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Research Article

ISOLATION OF A COMPOUND FROM THE BULBS OF *ELEUTHERINE BULBOSA* (MILLER) URBAN (IRIDACEAE)

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ABSTRACT

Objective: The present study is concerned with the detection, isolation, identification, and structural elucidation of the bioactive phytochemical from the bulbs of *Eleutherine bulbosa*.

Methods: Ethyl acetate is the solvent used in Soxhlet extraction. Column chromatography and thin layer chromatography is employed in the isolation procedure. Identification and structural elucidation of the bioactive compound is determined using ultraviolet-visible (UV-vis), Fourier transform-infrared (FTIR) spectroscopy, liquid chromatography–mass spectrometry (LCMS-MS), and ^{1}H nuclear magnetic resonance (^{1}HNMR) spectroscopy.

Results: A compound was isolated from the bulbs of *E. bulbosa* by solvent extraction and chromatography. UV-vis, FTIR spectroscopy, LCMS-MS, and ^{1}HNMR studies showed that the isolated compound was bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal.

Conclusion: An yellowish-brown crystalline needle-shaped compound was isolated from the bulbs of *E. bulbosa* and characterized chemically. Spectroscopic analysis identified the compound as bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal. Mass of the compound is m/z 244 (MH+=245).

Keywords: *Eleutherine bulbosa*, Chromatographic techniques, Naphthoquinone derivative, spectroscopy.

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INTRODUCTION

Eleutherine bulbosa (Miller) Urban belongs to the tribe Tigrideae of the family Iridaceae. It is an herbaceous seasonal perennial with plicate leaves, white stellate flowers, and fleshy bulbs covered with red tunics. Although the plant originated in the tropical American countries, it has got naturalized in several parts of the world such as South Africa, China, India, and the Philippines [1]. *E. bulbosa* is an important element of American Indian pharmacopeia and is often cultivated in gardens maintained by Indian tribes [2].

In the Indian subcontinent, *E. bulbosa* has been reported from the states of Odisha, Kerala, and Tamil Nadu [3,4]. It is interesting to note that even though *E. bulbosa* is an exotic ornamental, the local medical men in Kerala have identified its medicinal properties and the bulb is being used as a cure for various ailments. The plant is known by the local names "Vizhanarayani" or "Neerootikizhangu." The bulbs are used as an antidote to poisonous insect bites [4] and in regulating blood pressure [5]. The local tribals in Odisha use the bulb to treat diarrhea [3]. The antibacterial and cytotoxic potential of the crude ethyl acetate extract of the bulbs has been reported [6,7]. The bioactive compounds in the bulb extracts of *E. bulbosa* are inferred to be naphthoquinones, and a few of their derivatives have been isolated [8-12]. Since the bulbs of *E. bulbosa* are being used extensively among traditional medicinal practitioners, are rich in bioactive phytoconstituents, and are known to exhibit biological activities, the plant appears to be a good candidate for phytochemical study.

The present report is concerned with the detection, isolation, identification, and structural elucidation of the bioactive phytochemical from the bulbs of *E. bulbosa*.

MATERIALS AND METHODS

Bulbs of *E. bulbosa* (Figs. 1 and 2) were collected from different parts of Kerala and authenticated by the curator, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram. A voucher specimen (KUBH 5802) has been deposited in the department herbarium.

The bulbs of the plant were collected, cleaned, shade dried and powdered and stored in properly labeled, and air-tight bottles for further experiments.

Extraction procedure and isolation

Extraction

About 600 g of the bulb powder was extracted with 5l ethyl acetate for 10 h at 77°C using a Soxhlet apparatus [13]. The extract was concentrated under vacuum and reduced pressure in a rotary evaporator at 45°C. The amount of the residue so obtained was recorded.

Column chromatography

The crude ethyl acetate extract was further subjected to column chromatography using silica gel mesh (120-size) as adsorbent. Elution was done by hexane:ethyl acetate (3:1, 1:1, and 3:1) mixture. The fractions were collected in 50 ml beakers. Each of these fractions was evaporated to dryness.

Analytical thin-layer chromatography (TLC)

Analytical TLC was done using Silica gel 60 F 254 plates (Merck, Germany). The fractions obtained from column chromatography were dissolved in acetone and spotted on the TLC plates. The plates were developed using the solvent system toluene:ethyl acetate (9:1). The retention factor (R_f) value was calculated.

$$\text{Retention Factor } (R_f) = \frac{\text{Distance travelled by the solute front}}{\text{Distance travelled by the solvent front}}$$

Preparative TLC

The fractions which showed similar chromatograms were pooled together. When single spots were produced, corresponding to the

R_f value obtained from the analytical TLC, they were scraped out, centrifuged, and purified. Each of these steps was repeated at least 5 times.

Chemical characterization of the purified compound(s)

Physical properties

The colour, shape of crystals, and nature of solubility of the purified compound(s) in different solvents were noted. Melting point was determined using a melting point apparatus.

Structure determination

The ultraviolet (UV)-visible spectrum was taken using UV-2400 PC Series Shimadzu Spectrophotometer, wavelength 200–800 nm. Fourier transform-infrared (FTIR) analysis of the isolated compound was taken in KBr pellets using Shimadzu IR Prestige 21, spectrophotometer. Liquid chromatography–mass spectrometry (LC-MS/MS) analysis was done using AB Sciex API 2000 System. The scanning was performed using four different "declustering potentials" (DPs) such as 40V, 60V, 80V, and 100V. ^{1}H nuclear magnetic resonance (^{1}HNMR) spectra were recorded on a Bruker 400 MHz NMR spectrometer using deuterated acetone as the solvent.^{1}HNMR spectra were detected at δ ppm (0–10) scale with end sweep at 0 ppm. Molecular modeling was done using NWChem program, the structure and vibrational data have been generated using Density functional theory calculations. B3LYP methods were employed using 6-31+G* basis set.

Fig. 1: *Eleutherine bulbosa*

Fig. 2: Bulbs of the plant

RESULTS

Extraction

When 600 g of the dried bulb powder was extracted with 5 l of ethyl acetate, the extract yield was 60 g. This concentrated residue was subjected to column chromatography followed by TLC.

Isolation and quantification of the naphthoquinone compound

The different fractions obtained from column chromatography were collected in small glass vials. A total of about 12 fractions were obtained. The fractions obtained from column chromatography were subjected to analytical TLC. The only solvent mixture that revealed distinct bands in the analytical TLC was hexane:ethyl acetate (1:1). Three identical fractions *E. bulbosa* fraction (EBF) EBF8, EBF9, and EBF10, which eluted in hexane-ethyl acetate mixture (1:1) showed a similar pattern. These fractions were pooled together and concentrated *in vacuo.* The residue so obtained was an yellowish-brown crystalline compound. The crystalline compound weighed about 40mg and is hitherto referred to as the *E. bulbosa* isolated compound (EBIC).

TLC analysis

Analysis of the purified compound from *E. bulbosa* - EBIC by TLC (Fig. 3a and 3b) showed a single band with blue fluorescence at R_f value 0.85 under long UV (365 nm). Under visible light, the single band showed a yellow color. The solvent system was toluene:ethyl acetate (9:1)

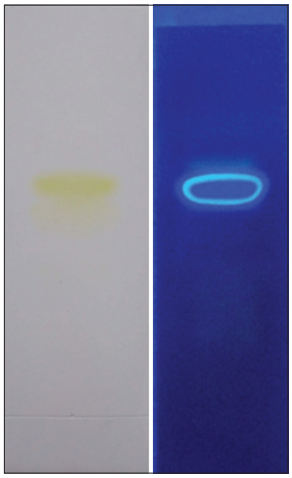

Fig. 3: (a) Thin-layer chromatography (TLC) of the purified compound from *Eleutherine bulbosa under* visible light. (b) TLC of the purified compound from *E. bulbosa under* long ultraviolet (365 nm)

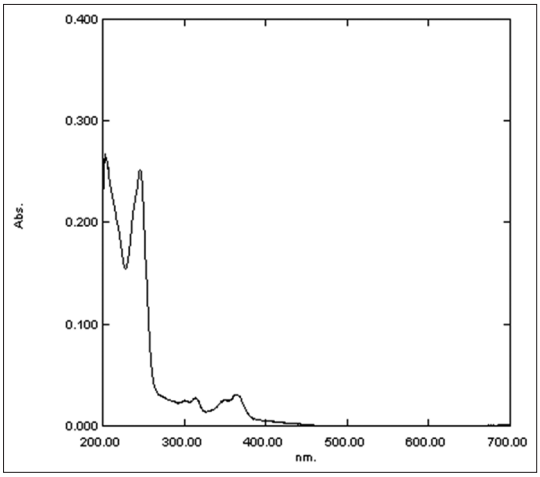

Fig. 4: Ultraviolet-visible spectrum of purified compound from *Eleutherine bulbosa*

Analysis of physical properties

The isolated compound was pure with an R_f value 0.85. The compound was an yellowish-brown crystalline needle-shaped solid with a melting point 195–196°C at 760 mm of Hg. Among the solvents (hexane, ethyl acetate, and water) tested, the compound was found to be the most soluble in ethyl acetate while it was insoluble in water.

Structure elucidation

UV-vis profile

The UV-vis profile of the purified compound showed peaks at 246 and 203 nm with absorbences, 0.251 and 0.268, respectively (Fig. 4).

FTIR analysis

FTIR (KBr) analysis showed the following peaks: 3487, 3062, 3034, 2873, and 1641 cm^{-1} and it is indicated in Fig. 5.

LC-MS/MS analysis

LC-MS/MS analysis showed the mass of the compound as *m/z* 244 (MH^+=245) and it is showed in Fig. 6.

^{1}HNMR analysis

^{1}HNMR showed peaks at 9.902, 7.169, 7.882, and 4.213 and between 0.9 and 1.5 ppm (Fig. 7).

From the computed (molecular modeling) and the above spectroscopic results, it is inferred that the purified compound, EBIC from *E. bulbosa,* in all probability is a naphthoquinone derivative with amino, carbonyl, and methyl substituents. The name of the compound was identified as bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal after comparison with chemical databases such as Guidechem Chemical Network and Chemical Manufacturers Dictionary. From the results of the different spectroscopic analyses and information from chemical databases, the chemical structure of the compound is as given in Fig. 8.

DISCUSSION

The UV-vis spectroscopy of the isolated pure compound showed that it was a naphthoquinone. Naphthoquinones are known to show UV absorption at a range between 200 nm and 300 nm [14]. The UV-vis profile of the compound showed peaks at 246 nm and 203 nm. The results of FT-IR indicated the possibility of aromatic (phenolic) compound with substituted amino and aldehyde groups. The FT-IR showed peaks at 3062.96 and 3034.03cm^{-1} indicating the presence of aromatic ring structure, peaks at 1641 cm^{-1} and 3487.3 cm^{-1} indicating the presence of amino group, and peaks at 2873 cm^{-1} indicating the presence of $-CH_3$ group [15]. The mass of the compound from LC-MS MS spectra at different DPs was *m/z* 244. Based on the spectra, the molecular formula assigned to the compound was $C_{13}H_{12}N_2O_3$. The spectral peak between 7.169 ppm and 7.882 ppm indicated the presence of hydrogen in the aromatic compound, a peak at 9.902 ppm indicated the presence of hydrogen in the aldehyde (-CHO) group, between 0.9 and 1.5 ppm indicated the CH_3 group, and a peak at 4.213 ppm indicated hydrogen in amino group (RNH_2).

The ^{1}HNMR study was helpful in the structural interpretation of the compound. A literature review showed that several naphthoquinones

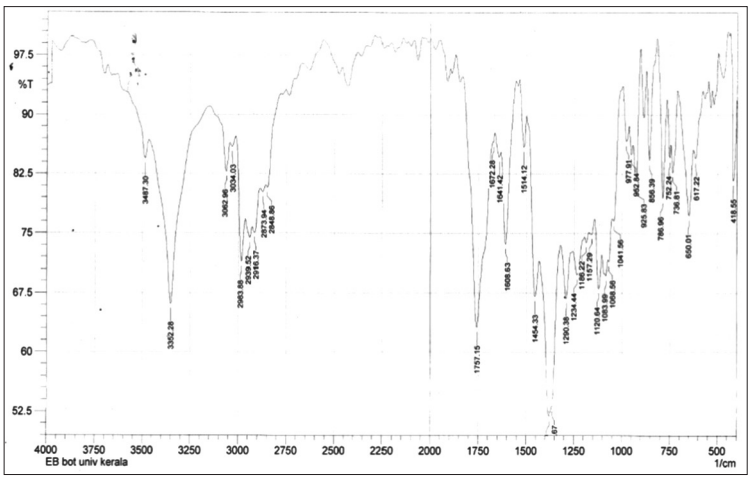

Fig. 5: Fourier transform-infrared spectrum of purified compound from *Eleutherine bulbosa*

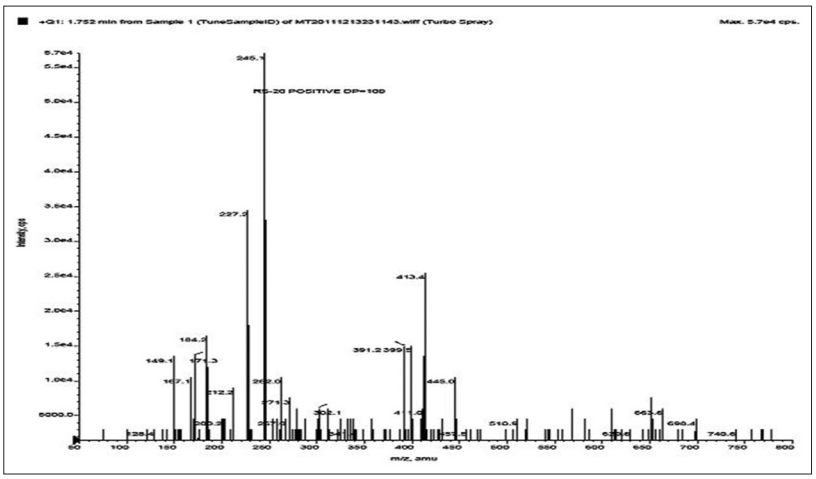

Fig.6: Liquid chromatography–mass spectrometry spectrum of the purified compound from *Eleutherine bulbosa*

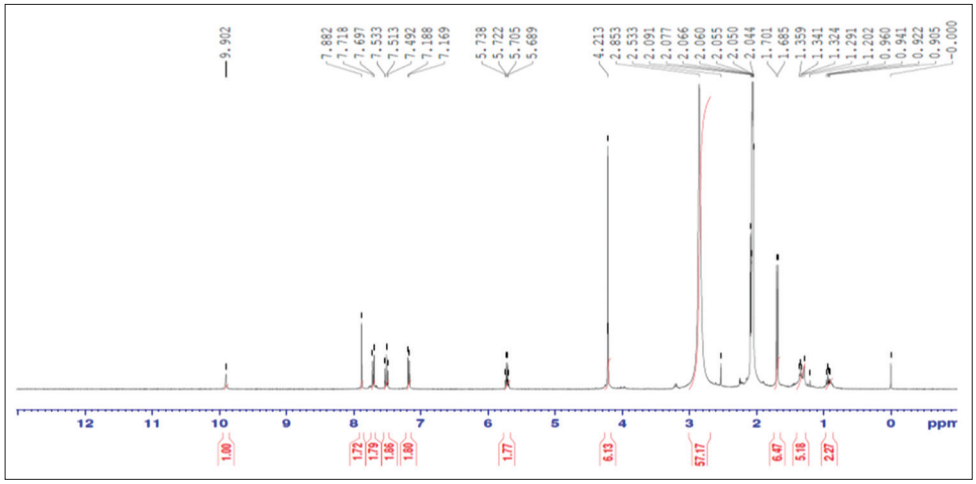

Fig. 7: ^{1}H nuclear magnetic resonance spectrum of purified compound from *Eleutherine bulbosa*

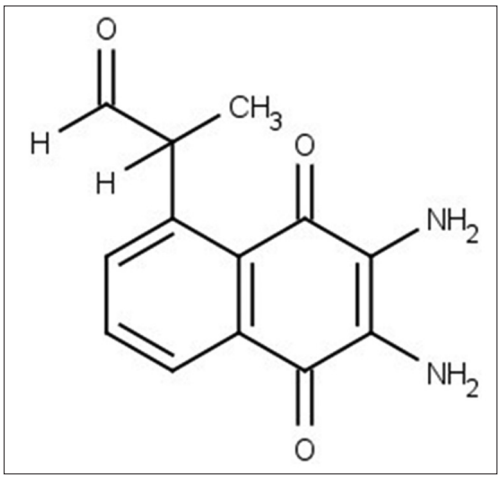

Fig. 8: Structure of the purified compound isolated from *Eleutherine bulbosa* [bis (2-6, 7-diamino-5, 8-dioxo naphthalene-1yl) propanal]

and their derivatives exhibit^{1}HNMR peaks between 7.169 and 7.882 ppm [16]. Earlier, a naphthoquinone, 11-hydroxyeleutherin isolated from the related genus, *Cipura paludosa* (Iridaceae) showed ^{1}HNMR peaks at 7.31 ppm, 7.68 ppm, and 7.74 ppm indicating the presence of hydrogen's in the naphthoquinone skeleton [17]. It could be interpreted from the above spectroscopic results that the compound, EBIC is a naphthoquinone derivative with amino, carbonyl, and methyl substituent.

The spectral characteristic interpretations are useful in the structural elucidation of bioactive compounds from plant extracts [18-20]. A few bioactive naphthoquinones and their derivatives have been isolated from the bulbs of *E. bulbosa*. A fungitoxic naphthoquinone, eleutherinone [21], polyketides such as eleutherin [22], and eleuthoside having α-glucosidase inhibitory activity [23] were isolated from this plant. The present study reports for the 1st time the presence of a novel naphthoquinone derivative from the bulbs of *E. bulbosa*.

CONCLUSION

An yellowish-brown crystalline needle-shaped compound was isolated from the bulbs of *E. bulbosa* and characterized chemically. Spectroscopic analysis identified the compound as bis (2-6,7-diamino-5,8-dioxo naphthalene-1yl) propanal.

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CONFLICTS OF INTEREST

The author has no conflicts of interest.

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