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Antifungal activity, phytochemical analysis and mode of action of *Atrichum undulatum* (Hedw.) P. Beauv.

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In the present study, the antifungal activity of a West Himalayan bryophyte *Atrichum undulatum* in different solvents (butanol, ethanol, methanol and aqueous) has been evaluated by agar well diffusion assay against six fungal strains (*Candida albicans, C. glabrata*, *C. tropicali*s, *C. parapsilosis, Issatchenkia orientalis* and *Kluyveromyces marxianus*). Among all the extracts, butanol extract showed potent antifungal activity against *I. orientalis*, *K. marxianus*, *C. parapsilosis* and *C. tropicalis* followed by ethanol and methanol extract. Fungal cells treated with butanol extract showed cell wall damage under scanning electron microscopy and confocal microscopy. GC-MS and FTIR analysis showed the presence of bioactive compounds which act as antimicrobial agents. The results support the traditional use of *A. undulatum* as an antimicrobial agent. The present research records for the first time the morphological alterations of fungal cells by the butanol extract of *A. undulatum* alone and synergistically with the antibiotic Amphotericin B (Amp B).

Keywords: Antifungal activity, *Atrichum*, *Candida*, Flow cytometry, Microscopy

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Introduction

Since ancient times, plant-derived products have been used as traditional medicines worldwide and are the basis of several pharmaceutical industries in recent times. Herbal medicines are now the major basis of many modern pharmaceutical industries. The urgent need for natural or plant-based medicines has increased due to the resistance of microbes to synthetic antibiotics. Also, plant-based medicines are reported to have low toxicity and more effectiveness[1].

Bryophytes have been used to cure various ailments. Bryophytes are small nonvascular cryptogams that grow as various growth forms on various substrata. Several bryophytes (especially mosses) have widely been used as medicinal plants in China. Attention towards the biochemical composition of bryophytes has increased due to the presence of their high bioactive compounds. Mosses are said to be resistant to infectious microbes, and due to this property, they have been used as medicinal plants to cure burns, bruises, external wounds, etc., in different parts of the world like China, America, Europe[2]. This property can be due to the presence of biologically active compounds which act as antimicrobials. Miller & Miller reported using *Marchantia polymorpha* to treat liver ailments due to its appearance like liver lobes[3]. Hu, investigated the use of *M. polymorpha* in jaundice and also to cure inflammation as a cooling and cleansing agent for the liver, as reported by Bland,[4,5].

Many medicinal bryophytes have been reported in literature[6-9]. During World War I, injured soldiers used *Sphagnum* moss to stop bleeding[10]. *Plagiochasma appendiculatum* has been used as traditional medicine in Kangra Valley by the Gaddi tribe to cure skin diseases, and the locals referred to it as 'Patarshali'[11].

Glime reported the use of *Atrichum* in the Chinese medicine market primarily under antibacterial and anti-inflammatory agents, *Rhodobryum giganteum* and *R. roseum* for the treatment of cardiac diseases, *Sphagnum* for the treatment of ocular diseases and *Polytrichum commune*, *Barbula unguiculata*, *Bryum capillare* and *Octoblepharum albidum* to cure inflammation and fever[12].

The effects of methanol extract, ethanol extract and aqueous extract of *A. undulatum* against *E. coli*, *B. subtilis* and *S. typhimurium* were investigated[13]. The ethanol extract was reported to show maximum activity, whereas the aqueous extract the least.

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The antibacterial effects of *Marchantia nepalensis*, *M. palmata*, *Plagiochasma appendiculatum*, *Asterella pathankotensis*, *Cyathodium cavernarum* and *Reboulia hemisphaerica* against *Escherichia coli*, *Staphylococcus aureus, Streptococcus pyogenes*, *Micrococcus luteus* and *Bacillus subtilis* were observed in our laboratory. Synergistic and antagonistic effects of selected liverworts were studied in combination with two medicinally very important plants, *Ocimum sanctum* and *Azadirachta indica*[14].

Sevim *et al*., found antibacterial activity in the methanol extracts of all the 23 bryophyte species tested against *Paenibacillus* larvae isolates[15].

Out of approx. 1.5 million fungal species, very small numbers of species that cause human diseases[16]. The fungus *Candida* is one of the challenges for health departments as it causes serious infections, spreads quickly, and is very opportunistic. *Candida* is one of the main reasons for invasive mycosis along with *Aspergillus fumigatus*[17]. The disease-causing frequency of *C. albicans* is maximum (50-70%) followed by *C. glabrata* (20-25%), *C. tropicalis*, *C. Krusei* and *C. parapsilosis*[18].

Bryophytes produce a wide variety of secondary metabolites which can be used in the pharmaceutical industry. Despite their worldwide distribution, bryophytes are ignored as a pharmaco-industrial resource. Hence, the present study attempts to study antifungal activity and mode of action of various extracts of *A. undulatum* on some fungal strains along with phytochemical analysis by GCMS and FTIR.

Materials and Methods

Collection of the plant material and preparation of extracts

Atrichum undulatum (Hedw.) P. Beauv. collected from Shimla (Himachal Pradesh, India) in North-West Himalaya during September 2017, has been identified by Prof. S. S. Kumar, Professor Emeritus, Department of Botany, Panjab University, Chandigarh and submitted to Herbarium of Department of Botany, Panjab University, Chandigarh for future reference (PAN6312). The fresh material was cleaned and washed under running water. The sample plant material was then shade-dried and crushed to powder form. The powdered plant material was extracted in each solvent, butanol, ethanol, methanol, and water. For this, 4 g of dried plant material was suspended in 20 mL of various organic solvents, maintained in a shaking incubator at 37°C for 18–24 h and filtered through Whatman filter paper no. 1. Different solvents alone were used as a negative controls.

Test fungi

Six *Candida* species had been procured from the National Collection of Pathogenic Fungi (NCPF), Post-Graduate Institute of Medical Education and Research (PGIMER), Chandigarh, India, and Microbial Culture Collection Centre (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India, i.e., i) *Candida albicans* (C. P. Robin) Berkhout, ii) *Candida glabrata* (H. W. Anderson) S. A. Mey. & Yarrow, iii) *Issatchenkia orientalis* Kudryavtsev, iv) *Candida parapsilosis* (Ashford) Langeron & Talice, v) *Kluyveromyces marxianus* (E.C. Hansen) Van der Walt., and vi) *Candida tropicalis* (Castell.) Berkhout.

Antifungal activity and Synergistic studies

The fungal strains were cultured in yeast extract-peptone-dextrose [YEPD broth and YEPD agar (HI Media, India)] and RPMI 1640 media (HI Media, India). The strains were stored as frozen stocks in 15% glycerol at -80°C. Before each experiment, the cells were freshly revived on respective agar plates from the stock.

The agar well diffusion method was used to determine the antifungal activity of various plant extracts against fungal strains and to determine the synergistic effect of selected plant extract with antibiotic Amphotericin B (Amp B), according to Clinical and Laboratory Standards Institute (CLSI) guidelines. The Minimum Inhibitory Concentration was determined by the broth microdilution method.

Mechanistic studies

The most efficient extract against the *Candida* species was further investigated using SEM, Confocal microscopy, flow cytometry, and phytochemical investigations utilising GC-MS and FTIR.

Scanning Electron Microscopy (SEM) of fungal cells

For SEM analysis, the suspensions of fungal cells had been prepared in YEPD broth from overnight grown cultures. The plant extract at inhibitory concentration had been added to the fungal cells (~1 x 10^4 CFU/mL). After treatment, the samples were observed under SEM[19].

Confocal Microscopy and Flow cytometry

The permeabilisation effect of selected plant extract on fungal cells was checked using a membrane-impermeable dye, Propidium Iodide (PI). Overnight grown colonies of fungal cells (~1 x 10^4 CFU/mL) were suspended in YEPD broth media containing plant extract at the MIC and PI

(1.42 µg/mL) at 30°C with constant shaking (200 rpm). Cells were harvested by centrifugation and suspended in Phosphate Buffer Saline (PBS, pH 7.4). Further, the cells were examined by Confocal Microscopy with a wavelength > 560 nm for PI. Fungal cells without treatment of plant extract served as the control[20].

Phytochemical analysis by GC-MS AND FTIR

The GC-MS analysis of the bioactive extract of the bryophytic sample was performed using Thermo Trace with TG 5MS (30 m X 0.25 mm, 0.25 µm) column. The injector temperature was set at 250°C, whereas the mass transfer line temperature was 280°C. The injection volume was 1.0 µL. The relative percentage of plant extract compounds was expressed as a percentage with peak area normalisation. For the identification of compounds present in the plant extract, the retention time and mass spectra fragmentation pattern were compared with those present in the National Institute of Standard and Technology (NIST), version 2015.

Fourier Transform Infra-Red (FTIR) spectroscopic analysis of fractioned plant extract was done using one drop of plant extract put between two plates of NaCl, the drop forming a thin film between the plates.

Statistical analysis

At least three separate experiments were carried out in triplicates. Data were expressed as Mean±Standard Deviation using GraphPad Prism (ver.8.0.1).

Results and Discussion

Antifungal activity of plant extracts

In the present study, the antifungal activity of *A. undulatum* was studied against selected fungal strains. Different extracts of *A. undulatum* exhibited distinct variations in antifungal activity. Hexane and aqueous extracts were not active against any of the fungal strains. Butanol extract showed the highest potency as an antifungal agent against *C. tropicalis*, *K. marxianus*, *C. parapsilosis* and *I. orientalis,* with zones of inhibition of 18, 15, 13, and 11 mm, respectively as shown in Fig. 1. Ethanol extract of *A. undulatum* was active against *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *I. Orientalis* and *K. marxianus* with zone of inhibition of 12, 11, 10, 10, and 10 mm, respectively, whereas methanol extract of *A. undulatum* was active against *C. glabrata*, *C. albicans*, *C. tropicalis*, *C. parapsilosis* and *K. marxianus* with the zone of inhibition of 11, 10, 10, 10, and 10 mm, respectively. Earlier, various phenolic compounds isolated from *Atrichum* had shown antimicrobial properties[21]. The antifungal activity of the DMSO extract of *A. undulatum* against *Aspergillus versicolor* and *A. fumigates* has also been reported[22].

Synergistic studies

Crude extract of *A. undulatum* prepared in butanol was tested with agar well diffusion assay for synergistic studies. The antifungal activity of butanol extract in combination with the antibiotic Amphotericin B resulted in varied degrees of zone of inhibition. There was an increase in the activity with a 16 mm zone of inhibition when plant extract was combined with antibiotic in comparison to an 11 mm inhibition zone when only plant extract was taken, as shown in Fig. 2. The present study is the first report on the synergistic effect of butanol extract of *A. undulatum* with antibiotic Amphotericin B (Amp B).

Mechanistic studies

Scanning electron microscopy

SEM was carried out to visualise the effects of the butanol extract of *A. undulatum* on the morphology of cells of *C. tropicalis*. There were visible signs of morphological alteration in the cells of *C. tropicalis* when treated with plant extract. Under control conditions, fungal cells had smooth walls (Fig. 3a), but after treatment with butanol extract, roughness in the walls of fungal cells was observed (Fig. 3b). This study is the first attempt to visualise morphological alterations in cell walls of *C. tropicalis* by butanol extract of *A. undulatum*.

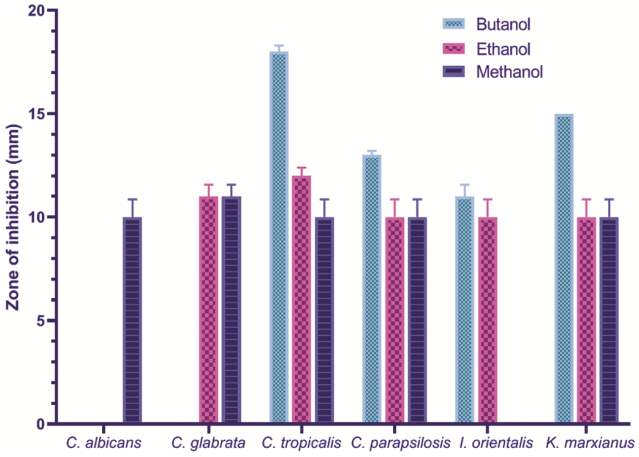

Fig. 1 — Histogram showing antifungal activity of butanol, ethanol and methanol extracts of *A. undulatum* against six fungal strains. Data were represented by the mean±standard deviation of three repetitive measurements.

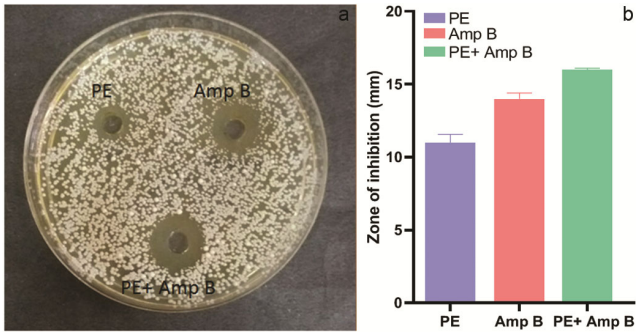

Fig. 2 — a) Synergistic effect of butanol extract of *A. undulatum*; and b) Histogram showing the synergistic effect of plant extract with Ampicillin B against *C. tropicalis*. The mean±standard deviation of three repeated measurements was used to represent the data. PE: Plant extract; Amp B: Ampicillin B.

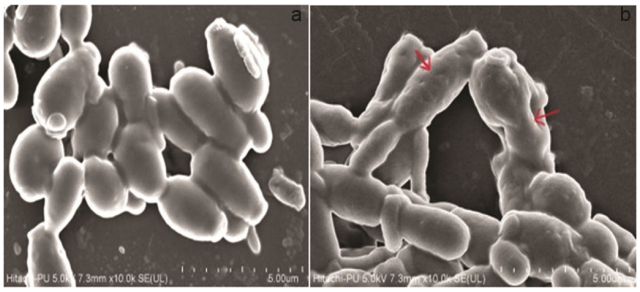

Fig. 3 — SEM images of cells of *C. tropicalis*, a) control (untreated cells); and b) treatment with butanol extract of *A. undulatum* (Arrows indicating rupturing of the cell wall).

Membrane permeabilisation of Candida tropicalis cells by butanol extract

Propidium iodide is the membrane-impermeable dye which binds to DNA after the permeabilization of the cells. Fungal cells treated with butanol extract were observed with red fluorescence, indicating cell damage (Fig. 4b) compared to the untreated fungal cells, which showed no signs of damage (Fig. 4a).

Effect of butanol extract on DNA of Candida tropicalis (Flow Cytometry)

Antifungal agents attack the cell membrane of fungal cells to make it permeable. The cell death of *C. tropicalis* was measured by using flow cytometry. Flow cytometric analysis confirmed the results displayed by the Agar well diffusion assay. A shift in intensity was observed from 10^2 in untreated cells (Fig. 5a) to 10^4 in butanol extract-treated cells (Fig. 5b), indicating entrapment of dye in the fungal cells due to cell wall damage. *C. tropicalis* cells showed 1.06% cell death, as revealed by flow cytometry results.

SEM and Confocal microscopic images and Flow cytometric analysis indicated the microbial membrane damage. Phenols act by initiating the leakage of intracellular components like K^+, the initial step for

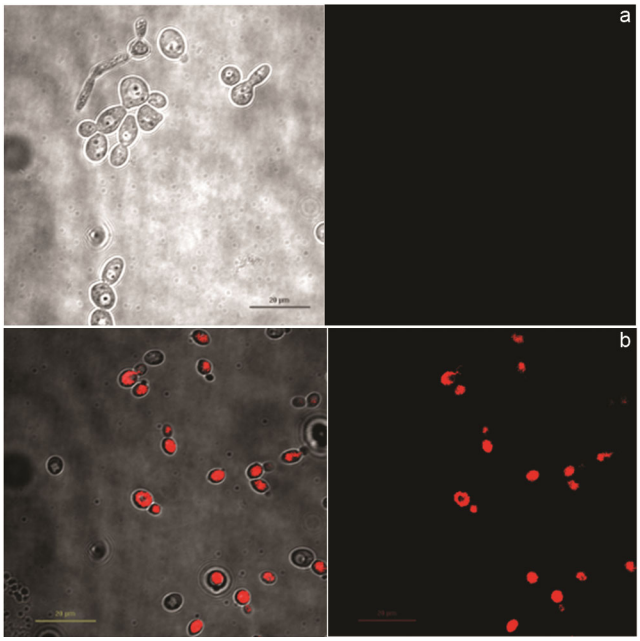

Fig. 4 — Confocal images of cells of *C. tropicalis* treated with butanol extract of *A. undulatum*. a) untreated cells; and b) treated cells.

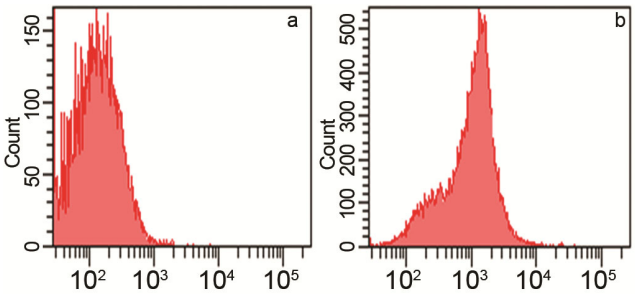

Fig. 5 — Detection of cell death in cells of *C. tropicalis* with butanol extract of *A. undulatum*. a) untreated *C. tropicalis* cells; and b) cells of *C. tropicalis* treated with butanol extract.

the membrane rupturing[23]. The secondary metabolites like phenols, flavonoids, terpenoids and aldehydes have antimicrobial potential due to the ability of these compounds to disrupt the cell cytoplasmic membrane of microbes[24-26]. Due to this property, the antimicrobial components present in the plant extract can easily penetrate the cell, leading to cell death.

Phytochemical analysis

Phytochemical analysis of *A. undulatum* was done by FTIR and GC-MS analysis.

FTIR Analysis

FTIR spectrum of *A. undulatum* is given in Fig. 6. The common IR absorption frequency values of *A. undulatum* suggested that N-H and O-H stretching of amides correspond to the presence of alkaloids, phenolics, saponins and tannins; C-O stretching in

carboxylic acids; C=O stretching of amides, esters, carboxylic acids, aldehydes and ketones attributed to the presence of carbohydrates, flavonoids, phenolics and tannins and S=O stretching in sulphones, sulphonyl chlorides, sulphates and sulphonamides is characteristic for the presence of carbohydrates, reducing sugars and saponins, whereas C-O stretching in alcohols, esters and ethers corresponds to the presence of carbohydrates, flavonoids, glycosides, gums and mucilages and reducing sugars.

GC-MS analysis

The results of GC-MS analysis of the crude butanol extract of *A. undulatum* revealed the presence of some bioactive compounds (Fig. 7). The various components detected in the butanol extract are presented in Table 1.

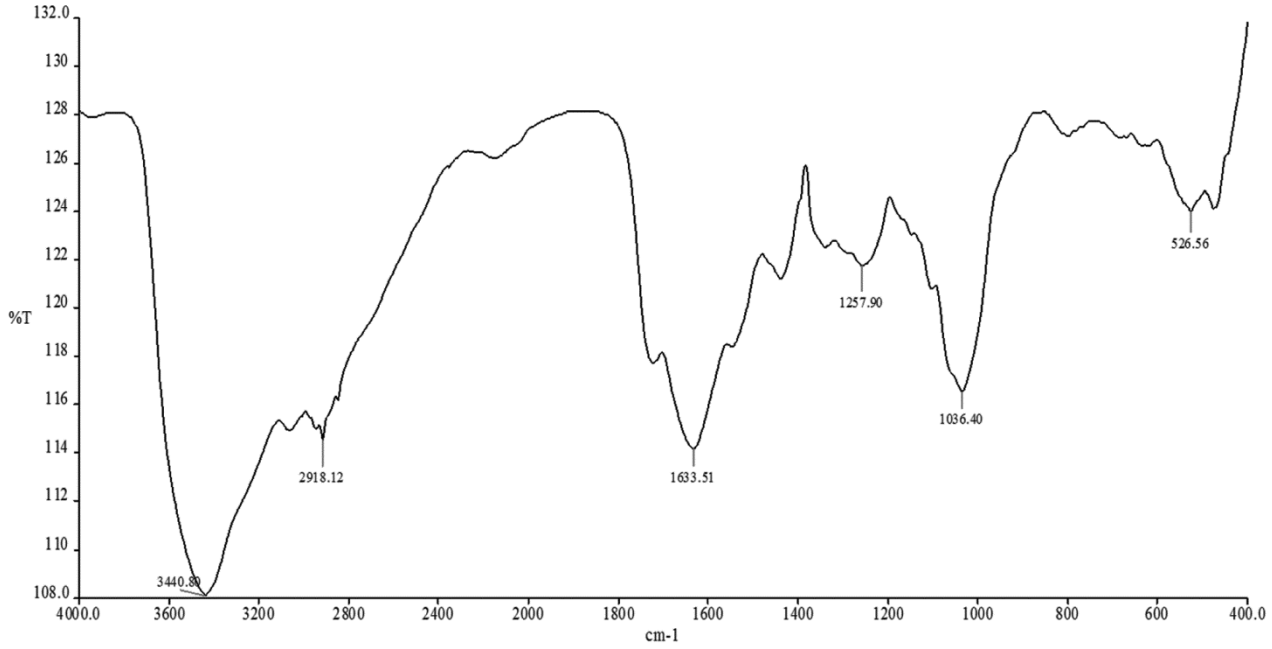

Fig. 6 — FTIR spectrum of butanol extract of *A. undulatum* showing peak frequencies in IR region.

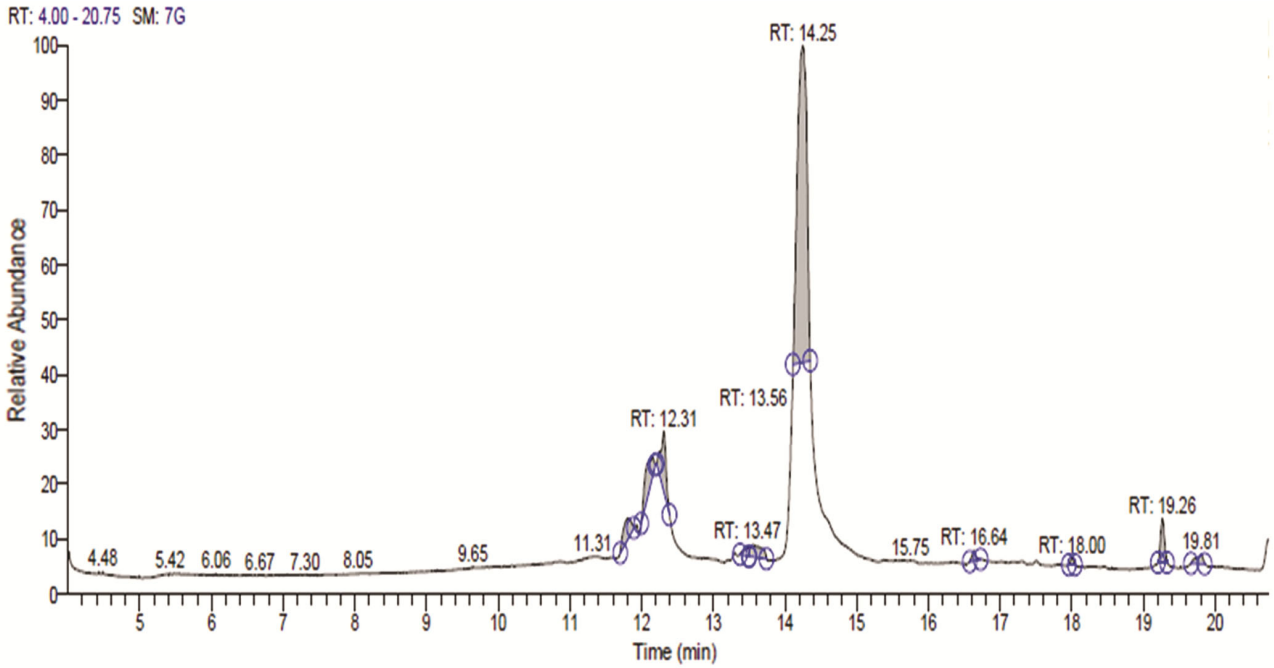

Fig. 7 — GC-MS profile of butanol extract of *A. undulatum*.

Table 1 — Phytochemical identification of compounds in butanol extract of *A. undulatum* by GC-MS

S.No.	% Peak area	RT (min.)	Compounds detected	CAS No.	Mol. Formula
1	4.14	11.78	Palmitoleic acid	373-49-9	$C_{16}H_{30}O_2$
2	4.14	11.78	Cis-9-Hexadecenoic acid	NA	$C_{16}H_{30}O_2$
3	4.14	11.78	9-Hexadecenoic acid	2091-29-4	$C_{16}H_{30}O_2$
4	6.33	12.07	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$
5	6.33	12.07	l-(+)-Ascorbic acid2, 6 dihexadecanoate	28474-90-0	$C_{38}H_{68}O_8$
6	6.33	12.07	Pentadecanoic acid	1002-84-2	$C_{15}H_{30}O_2$
7	7.48	12.31	n-Hexadecanoic acid	57-10-3	$C_{16}H_{32}O_2$
8	7.48	12.31	l-(+)-Ascorbic acid 2, 6 dihexadecanoate	28474-90-0	$C_{38}H_{68}O_8$
9	7.48	12.31	Pentadecanoic acid	1002-84-2	$C_{15}H_{30}O_2$
10	1.09	13.47	9-Octadecenoic acid (Z) -,methyl ester	112-62-9	$C_{19}H_{36}O_2$
11	1.09	13.47	10-Octadecenoic acid, methyl ester	13481-95-3	$C_{19}H_{36}O_2$
12	1.09	13.47	6-Octadecenoic acid, methyl ester, (Z)-	2777-58-4	$C_{19}H_{36}O_2$
13	3.43	13.56	9-Octadecenoic acid (Z) -,methyl ester	112-62-9	$C_{19}H_{36}O_2$
14	3.43	13.56	trans-13-Octadecenoic acid, methyl ester	NA	$C_{19}H_{36}O_2$
15	3.43	13.56	cis-13-Octadecenoic acid, methyl ester	NA	$C_{19}H_{36}O_2$
16	70.32	14.25	12-Methyl-E, E-2,13-octadecadien1ol	NA	$C_{19}H_{36}O$
17	70.32	14.25	Cis-9-Hexadecenal	56219-04-6	$C_{16}H_{30}O$
18	70.32	14.25	17-Octadecynoic acid	34450-18-5	$C_{18}H_{32}O_2$
19	0.99	16.64	Hexadecanoic acid, 1-(hydroxymethyl)-1, 2-ethanediyl ester	761-35-3	$C_{35}H_{68}O_5$
20	0.99	16.64	Docosanoic anhydride	55726-23-3	$C_{44}H_{86}O_3$
21	0.99	16.64	Eicosanoic acid	506-30-9	$C_{20}H_{40}O_2$
22	0.54	18.00	Tricaproin	621-70-5	$C_{21}H_{38}O_6$
23	0.54	18.00	Pentanoic acid, 2methyl-,1,2,3-propanetriyl ester	56554-55-3	$C_{21}H_{38}O_6$
24	0.54	18.00	Spiro(1,3-dioxolane)-2, 3'-[5'androsten-16'-trimethylsilyloxy)-	NA	$C_{24}H_{40}O_3Si$
25	4.12	19.26	9-Octadecenoic acid (Z) -,2-hydroxy-1-(hydroxymethyl)ethyl ester	3443-84-3	$C_{21}H_{40}O_4$
26	4.12	19.26	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	537-39-3	$C_{57}H_{10}4O_6$
27	4.12	19.26	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	NA	$C_{19}H_{34}O_2$
28	1.56	19.81	Methyl 11-docosenoate	NA	$C_{23}H_{44}O_2$
29	1.56	19.81	Erucic acid	112-86-7	$C_{22}H_{42}O_2$
30	1.56	19.81	13-Docosenoic acid, methyl ester	56630-69-4	$C_{23}H_{44}O_2$

GC-MS analysis of the butanol extract of *A. undulatum* revealed the presence of thirty compounds with a retention time of 11.78–19.81 min and a maximum peak area of 70.32%. The butanol extract showed the presence of bioactive compounds like Palmitoleic acid, n-hexadecanoic acid; l-(+)-Ascorbic acid 2,6 di-hexadecanoate; 9-Octadecenoic acid (Z)-, methyl ester; 10-Octadecenoic acid, methyl ester; trans-13-Octadecenoic acid, methyl ester; cis-13-Octadecenoic acid, methyl ester; Eicosanoic acid; Tricaproin; E, E,Z-1,3,12-Nonadecatriene-5,14-diol and Erucic acid.

GC-MS analysis revealed the presence of mostly fatty acids. n-Hexadecanoic acid- a fatty acid is known to be an anti-inflammatory compound[27]. The fatty acid decanoic acid has antibacterial potency[28]. The antimicrobial potential of fatty acids against *E. coli* has already been reported[29]. Tatipamula *et al.* reported the fatty acids as the main components in the ethanol extract of *Taxithelium nepalense* (Schwagr.) Broth. in GC-MS analysis[30].

Conclusion

The results indicate the antifungal potential of *A. undulatum* as its butanol extract caused degradation of the cell wall of the treated fungal strains. GC-MS analysis revealed the presence of bioactive compounds having antimicrobial, anti-inflammatory, cytotoxic and antioxidant properties. FTIR analysis proved the presence of secondary metabolites like saponins, terpenoids, alkaloids and phenolics, which also have antimicrobial properties. The purified bioactive compounds from *A. undulatum* can be used significantly as an antibiotic. The results justify the traditional use of *A. undulatum* as an antimicrobial plant.

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Conflict of interest

No potential conflict of interest was reported by the author(s).

References

1 Vidal C A S, Sousa E O, Rodrigues F F G, Campos A R, Lacerda S R, *et al.*, Phytochemical screening and synergistic interactions between aminoglycosides, selected antibiotics, and extracts from the bryophyte *Octoblepharum albidum* Hedw (Calymperaceae), *Arch Biol Sci*, 2012, **64**(2), 465-470, doi: org/10.2298/ABS1202465V.

2 Asakawa Y, Biologically active substances from Bryophytes, in *Bryophyte development: Physiology and biochemistry*, R N Chopra and S C Bhatla (Eds.), (Boca Raton: CRC Press), 1990, 259-287.

3 Miller N G and Miller H, Make ye the bryophytes, *Horticulture*, 1979, 57, 40-47.

4 Hu R, *Bryology*, (Higher Education Press, Beijing, China), 1987, 465.

5 Bland J, *Forests of Lilliput*, (Prentice- Hall, Inc., Englewood Cliffs), 1971, 210.

6 Madsen G C and Pates A L, Occurrence of antimicrobial substances in chlorophyllose plants growing in Florida, *Bot Gaz*, 1952, **113**(3), 293-300, doi: org/10.1086/335721.

7 Chandra S, Chandra D, Barh A, Pandey R K and Sharma I P, Bryophytes: Hoard of remedies, an ethno-medicinal review, *J Tradit Complement Med*, 2017, **7**(1), 94-98, doi: org/10.1016/j.jtcme.2016.01.007.

8 Mishra R, Pandey V K and Chandra R, Potential of bryophytes as therapeutics, *Int J Pharm Sci Res*, 2014, **5**(9), 3584-3593, doi: org/10.13040/IJPSR.0975-8232.5(9).3584-93.

9 Ando H, Use of bryophytes in China 2. Mosses indispensable to the production of Chinese gallnut, *Proc Bryol Soc Jap*, 1983, **3**(1), 124-125.

10 Jones M L, A short history of the development of wound care dressings, *Br J Healthc Assists*, 2015, **9**(10), 482-485, doi: org/10.12968/bjha.2015.9.10.482.

11 Kumar K, Singh K K, Asthana A K and Nath V, Ethno therapeutics of Bryophyte *Plagiochasma appendiculatum* among the Gaddi Tribes of Kangra Valley, Himachal Pradesh, India, *Pharmaceutica Biol*, 2000, **38**(5), 353-356, doi: org/10.1076/phbi.38.5.353.5963.

12 Glime J M, Bryophyte ecology, in *Physiological Ecology*, vol 1, Houghton. MI: Michigan Technological University and The International Association of Bryologists. Retrieved from http://www.bryoecol.mtu.edu. 2007.

13 Vats S and Alam A, Antibacterial activity of *Atrichum undulatum* (Hedw.) P. Beauv. against some pathogenic bacteria, *J Biol Sci*, 2013, **13**(1), 427-431, doi: 10.3923/jbs.2013.427.431.

14 Devi K and Kapila S, Antibacterial effect of some Indian liverworts, *Int J Pharm Sci Rev Res*, 2013, **20**(2), 219-221.

15 Sevim E, Baş Y, Çelik G, Pinarbaş M, Bozdeveci A, *et al.*, Antibacterial activity of bryophyte species against *Paenibacillus l*arvae isolates, *Turk J Vet Anim Sci*, 2017, **41**(4), 521-531, doi: org/10.3906/vet-1611-70.

16 Hawksworth D, The magnitude of fungal diversity: The 1.5 million species estimate revisited, *Mycol Res*, 2001, **105**(12), 1422-1432, doi: org/10.1017/S0953756201004725.

17 Brakhage A A, Systematic fungal infections caused by *Aspergillus* species: Epidemiology, infection process and virulence determinants, *Curr Drug Targets*, 2005, **6**(8), 875-886, doi: org/10.2174/138945005774912717.

18 Pfaller M A and Diekema D J, Epidemiology of invasive candidiasis: A persistent public health problem, *Clin Microbiol Rev*, 2007, **20**(1), 133-163, doi: org/10.1128%2FCMR.00029-06.

19 Mares D, Electron microscopy of *Microsporumcookei* after *in vitro* treatment with protoanemonin: A combined SEM and TEM study, *Mycopathologia*, 1989, **108**(1), 37-46, doi: org/10.1007/bf00436782.

20 Kim D, Lee D G, Kim K L and Lee Y, Internalization of tenecin 3 by a fungal cellular process is essential for its fungicidal effect on *Candida albicans*, *Eur J Biochem*, 2001, **268**(16), 4449-4458, doi: org/10.1046/j.1432-1327.2001.02364.x.

21 Nikolajeva V, Liepina L, Petrina Z, Krumina G, Grube M, *et al.*, Antibacterial activity of extracts from some bryophytes, *Adv Microbiol*, 2012, **2**(3), 345-353, doi: org/10.4236/aim.2012.23042.

22 Sabovljević A, Soković M, Glamočlija J, Ćirić A, Vujičić M, *et al.*, Bio-activities of extracts from some axenically farmed and naturally grown bryophytes, *J Med Plants Res*, 2011, **5**(4), 56-671.

23 Lambert P A and Hammond S M, Potassium fluxes, first indicators of membrane damage in microorganisms, *Biochem Biophys Res Commun*, 1973, **54**(2), 796-799, doi: org/10.1016/0006-291x(73)91494-0.

24 Tsuchiya H, Sato M, Miyazaki T, Fujiwara S, Tanigaki S, *et al*, Comparative study on the antibacterial activity of phytochemical flavones against methicillin-resistant *Staphylococcus aureus*, *J Ethnopharmacol*, 1996, **50**(1), 27-34, doi: org/10.1016/0378-8741(96)85514-0.

25 Nazzaro F, Fratianni F, De Martini L, Coppola R and De Feo V, Effect of essential oils on pathogenic bacteria, *Pharmaceuticals*, 2013, **6**(12), 1451-1474, doi: org/10.3390%2Fph6121451.

26 Saritha K, Angireddy R, Khanapur M, Oruganti S and Yenugu S, Mechanism of antibacterial action of the alcoholic extracts of *Hemidesmus indicus* (L.) R. Br. Ex Schult, *Leucas aspera* (Wild.), *Plumbago zeylanica* L. and *Tridax procumbens* (L.) R. Br. Ex Schult, *Front Microbiol*, 2015, **6**(1), 1-9, doi: org/10.3389/fmicb.2015.00577.

27 Aparna V, Dileep K V, Mandal P K, Karthe P, Sadasivan C, *et al.*, Anti-inflammatory property of n-hexadecanoic acid: Structural evidence and kinetic assessment, *Chem Biol Drug Des*, 2012, **80**(3), 434-439, doi: org/10.1111/j.1747-0285.2012.01418.x.

28 Petschow B W, Batema P and Ford L L, Susceptibility of *Helicobacter pylori* to bactericidal properties of medium chain monoglycerides and free fatty acids, *Antimicrob Agents Chemother*, 1996, **40**(2), 302-306, doi: org/10.1128%2Faac.40.2.302.

29 Marounek M, Skrivanova E and Rada V, Susceptibility of *Escherichia coli* to C_2-C_{18} fatty acids, *Folia Microbiol*, 2003, **48**(6), 731-735, doi: org/10.1007/bf02931506.

30 Tatipamula V B, Killari K N, Gopaiah K V and Ketha A, GC-MS analysis of ethanol extract of *Taxithelium nepalense* (Schwaerg) Broth along with its alpha Glucosidase inhibitory activity, *Indian J Pharm Sci*, 2019, **81**(3), 569-574, doi: org/10.36468/pharmaceutical-sciences.547.