active compounds is limited to medicinal plants referred in ancient texts. At present, weeds also considered as a source for bioactive compounds. An analysis of 101 species from which 119 contemporary pharmaceuticals derived showed that 36 of these plants were weeds. These results are an order of magnitude higher than what would be predicted by random occurrence of weeds in the modern pharmacopeias (Stepp, 2004). Though the search for active compounds, triggered significantly in terrestrial weeds, the work in aquatic weeds is still under consideration. These plants have not yet been considered for isolation of pharmacological compounds produced by them. Duckweeds invade the aquatic environment within a short span of time. The rapid growth of duckweeds finds application in bioremediation of polluted waters and as a test organism for environmental studies. Duckweeds are useful as expression systems for economical production of complex biopharmaceuticals. However, until today, the research for active compounds in duckweeds is a question. The minor duckweed *Lemna paucicostata* is selected in the present study for the isolation and characterization of active compounds. The adaptation of *Lemna paucicostata* to polluted waters, suggested that it might consists of active compounds in it and this opinion driven us to analyze this plant for investigation of phytocompounds.

Lemna paucicostata Hegelm is lower duckweed and belongs to the family Lemnaceae of Angiosperms. It is a short-day plant and grows both in fresh and salt waters. This plant is useful against coryza, colds and running eyes. It generally accumulates arsenic, cadmium, manganese and copper from the polluted waters (Yutaka *et al*., 1984). *Lemna paucicostata* is useful as a feed for cattle, fish, broiler and as a valuable plant for biomanufacturing of proteins, polymers and vitamin A (Skillicorn *et al.*, 1990; Alese *et al.*, 2006). Investigations were earlier carried out on the levels of cyclic AMPs and nutritional content (Lakshman *et al.*, 1991; Mbagwu and Adenij, 1998). Many biological tests have also been performed on *Lemna paucicostata* (Hillman and Susan, 1981; Beppu and Takimoto, 1983). All species in the genus are useful to treat waste water and to remove metals. Studies on other species of this genus revealed the presence of oxygenated fatty acids and diterpenoid diols (Lucio and Monaco, 1984). Lemnan and pectic polysachharides were isolated form *Lemna minor* (Popov *et al.*, 2006). To date, no report is available on the isolation and identification of bioactive

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compounds from this genus. Very recently, Kenji reported the identification of C_{14} oxlipin glucosides (Kenji *et al*., 2010) from *Lemna paucicostata*. But no other report exists on the isolation of any aromatic compounds. We report here for the first time the identification of antibacterial aromatic compound and a steroidal glycoside from *Lemna* genus. Whole plant of *Lemna paucicostata* was dried under shade for 4 days and powdered. The material then was immersed in hexane for 7 days to remove fatty compounds. Repeatedly, methanolic extraction was carried out till the entire compound was extracted into the solvent. The extract was concentrated and subjected to column chromatography on silica gel. The compounds, thus, collected in different fractions with increasing ethyl acetate percent in hexane were tested on TLC. The fraction isolated with hexane:ethyl acetate (70:30) showed the presence of one compound in major quantity. To isolate this compound, the fraction was concentrated, dried and washed with chloroform. The remaining compound was tested on TLC for its purity.

2. Materials and Methods

2.1 General isolation procedures

The IR spectra were recorded on a Bruker Tensor 27 FT-IR spectrometer with KBr pellets. The ^{1}H NMR spectra were recorded in Varian 400 MHz. EI-MS were obtained at 70 ev and FAB–MS using Argon (6kv) as the FAB gas. Silica gel (60-120 mesh and 100-200 mesh, Merck, Darmstadt, Germany) was employed for column chromatography. TLC was carried out on precated kiesel gel 60 F_{254} of 0.25 thick, (Merck, Darmstadt, Germany) plates, with hexane-ethyl acetate and chloroform-ethylacetae as solvent systems. Coloured spots were visualized by exposure to I_2 vapours followed by spraying with 10% H_2SO_4 solution. Dragendorff"s reagent was used for the detection of alkaloids. Methanol, chloroform, ethyl acetate and hexane, used for extraction of compounds were purchased from the Finar chemicals, Hyderabad India. Methanol-sulfuric acid, Dragandroff reagent, ethanol-sulfuric acid were used to detect the compounds. These reagents were prepared with the sources supplied by Finar Chemicals. Silica coated glass plates for TLC were purchased from Merck. All other reagents and chemical used were of analytical grades supplied by Finar chemicals.

2.2 Plant material

Fresh plants of *Lemna paucicostata* Hegelm were collected from the ponds of Chidambaram area, Tamil Nadu, India. The voucher specimen was deposited in the Department of Botany, Osmania University, Hyderabad, India. The plants were dried under shade for 4 days and ground to fine powder. Plant material was stored at room temperature until extraction.

2.3 Extraction and isolation

The dried powdered plant material (3 kg) was extracted with 10 liters of hexane with frequent shaking at room temperature for 7 days. The material was exhaustively re-extracted with 10 liters of distilled methanol in order to isolate polar compounds. All the extractions combined and concentrated under reduced pressure. The crude methanolic extract contained two major compounds as judged by TLC, which was carried on silica support with the solvent system of hexane and ethyl acetate. The column (3 feet) was washed with distilled acetone to remove impurities. The methanolic extract was primarily fractionated on silica gel (60:120) in a glass column. The extract was eluted with a gradient of increasing polarity (hexane: ethyl acetate: ethyl acetate: methanol) with 10% increments of ethyl acetate and methanol from 20% to 100%. The elute was divided into 3 major fractions, which were further purified on silica gel of 100:200 mesh. The fractions, which were eluted in hexane:ethyl acetate were spotted on TLC plate and developed with methanol-sulfuric acid reagent. Fractions showing similar patterns on the TLC plate were combined to give 3 larger fractions. The fraction, which contained one major compound giving lemon yellow color with 10% (v/v) methanol-sulfuric acid reagent, together with two other minor compounds was designated as fraction 1. The above major compound (50 mg) in fraction 1 was eluted with hexane and ethyl acetate (70:30) and designated as compound 1. This compound was viewed under short UV light as black-colored spot. Fraction 2 did not contain any major compound. In fraction 3, the compound which gave deep-violet color when exposed to iodine vapours and sprayed with methanol-sulfuric acid was eluted with ethyl acetate and methanol (70: 30). It was designated as compound 2 (75 mg). This compound could not be detected under UV light.

2.4 Compounds 1 and 2

Compound 1 is a brownish white amorphous powder (80% pure) and ^{1}H NMR (DMSO): δ 5.5 to δ 7.0. These characters indicate that the compound is aromatic. The M+ spectrum exhibited signals at 321, 325, 349, 378, 399, 416, 442, 532, and 664. Compound 2: It is a greenish-white solid (85% pure), and has IR *Vmax* (film) cm-^{1} 3415 (OH), 2933 (C-H) 1618, 1380 (doublet - isopropyl, *t*-butyl), 1073 (CO) cm-1 and 620 (C-H). ^{1}H NMR (CD_3OD) signals appeared at: 5.5, 5.0, 4.95, 3.90, 3.87, 3.54, 3.31, 2.27, 2.23, 2.18, 2.15, 2.09, 2.05, 2.04, 2.03, 2.02 x 2, 2.00, 1.96.

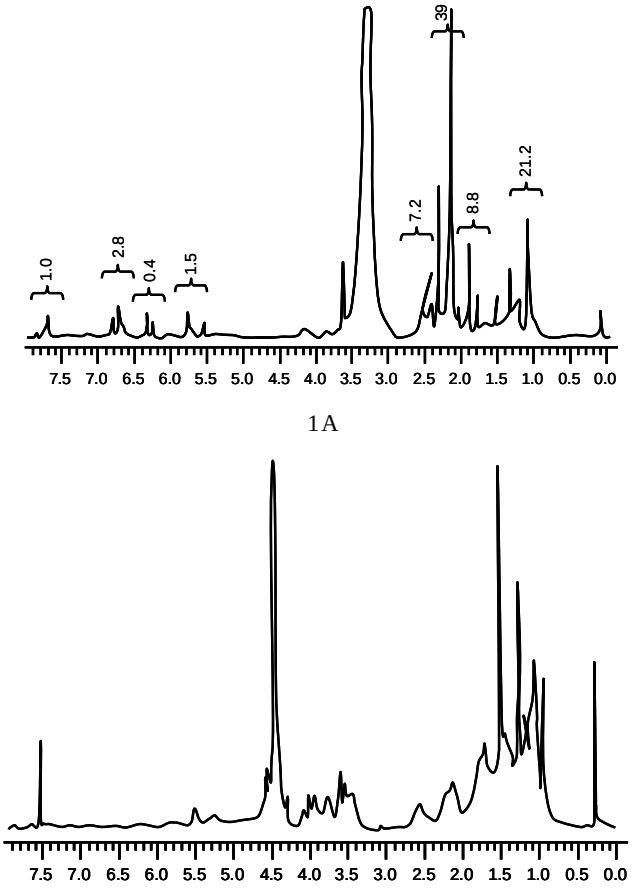
2.5 Antibacterial testing

Antibacterial activity was tested against Gram-negative bacteria *Pseudomonas aeuriginosa* by single disc method (Bauer *et al.*, 1966). The bacterial strain was cultured in sterilized Luria Bertani (LB) broth for 16-18 hours at 37^{0}C on rotary shaker. LB media (pH 5.7) with agar as gelling agent was prepared and sterilized at 121^{0}C for 15-20 minutes in an autoclave. Plating was carried out under aseptic conditions. A loop full of liquid broth of bacterial culture was spread over solidified LB medium in a Petri plate. Sterilized discs (Whattmann filter paper) were equidistantly placed on the agar media. The above compound each of 5 mg was weighed and dissolved in 5 ml of DMSO. The dissolved compound (3µl) was put on the disc with a micropipette and plates were wrapped tightly with parafilm and incubated at 37^{o}C for 24 hours.

3. Results and Discussion

Only single band was observed on TLC plate and this compound is submitted for ^{1}H NMR analysis. The appearance of signals between δ 5.5 to δ 7.9 (1A) in ^{1}H NMR revealed the presence of aromatic protons present in it and it was designated as compound 1. The ^{1}H NMR spectra of the compounds 1 and 2 (Figures 1A and 1B) in the extracts of *Lemna paucicostata* are shown in Figure 1. The mass spectrum of compound 1, and the IR spectrum of compound 2 are shown in the Figures 2 and 3, respectively. As Lemnaceae have rich flavonoid chemistry (Zennei and Clure, 1977), we judge that the compound 1 might be a flavonoid because it gave yellow color

upon spraying the methanolsulfuric acid reagent. It also exhibited significant antibacterial activity against Gram-negative bacteria *Pseudomonas aeruginosa*. To the best of our knowledge, this is the first report on the identification of an antibacterial activity of an aromatic compound from the genus *Lemna*. It is also evident that shikimate pathway which plays an important role in the biosynthesis of aromatic amino acids (Klauss, 1995) is producing such an aromatic compound in *Lemna* species.



1A

1B

Figure 1: [1]H NMR spectra of the compounds 1 and 2 (1A, 1B) in the extracts of *Lemna paucicostata*

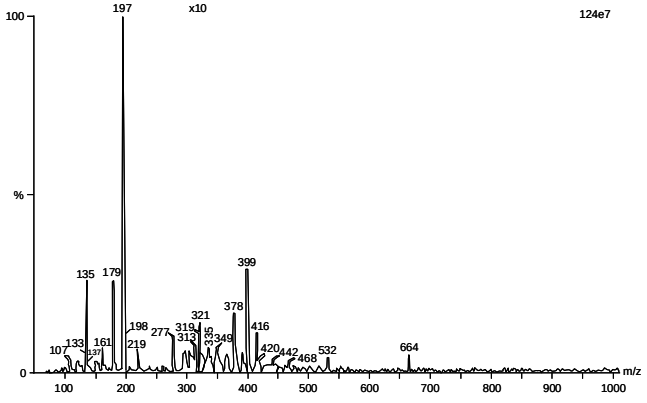


Figure 2: Mass spectrum of compound 1

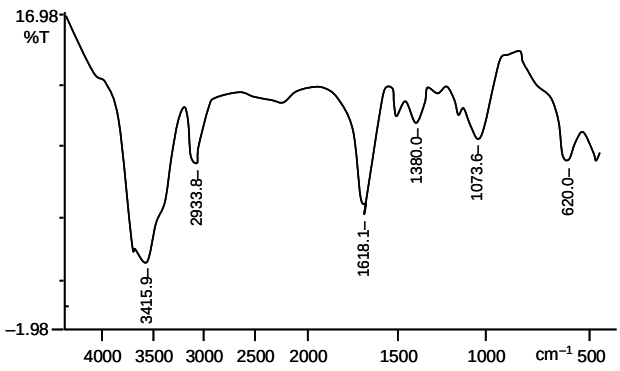


Figure 3: IR spectrum of compound 2

Figure 4: Antibacterial activity testing of aromatic compound

Fraction 3 was eluted with 70% ethyl acetate and 30% methanol which gave a greenish white solid in high quantity, but with impurities. Upon repeated purification in column chromatography, this fraction produced compound 2 with 80% purity. When charred with methanol and sulfuric acid, it turned into a violet colored spot on TLC. Based on the [1]H NMR (Figure 1), and IR (Figure 3) spectral data, we conclude that the compound is a steroid glycoside. The [1]H NMR signals in the spectrum stresses the need for further purification and structural investigation of the compound. The [1]H NMR and IR spectra revealed that the compound 2 is a glycoside fatty acid. Signals at δ 5.46 (1H b r d = 5.4 Hz, H-6) suggested the presence of steroid nucleus. The signals at δ 4.95, 3.90, and 3.87 allowed us to conclude that the compound is having a glycoside moiety. In the IR spectra, the signal at 1380 cm-1 indicates that the CH_3 umbrella deformation is present in the compound (Figure 3). The aromatic compound was used against *Pseudomonas aeruginosa* to find out if it has any antibacterial activity (Figure 4). Interestingly, significant inhibition of bacterial growth (10 mm) against Gram-negative bacteria, *Pseudomonas aeruginosa* was observed. This indicates that this compound has antibacterial activity.

4. Conclusions

Microbes are becoming resistant to the existing antibiotics (Roger and Timothy, 2008), a situation creating a need for the isolation and identification of novel molecules that can act against microbes. Our report on the identification of antimicrobial activity of the aromatic compound of *L. paucicostata* is the first report from this genus. This will encourage phytochemists to search for bioactive compounds in aquatic weeds too. This study will also become an evidence to consider *Lemna paucicostata* as medicinal plant rather than an aquatic weed. This investigation on *L. paucicostata* surely will trigger more research on many other aquatic weeds for identification of pharmaceutical compounds. As the structural and medicinal properties of the steroidal glycoside from *L. paucicostata* are still under investigation, this report predicts that it may also serve as cardiac glycoside. Studies to investigate the other compounds and their activity in *Lemna paucicostata* will aid in future discovery of new antibiotics from other species of *Lemna*. This study strongly suggests that further chemical investigation of this plant is necessary to isolate more bioactive compounds.

Acknowledgments

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Conflict of interest

We declare that we have no conflict of interest.

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