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Research Article

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In-vitro Anti-Inflammatory and Anticancer activities of *Octoblepharum albidum* Hedw.

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ABSTRACT

The chemical investigation of ethyl acetate extract of stressed moss *Octoblepharum albidum* Hedw. **(EA)** yielded two known metabolites, namely daucosterol **(1)** and friedelin **(2)**, which are characterized by using elemental and spectral analysis, also these metabolites are for the first conveyed from this species. **EA** and its secondary metabolites **(1-2)** were screened for *in vitro* anti-inflammatory (protein denaturation method), and anticancer (SRB assay using Ovary (PA1), Cervical (C-33A), and Lung (NCI-H358) cancer cell lines). The outcomes of bioassays displayed that **1** and **2** have effective inhibitory outlines against bovine albumin protein denaturation with a 50% inhibitory values of 435 and 403 µg/mL, respectively. Furthermore, compounds **1** and **2** demonstrated a noteworthy degree of specificity against PA1, C-33A, and NCI-H358, also they revealed a low degree of specificity against normal (NHME) cell line states non-lethal to normal human cells. The present study is the first report on the chemical examination of moss *O. albidum*.

Keywords: *Octoblepharum albidum*; Moss; Protein Denaturation; Anti-inflammatory; Anti-cancer, Acute toxicity studies.

1. INTRODUCTION

Mosses (non-vascular plants) persist in both humid and wet locations. About 17,000 moss species falling in 89 families and around 898 genera are identified across the world, of which 1786 moss species are identified and reported from Indian origin, till now (Mägdefrau, 1982; Proctor, 1990). Due to issues in collection of mosses, they are neglected for chemical and biological investigations, however, from the past two decades, mosses have been highly acknowledged (by chemists, biologists, and pharmacologists) to possess prominent biological activities due to their specific functional compounds (which are not available in higher plants) (Wang *et al.,* 1981; Ingólfsdóttir, 2000; Montenegro *et al.,* 2009; Kato-Noguchi *et al.,* 2010; Klavina *et al.,* 2015; Tatipamula *et al.,* 2017).

Based on the physiological adaptation, epiphytes (especially mosses) show a difference in their biological components, namely benzoic acid derivatives, carbohydrates, fatty acids, flavonoids, lipids, polyphenols, steroids, and terpenoids (Klavina *et al.,* 2015). Mosses extracts possess to have anti-diabetic, antifeedant, anti-HIV, antimicrobial, antioxidant, cytotoxic, sedatives, and nematocidal activities (Pejin and Bogdanović-Pristov, 2012; Klavina *et al.,* 2015; Tatipamula *et al.,* 2017). Furthermore, a like mangals, the species habituated in their surroundings also adapt to a stressful environment, thereby attain a great possibility to differ in their phytochemicals due to differences in their predator mechanism

(Bharadwaj *et al.*, 2018; Sastry and Bharadwaj, 2018).

Moss *Octoblepharum albidum* Hedw. (family: Calymperaceae) possess to have analgesic, antipyretic, antioxidant, antibiotic, and antimicrobial activities (Vidal *et al.,* 2012; Mukhopadhyay *et al.,* 2013). Also, there is no proper chemical evaluation has been carried out on stressed moss *O. albidum*, to date. So in the quest to identify bioactive metabolites from *O. albidum,* the current study was aimed to identify the chemical compounds present in stressed moss *O. albidum* by using chromatography techniques.

2. MATERIALS AND METHODS

Collection

From the trunks of Cocus species, the specimens of moss *Octoblepharum albidum* Hedw was collected at Bhitharkanika Island, Odisha, India, in March 2019. Dr. Ankita Srivastava determined the species, and a voucher specimen (LWG-6/VB-Orissa-2016) was deposited at Lucknow Bryophyte Herbarium, National Botanical Research Institute, Lucknow, India (Sastry and Bharadwaj, 2018).

Extraction

The collected stressed moss *O. albidum* (50 g) was dried in the shade and extracted thrice with ethyl acetate. All the obtained filtrates are combined and concentrated under reduced pressure to yield ethyl acetate extract of *O. albidum* (EA, 2.60 g, 5.2%w/w) as a dark greenish solid. EA (2.0 g) laid open to column chromatography of 230-400 mesh size and eluted with increasing polarity of hexane in ethyl acetate, to obtain two fractions (F-1 & F-II). F-I was laid open to column chromatography of 230-400 mesh size and eluted with increasing polarity of hexane in ethyl acetate yielded 1 (500 mg, 4.0%ww) as white solid. F-II lay open to column chromatography of 230-400 mesh size and eluted with increasing polarity of dichloromethane ethyl acetate yielded 2 (200 mg, 1.0%w/w) as pale yellow solid.

In-vitro Anti-inflammatory activity

Employing the protein denaturation method (Tatipamula and Vedula, 2018), the EA and isolated compounds (1-2) were determined for their in vitro anti-inflammatory activity in triplicate. Albumin protein from bovine serum was used as a protein. By using sodium phosphate buffer, the protein was solubilized. To the 0.2 mL of the prepared protein sample, added know concentrations of the test sample, and finally made up the volume to 5 mL with the above buffer. All the prepared samples are then incubated at 37 ˚C for 20 min, then boiled at 95 ˚C for 20 and chilled to 25 ˚C, noted absorbance at 562 nm against the blank.

Sulforhodamine B (SRB) colorimetric assay

Utilizing the SRB assay (Haritha *et al.,* 2019), the EA and isolated compounds (1-2) were implemented for their in vitro anticancer activity in triplicate (n=3) using three cancer cells: Ovary (PA1), Cervical (C-33A), and Lung (NCI-H358), and one normal cell line: normal human mammary epithelial (NHME). All the cells are purchased in good order from National Centre for Cell Science, Pune, and preserved according to the procedures of Haritha *et al.,* 2019. To 190 µL suspension of cancer cells added test samples (dissolved in DMSO) and incubated at 37˚C for 3 h in the presence of 5% CO_2. Later, added cold TCA (100 µL) and incubate at 4˚C for 1 h, then washed and dried to room temperature. To it added 0.057% SRB solution (100 µL) and incubated for 30 min. Finally, the 96-well plate is stained and detects the optical density at 510 nm.

% Growth Inhibition = 100–(Sample absorbance/Control absorbance) X 100

Statistical analysis

All in vitro assay test results were note as mean ± SD. By using a one-way analysis of variance (ANOVA) followed by a t-test test with $p < 0.05$ was measured to be statistically significant.

3. RESULTS

Chemistry

Chemical investigation of ethyl acetate extract of stressed moss *O. albidum* (EA) yielded two compounds, namely 1 and 2 (Figure.1), which are found to be daucosterol and friedelin, respectively by characterized using spectral data.

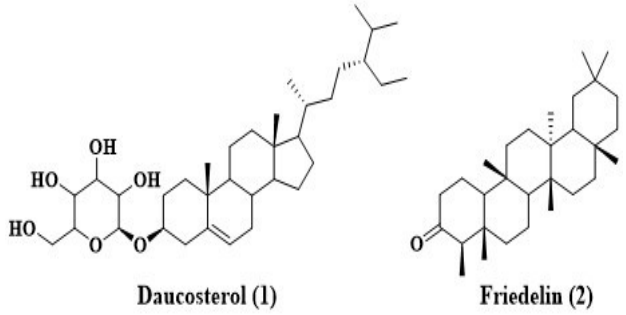

Figure 1: Chemical structures of known secondary metabolites identified from ethyl acetate extract of *Octoblepharum albidum*.

1 (Daucosterol): White solid, R_f: 0.6 (Hex:EA, 1:1), Mol. For.: $C_{35}H_{60}O_6$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.83 (1H, s, CH-33), 0.97 (1H, s, CH-14), 1.00 (3H, s, CH_3-25), 1.01 (3H, t, *J* = 0.8 Hz, CH_3-19), 1.03 (3H, t, *J* = 0.8 Hz, CH_3-26), 1.03 (6H, t, *J* = 0.8 Hz, CH_3-28 & 29), 1.08 (1H, s, CH-35b), 1.10 (1H, s, CH-23), 1.11 (1H, s, CH-17), 1.13 (3H, s, CH_3-18), 1.15 (1H, s, CH-34), 1.17 (1H, s, CH-12b), 1.18 (1H, s, CH-21a), 1.23 (1H, s, CH-1a), 1.24 (1H, s, CH-24a), 1.29 (1H, s, CH-22a), 1.33 (2H, s, CH-16a & 22b), 1.35 (1H, s, CH-24b), 1.37 (2H, s, CH-11a & 12b), 1.38 (1H, s, CH-21b), 1.39 (1H, s, CH-20), 1.41 (1H, s, CH-8), 1.42 (1H, s, CH-2a), 1.46 (1H, s, CH-32), 1.48 (1H, s, CH-1b), 1.49 (1H, s, CH-27), 1.58 (1H, s, CH-16b), 1.61 (1H, s, CH-15b), 1.73 (1H, s, CH-2a), 1.77 (1H, s, CH-7b), 1.82 (1H, s, CH-11a), 1.85 (1H, s, CH-4b), 1.86 (1H, s, CH-15a), 2.00-2.04 (1H, m, CH-7a), 2.17-2.19 (1H, m, CH-9), 2.24-2.28 (1H, m, CH-4a), 3.40 (1H, s, CH-31), 3.45 (1H, dd, *J* = 0.4, 0.8 Hz, OH-32), 3.47 (1H, d, *J* = 1.6 Hz, H-35a), 3.62-3.64 (1H, s, dd, *J* = 1.2, 1.6 Hz,OH-34), 3.69-3.70 (1H, dd, *J* = 1.2, 1.6 Hz, OH-33), 3.72 (1H, d, *J* = 0.8 Hz, OH-35), 3.79-3.83 (1H, m, CH-3), 5.40 (1H, d, *J* = 1.2 Hz, OH-30), 5.46-5.48 (1H, t, *J* = 4.8 Hz, OH-6). ^{13}C NMR (400 MHz, DMSO-d_6): δ 12.11 (C-25), 13.94 (C-19), 18.77 (C-26), 19.64 (C-18), 19.87 (C-28/C-29), 22.38 (C-11), 24.86 (C-15), 24.95 (C-24), 28.15 (C-16), 28.30 (C-22), 29.65 (C-2), 31.13 (C-27), 31.88 (C-7), 35.12 (C-8), 35.41 (C-21), 36.29 (C-20), 36.95 (C-1), 38.15 (C-10), 39.10 (C-4/C-12), 43.44 (C-13), 45.01 (C-23), 50.51 (C-9), 56.86 (C-17), 58.13 (C-14), 62.26 (C-35), 70.46 (C-32), 73.84 (C-34), 74.80 (C-33), 76.65 (C-31), 78.16 (C-3), 101.49 (C-30), 122.08 (C-6), 141.21 (C-5). Elemental analysis: found C-72.66, H-10.62(%), calcd. C, 72.87, H, 10.48(%). ESI-MS negative mode: *m/z m/z* 577.50 [(M-H$^+$), 45.47%] calcd. 576.86.

2 (Friedelin): Pale yellow solid, R_f: 0.4 (Hex:EA, 1:1), Mol. For.: $C_{30}H_{50}O$; ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (1H, s, CH-8), 0.94 (1H, s, CH-10), 0.98 (1H, s, CH-11b), 1.00 (1H, s, CH-12b), 1.00 (6H, s, CH_3-29 & 30), 1.01 (6H, s, CH_3-24 & 28), 1.02 (9H, s, CH_3-25, 26 & 27), 1.03 (1H, s, CH-19a), 1.22 (1H, s, CH-19b), 1.23 (3H, s, CH_3-23), 1.37 (5H, m, CH-6a, 15b, 16b, 21b & 22b), 1.42 (1H, s, CH-7b), 1.62 (6H, m, CH-6b, 7a, 15a, 16a, 21a & 22a), 1.68 (1H, m, CH-11a), 1.75 (1H, m, CH-18), 1.77 (1H, m, CH-12a), 1.84 (1H, m, CH-1a), 2.01 (1H, m, CH-1b), 2.12 (1H, m, CH-4), 2.15 (2H, m, CH-2). ^{13}C NMR (400 MHz, DMSO-d_6): δ 9.57 (C-23), 15.78 (C-24), 16.08 (C-25), 17.47 (C-26/C-27), 19.34 (C-7), 22.76 (C-1), 25.29 (C-28), 28.60 (C-29/C-30), 30.00 (C-12), 30.13 (C-20), 30.45 (C-15), 31.18 (C-17), 35.34 (C-21), 35.93 (C-16), 36.56 (C-11), 37.21 (C-19), 37.70 (C-9), 38.85 (C-22), 40.00 (C-13), 40.22 (C-6), 40.37 (C-2), 40.96 (C-5), 41.70 (C-14), 43.59 (C-18), 50.83 (C-8), 57.39 (C-4), 57.70 (C-10), 212.18 (C-3). Elemental analysis: found C-88.64, H-11.52(%), calcd. C-88.44, H-11.81(%). ESI-MS negative mode: *m/z* 427.30 [(M-H$^+$), 42.15%], calcd. 426.73.

3.2 In-vitro Anti-inflammatory activity

In general, inflammation is caused by denaturation of biological protein (Tatipamula and Vedula, 2020). In the present study, EA and secondary metabolites (1-2) have investigated for their restraint of bovine serum albumin protein denaturation influenced by heat. The outcomes of

the study indicated that all tested samples exhibited noteworthy anti-inflammatory action. The 50% inhibition of 1, 2, and EA on bovine serum albumin protein denaturation were found to be 462.0±10.25, 790.0±14.52, and 220.0±12.14 µg/mL, respectively, while indomethacin with 110±10.24 µg/mL (Figure 2).

3.3 Anticancer activity

Generally, prolonged inflammation many dangerous diseases, as well as cancer. As EA and its secondary metabolites (1-2) revealed well anti-inflammatory actions, we additional estimated for their anti-cancer properties by the SRB assay, using three cancer cells, namely PA1, C-33A and NCI-H358 and one normal cell line (NHME). The results of percentage of inhibition of cell growth against respective concentrations are designed to attain IC_{50} values.

All the tested samples demonstrated a sensible degree of specificity against PA1, C-33A, and NCI-H358. Moreover, EA and its secondary metabolites (1-2) exhibited a very slight degree of specificity against NHME designates non-toxic. Among all the samples, EA displayed a more prominent degree of specificity against PA1, C-33A and NCI-H358 with IC_{50} values of 41.0±2.14, 59.20±2.11, and 38.5±3.47 µg/mL, respectively while doxorubicin with 4.5±0.12, 3.8±0.24 and 6.3±0.14 µg/mL, respectively (Fig. 3). Compounds 1 and 2 revealed IC_{50} value of 77.50±4.17 and 88.50±7.10 µg/mL on PA1, respectively; 80.50±6.57 and 95.0±6.55 µg/mL on C-33A, respectively; and 92.0±5.86 and 97.50±7.89 µg/mL on NCI-H358, respectively (Figure 3).

4. DISCUSSION

In the current research work, the column chromatographic inspection of ethyl acetate extract of *O. albidum* (EA) yielded two compounds (1-2), which are characterized by spectral and elemental analysis (Fig. 1). EA and its secondary metabolites (1-2) were exposed to *in-vitro* studies of anti-inflammatory and anticancer activity using by using the albumin protein denaturation method and the SRB assay, respectively.

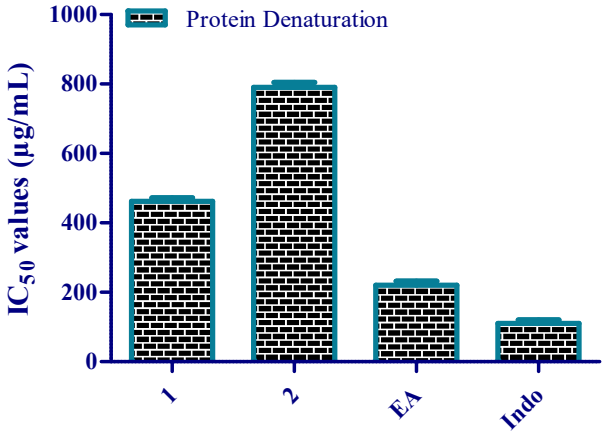

Figure 2: IC50 values of EA and secondary metabolites (1-2) against albumin protein denaturation.

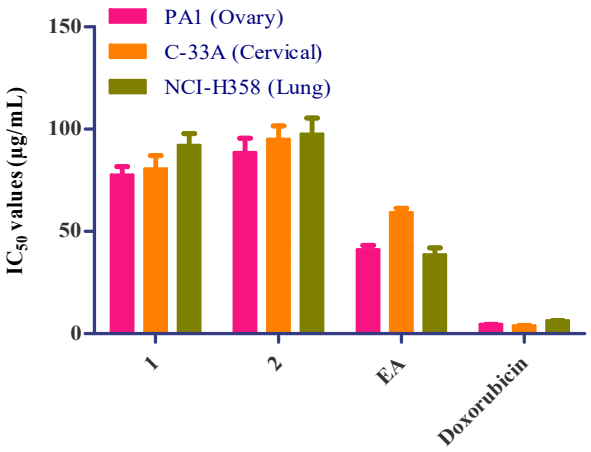

Figure 3: IC50 values of EA and secondary metabolites (1-2) against a series of cancer cell lines

From the results of the protein denaturation method, it can be found that EA and its secondary metabolites (1-2) exhibited noticeable inhibitory abilities against bovine serum albumin protein denaturation (Figure 2). From the data, it is proposed that EA may act by blocking the biosynthesis of Interleukin-8, prostanoids, and thromboxane (Tatipamula *et al.,* 2019). Hence, EA has noticeable anti-inflammatory competence

against both acute and chronic simulations of inflammation connected with arthritis (Tatipamula *et al.,* 2020).

Additionally, as the EA and its secondary metabolites (1-2) represented substantial anti-inflammatory possessions, we assessed compounds 1, 2 along with EA for their anti-cancer properties by SRB assay using PA1, C-33A, and NCI-H358 cancer cells and NHME cells. Form Fig. 3, it can be concluded that crucial elements responsible for the anti-cancer activity of EA were found to be 1 and 2. Also, as stated earlier, chronic inflammation eventually causes cancer, the anticancer action of EA and its secondary metabolites (1-2) was due to their anti-inflammatory abilities. Furthermore, EA and its secondary metabolites (1-2) displayed a slight degree of specificity against NHME conditions indicates non-lethal to human cells.

Conclusion

The present research work is a basic research study of the chemical and pharmacological investigation of stressed moss *O. albidum*. Chemical investigation of ethyl acetate extract of *O. albidum* (EA) results in the isolation of two known secondary metabolites, namely daucosterol (1) and friedelin (2), which were characterized by elemental analysis and spectral data. The pharmacological investigation exposed the inhibitory abilities of EA and its secondary metabolites (1-2) against albumin protein denaturation, PA1, C-33A, and NCI-H358. Furthermore, the key secondary metabolites accountable for its natural action are 1 and 2. The current study impetus the researchers to identify pharmacological usage of under-investigated bryophytes including mosses.

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Conflicting of Interests

None to declare.

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