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PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT ACTIVITY OF AN INDIAN LIVERWORT *DUMORTIERA HIRSUTA* SUBSP. *HIRSUTA* (SW.) NEES

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ABSTRACT

The objective of the present study was to investigate the phytochemical composition and antioxidant activity of various solvent extracts of *Dumortiera hirsuta*. The highest phenols, flavonoids and terpenoids content were present in methanol extract. Also, it exhibited the highest antioxidant activity, whereas the least was observed in aqueous extract. FTIR and GC-MS confirmed the presence of the functional groups and volatile composition of the liverwort. Results revealed that *D. hirsuta* acts as a potent antioxidant agent due to its high phenolic content and free radical scavenging activity and can be explored pharmaceutically.

Key words : Antioxidant, Liverwort, DPPH, GC-MS, FTIR.

Introduction

Inability of the endogenous antioxidative defense system of the body to fight against the generated free radicals or reactive oxygen species, causes a life-threatening condition known as oxidative stress. Free radicals are extremely unstable compounds with unpaired electrons rotating around the nucleus in their peripheral layer. These molecules can damage proteins, DNA and lipids in cells. Extreme accumulation of ROS in the body lead to tissue damage, cell death and the onset of numerous pathological disturbances like inflammation, stroke, atherosclerosis, hypertension, diabetes mellitus, cancer, Alzheimer's disease and Parkinson's disease (Rahman *et al.*, 2012; Doughari *et al.*, 2009). Antioxidants are the substances that have the ability to scavenge the free radicals and protect the biomolecules from the oxidative damage. Hence, during the inadequate antioxidative defense system of our body, we can reduce the deleterious effects of oxidative damage by adding antioxidant supplements to our diets. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are among the commonly employed synthetic

antioxidants in the food and pharmaceutical sectors. But due to the carcinogenic effects of synthetic antioxidants, there has been a surge of interest in natural antioxidants derived from plants for application in health sector (Pejin and Bogdanoviæ-Pristov, 2012). Flavonoids, phenols, ascorbic acid, carotenoids, tocopherols, tocotrienols, cinnamic acid, folic acid and benzoic acids are some key antioxidants found in plants (Ghasemzadeh *et al.*, 2010).

Bryophytes, the earliest land plants, are the second largest plant group after angiosperms. They include Bryophyta (mosses), Marchantiophyta (liverworts), and Anthocerotophyta (hornworts) and bridge the evolutionary gap between algae and vascular plants. Since ancient period, bryophytes have been utilized in Indian, European, North American and Chinese traditional medicine to treat skin diseases, burns, bronchitis, tonsillitis, tympanitis and cardiovascular disorders (Khanam *et al.*, 2011). Bryophytes possess a diverse range of biological activities including, antioxidant, antitoxic, antimicrobial, NO production inhibitory, and muscle relaxing activities, attributed to their phytochemical composition (Asakawa, 2007).

Dumortiera hirsuta, a member of the family Dumortieraceae, is a thallose liverwort and is widely distributed in the tropical and subtropical regions of the globe. The liverwort is most found at the places where water is easily available. *D. hirsuta* has a dark green dichotomously branched thallus, with dorsal surface lacking vestigial air chambers and with few dorsal papillae. Due to the absence of well differentiated thallus into different regions as constituted by the other members of Order Marchantiales, *D. hirsuta* is now not classified under family Marchantiaceae. Even it is the only genus to own the family Dumortieraceae (Durant-Archibold *et al.*, 2018). Distribution of *D. hirsuta* across India includes several parts of Himachal Pradesh, Arunachal Pradesh, Andhra Pradesh, Odisha, Manipur, Nagaland, Sikkim, Assam, Meghalaya, Jammu and Kashmir, Uttarakhand, Goa, Madhya Pradesh, Kerala, Tamil Nadu and West Bengal (Yadav *et al.*, 2022). So far, majority of the studies focused on the distribution of *D. hirsuta* throughout India, with just a few focusing on identification of its biochemical components (Alam, 2012; Asakawa, 1984; Alam *et al.*, 2011). The volatile composition of *D. hirsuta* subsp. *Nepalensis* and *D. hirsuta* sub sp. *hirsuta* was reported by Durant-Archibold *et al.* (2018). A study examined the effects of seasonal variation on the antioxidative defense system of *D. hirsuta* (Yadav *et al.*, 2022)

Detailed studies determining the phytochemical composition and free radical quenching activity are scarce for *Dumortiera hirsuta*. The objective of this work is to study the effect of solvent's polarity on phytochemical composition and antioxidative activity of *D. hirsuta*. The findings of this study may add some significant value to prior reports and enhance the therapeutic efficacy of the liverwort.

Materials and Methods

Collection of plant material and study area

The fresh thalli of liverwort *D. hirsuta* were collected from wet walls of Bhagsunag waterfall (latitude 32.24798°N and longitude 76.33757°E), Dharamshala, Himachal Pradesh (India) in the month of September 2020. The waterfall is situated at an altitude of 2120 m. Several other important factors like precipitation (107mm), humidity (77%) and temperature (20°C) were also noted on the spot during collection. Identification was validated by morphological features using keys and a voucher sample (PAN 6412) was deposited at the herbarium of Department of Botany, Panjab University, Chandigarh,

India.

Preparation of plant extracts

The soil and other unwanted plant species attached to the thallus were removed manually with the help of forceps. Thereafter, the collected plant material was first washed with tap water to remove the major impurities and then finally washed twice with distilled water. The plant was shade dried and then crushed into powder. Extracts of powdered sample were prepared in different polarity solvents using soxhlet apparatus. Solvents were heated up to their boiling point *i.e.*, water (100°C), methanol (64.7°C), acetone (56°C), petroleum ether (55°C) and hexane (68°C) and the plant sample was extracted for 5-7 hours at 2-4 cycles per hour. Whatman filter paper no. 1 was used to filter the extract. Thereafter, solvents were completely evaporated using rotary evaporator and the crude extracts obtained were stored (4°C) for further use.

Phytochemical analysis

Total phenol estimation

Total phenol content was estimated following Folin-Ciocalteu colorimetric method with few modifications (Daniel and Krishnakumari, 2015). To 1 mL of sample, 0.5 mL of Folin-Ciocalteu reagent was added and incubated at room temperature for 5 minutes. Thereafter, 2 mL of 20% sodium carbonate was added and the solution was incubated for 1 hour in dark. Absorbance was measured at 650 nm. Gallic acid was used as standard and total phenolic content was expressed as mg Gallic Acid Equivalents per gram (GAE/g) of sample in dry weight.

Total flavonoid estimation

Flavonoid content was estimated following aluminium chloride method (Sembiring *et al.*, 2018). To 100 µL of extract, 1 mL of 10% aluminium chloride and 1 mL of 1M sodium acetate were added. After 45 minutes of incubation at room temperature, absorbance was measured at 415 nm. Rutin was used as standard and the flavonoid content was expressed as mg Rutin Equivalents per gram (RE/g) of sample in dry weight.

Total terpenoid content

Estimation of terpenoid content was estimated following the method of Ghorai *et al.* (2012). To 200 µL of plant extract, 1.5 mL of chloroform was added and the solution was vortexed thoroughly. After rest of 3 minutes, 100 µL of concentrated sulphuric acid was added and the solution was incubated for 1.5-2 hours at room temperature in dark. After incubation, a reddish-brown

precipitate was formed, supernatant was collected carefully from this solution. 1.5 mL of methanol was added to the precipitate and mixed thoroughly. Absorbance was measured at 538 nm using spectrophotometer and linalool was used as standard. The terpenoid content was expressed as mg Linalool Equivalents per gram (LE/g) of sample in dry weight.

Antioxidant activity

DPPH radical scavenging assay

Free radical scavenging potential of the plant extracts against DPPH radical was investigated following the method described by Wang *et al.* (2016). To 1 mL of plant extract, 1 mL 0.1mM DPPH solution was added. The mixture was vortexed thoroughly and after incubating it for 30 minutes in dark, absorbance was measured at 517 nm. The inhibitory (%) was calculated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[\frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100 \right]$$

FRAP (Ferric reducing antioxidant power) assay

The ferric ion reducing ability of the extracts was measured using FRAP assay (Benzie and Strain, 1999). The FRAP reagent was prepared by dissolving 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl solution, and 20 mM FeCl₃, at a ratio of 10:1:1 (v/v/v). Thereafter, 3 mL of FRAP reagent was added to 100 µL of plant extract and mixed thoroughly. The absorbance was measured at 593 nm after 30 minutes of incubation. FeSO₄ was used to generate a standard calibration curve and reducing ability of the samples was expressed as µM FeSO₄ E/g of sample in dry weight.

Metal chelating assay

The metal chelating activity was calculated according to Dinis *et al.* (1994). To 0.1 mL of plant extract, 50µL of 2 mM FeCl₂ was added. The reaction initiated with the addition of 200 µL of 5 mM Ferrozine. To this, 2 mL of methanol was added and the solution was allowed to stand for 10 minutes at room temperature. Metal chelating activity was determined by measuring the decrease in absorbance of Fe²⁺-Ferrozine complex at 562 nm. The metal chelating activity was calculated using the following formula:

$$\text{Metal chelating activity (\%)} = \left[\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100 \right]$$

Total antioxidant activity

The total antioxidant activity was determined using Phosphomolybdenum method of Prieto *et al.* (1999). 0.1 mL of extract was mixed with 1 mL of phosphomolybdate

reagent solution (28 mM sodium phosphate, 4 mM ammonium molybdate and 0.6 M sulphuric acid). The sample tubes were sealed and incubated in boiling water bath at 90-95°C for 90 minutes. After incubation, the sample was cooled to room temperature and absorbance measured at 695 nm. The total antioxidant activity was calculated using the following formula:

$$\text{TAA (\%)} = \left[\frac{(A_{\text{sample}} - A_{\text{control}})}{A_{\text{sample}}} \times 100 \right]$$

FTIR Spectroscopy

The functional groups present in a compound were determined using Fourier Transform Infrared Spectroscopy. The FTIR spectroscopy of the liverwort was performed at Central Instrumentation Laboratory (CIL), Panjab University, Chandigarh. The powdered methanolic extract of the liverwort was combined with KBr. The sample was placed in an FTIR spectrometer with a scanning range of 400 to 4000 cm⁻¹. The spectral bands obtained above 1200 cm⁻¹ are caused by vibrations of individual functional groups, whereas the bands obtained below 1200 cm⁻¹ are caused by vibrations of entire molecule and are recognized as the fingerprint region.

Gas chromatography-Mass spectrometry (GC-MS)

The GC-MS analysis of methanol extract of the liverwort was performed at Central Instrumentation Laboratory (CIL), Central University of Punjab, Bathinda. The sample's volatile composition was determined using a Shimadzu triple-quad GCMS-TQ8050 NX with standard capillary column. With a flow rate of 1 mL min⁻¹, helium was used as the carrier gas. 2 µL of the syringe filtered (0.22µm) sample was analyzed with the column held initially at 70°C for 2 minutes and then increased at a rate of 5°C min⁻¹ to 300°C, which was held constant for 10 min. ACQ mode was used for GC-MS spectral detection at 70eV with a scan time of 0.5 sec, and fragments were recorded in the 40 to 850 m/z range. The temperature of the ion source was kept constant at 200!. The components of the sample were identified by comparing the retention time of chromatographic peaks to mass spectra libraries from NIST17R.

Statistical analysis

All the experiments were performed in triplicates and the data were expressed as Mean ±SD. The mean values of all the parameters were determined for significance by analysis of variance (ANOVA) using Graph pad prism (ver.8.0.1).

Results

Total phenol content

Total phenolic content (TPC) of the extracts was measured using F-C reagent and was expressed as mg GAE/g DW. The concentration of TPC in all the extracts was in the following order: methanol (138.07 ± 0.69) > acetone (32.25 ± 0.09) > hexane (26.05 ± 0.21) > petroleum ether (18.96 ± 0.10) > aqueous (15.25 ± 0.12).

Total flavonoid content

Total flavonoid content (TFC) of the plant was measured by aluminium chloride method and expressed as mg RE/g DW. The concentration of TFC in the studied plant extracts was in the order: methanol (91.14 ± 0.71) > acetone (21.6 ± 0.74) > petroleum ether (15.2 ± 1.07) > hexane (11.57 ± 5.26) > aqueous (7.76 ± 0.55).

Total terpenoid content

Estimated terpenoid content was expressed as mg LE/g DW. Total terpenoid content of the plant was in the following order: methanol extract (4.15 ± 0.16) > hexane extract (1.09 ± 0.20) > acetone extract (0.98 ± 0.17) >

petroleum ether extract (0.53 ± 0.28) > aqueous extract (0.46 ± 0.17).

In-vitro antioxidant activity

The antioxidant potential of the liverwort was evaluated in five solvent extracts using DPPH radical scavenging activity, FRAP assay, metal chelating activity, and total antioxidant activity. DPPH radical is a purple-colored stable free radical which attains yellow-golden color on accepting electrons. During the study, a successive increase in the DPPH radical quenching activity was observed in dose-dependent manner (from 25-400 $\mu\text{g/ml}$). DPPH radical scavenging activity of all extracts ranged between 0.83 ± 0.42 to $71.19 \pm 0.55\%$ (Fig. 1A). Methanol extract was found with most electron donating potential, at highest studied concentration *i.e.*, 400 $\mu\text{g/ml}$, it was able to scavenge $71.19 \pm 0.55\%$ DPPH radicals, whereas at the same concentration, the least potential to scavenge DPPH radicals was observed in

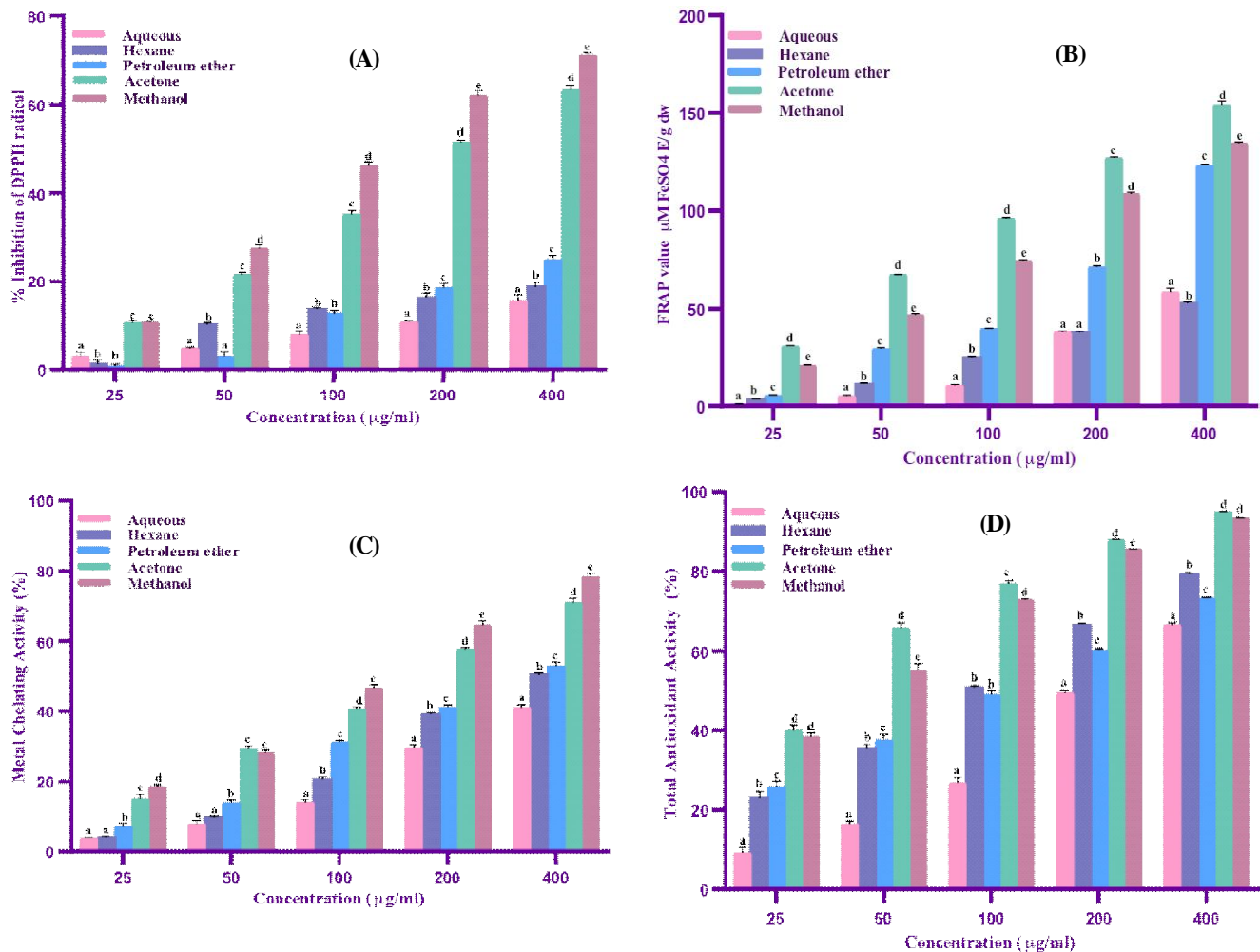


Fig. 1 : Antioxidant activity of *Dumortiera hirsuta* in different solvent extracts. (A) DPPH radical scavenging activity; (B) Reducing power; (C) Metal chelating activity; (D) Total antioxidant activity. Values with different letters (a,b,c,d,e) are significantly ($p < 0.05$) different from each other among the same concentration.

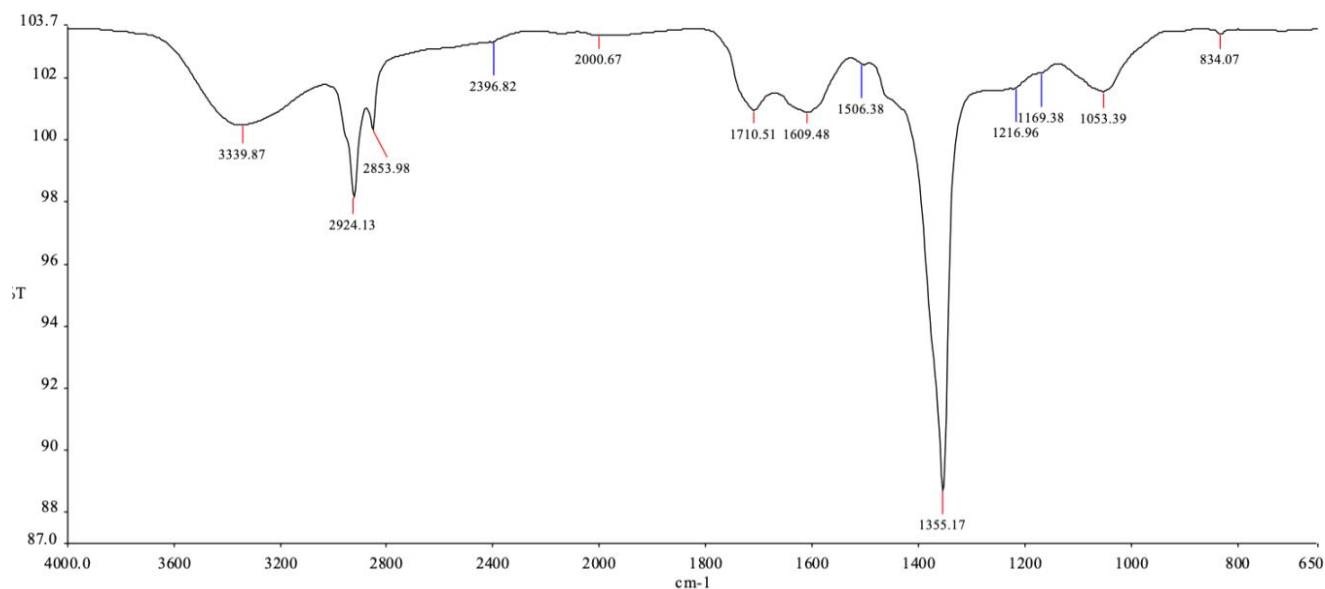


Fig. 2 : FTIR spectrum of methanolic extract of *D. hirsuta*.

Table 1 : FTIR peak values of methanolic extract of *Dumortiera hirsuta*.

S. no.	Wave number (cm ⁻¹)	Functional Group/Assignment
1	3339.87	O-H stretch
2	2924.13	C-H asym./sym. stretch
3	2853.98	C-H asym./sym. stretch
4	2000.67	Cyanide/Thiocyanate ion
5	1710.51	C=O stretching of esters, carboxylic acids, ketones and aldehydes
6	1609.48	C=C-C Aromatic ring stretch/Imino(-C=N-)/Azo(-N=N-)/Primary/Secondary amine, N-H bend
7	1506.38	C=C-C Aromatic ring stretch/Aromatic nitro compounds
8	1355.17	Phenol or alcohol, O-H bend/C-N stretch/Nitro compounds/Nitrate ion/Carboxylate/Sulfonates
9	1216.96	C-C vibrations/C-H in-plane bend/P-O-C stretch
10	1169.38	C-N stretch/Dialkyl/aryl sulfones/Sulfonates/Cyanates/C-H in-plane bend
11	1053.39	C-C vibrations/C-N stretch/C-O stretch/C-H in-plane bend/Phosphate ion/Si-O-Si/C-F stretch
12	834.07	C-H 1,4-Disubstitution (para)/C-H out-of-plane bend/Nitrate ion/Peroxides, C-O-O- stretch/Epoxy and oxidant rings

aqueousextract with $15.71 \pm 1.29\%$ inhibition.

Reducing potential of the liverwort provided the direct assessment of its antioxidant activity. It was reported that the reducing power increased with the increasing concentration and at highest studied concentration i.e. $400 \mu\text{g/ml}$, maximum value was observed in case of acetone ($154.19 \pm 1.94 \mu\text{M FeSO}_4 \text{ E/g DW}$) whereas minimum was observed in hexane ($52.65 \pm 0.81 \mu\text{M FeSO}_4 \text{ E/g DW}$) (Fig. 1B). Similarly, the metal chelating activity of *D. hirsuta* extracts was assessed at various concentrations ranging from $25\text{-}400 \mu\text{g/ml}$ (Fig. 1C). The metal chelating activity of the extracts ranged between

3.95 ± 0.22 to $78.38 \pm 1.01\%$. At maximum concentration i.e., $400 \mu\text{g/ml}$, the highest chelation was reported in methanol extract ($78.38 \pm 1.01\%$), whereas lowest was observed in aqueous extract ($41.09 \pm 0.89\%$). The Total Antioxidant Activity (TAA) ranged between 9.22 ± 1.40 to $95.03 \pm 0.12\%$ (Fig. 1D). At $400 \mu\text{g/ml}$, the highest TAA was reported in acetone extract ($95.03 \pm 0.12\%$), whereas the lowest value was obtained in case of aqueous extract ($66.53 \pm 0.60\%$).

FTIR analysis

Bioactive compounds can be investigated for their presence using FTIR. Different peaks obtained in the

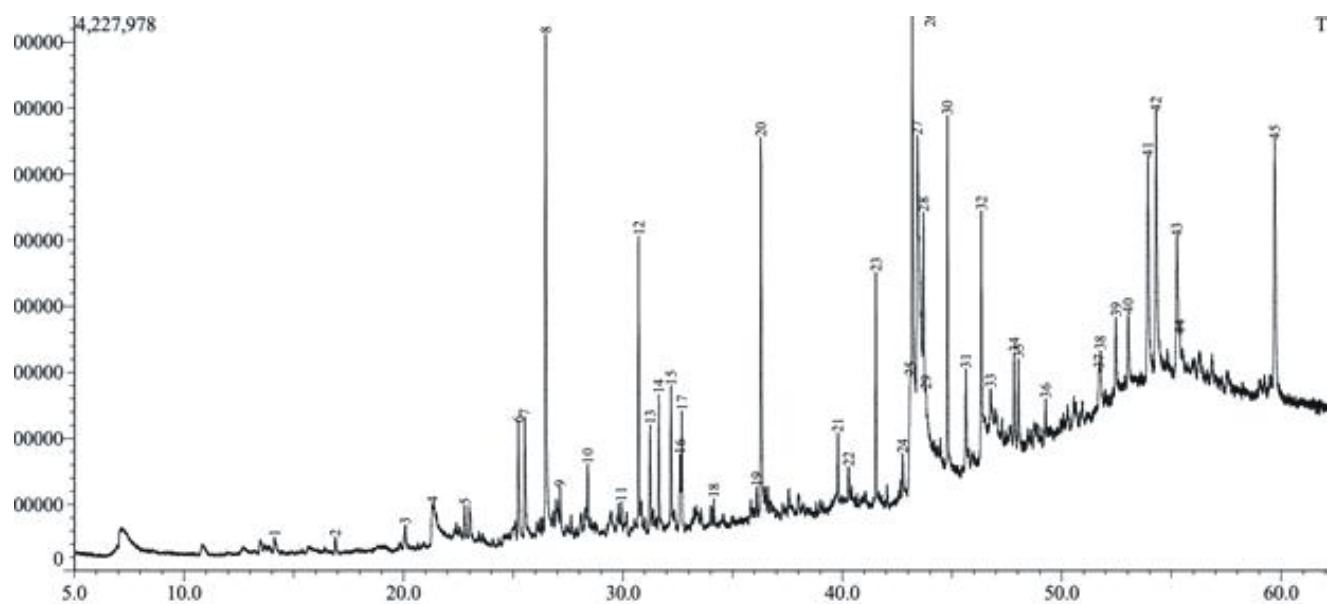


Fig. 3 : GC-MS chromatogram of methanolic extract of *D. hirsuta*.

Table 2 : The GC-MS profile of the methanol extract of *Dumortiera hirsuta*, compounds reported with retention time, % peak area, molecular formula and molecular weight.

S. no.	RT	Compounds	Molecular Formula	Molecular Weight	Area %
1	43.423	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C ₁₉ H ₃₈ O ₄	330	9.59
2	26.473	Longipinene	C ₁₅ H ₂₄	204	8.16
3	43.192	Dotriacontane	C ₃₂ H ₆₆	450	6.45
4	59.707	Tris(2,4-di-tert-butylphenyl) phosphate	C ₄₂ H ₆₃ O ₄ P	662	6.22
5	36.294	Phytol	C ₂₀ H ₄₀ O	296	5.82
6	54.298	Stigmasterol	C ₂₉ H ₄₈ O	412	5.43
7	53.924	Lathosterol	C ₂₇ H ₄₆ O	386	4.68
8	21.341	Ethriol	C ₆ H ₁₄ O ₃	134	4.20
9	43.703	Phthalic acid, bis(2-ethylhexyl) ester	C ₂₄ H ₃₈ O ₄	390	4.09
10	30.722	Phetyl stearate	C ₃₈ H ₇₄ O ₂	562	3.68
11	55.248	Stigmast-5-en-3-ol, oleate	C ₄₇ H ₈₂ O ₂	678	3.03
12	46.339	Tetracosane	C ₂₄ H ₅₀	338	2.94
13	25.526	Spathulenol	C ₁₅ H ₂₄ O	220	1.70
14	43.100	Norfentanyl	C ₁₄ H ₂₀ N ₂ O	232	1.69
15	32.690	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	C ₁₈ H ₂₈ O ₃	292	1.56
16	45.627	Octocrylene	C ₂₄ H ₂₇ NO ₂	361	1.53
17	52.465	Tocopherol acetate	C ₃₁ H ₅₂ O ₃	472	1.38
18	53.029	5,6-Dihydroergosterol	C ₂₈ H ₄₆ O	398	1.30
19	48.025	Squalene	C ₃₀ H ₅₀	410	1.17
20	32.610	Hexadecanoic acid, methyl ester	C ₁₇ H ₃₄ O ₂	270	0.86

spectra suggested the presence of various functional groups which further specify various bioactive compounds. The FTIR spectrum of methanol extract of *D. hirsuta* is shown in Fig. 2. Wavenumber ranges, functional groups of each FTIR spectral peak in comparison with previous reports are given in Table 1 (Nandiyanto *et al.*, 2019).

GC-MS analysis

One of the most used methods to study the phytochemicals *i.e.* Gas Chromatography-Mass Spectroscopy was performed to investigate the volatile composition of the methanol extract of *D. hirsuta*. The chromatograph (Fig. 3) revealed the presence of forty-five compounds, the major peaks were of the following compounds: Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (9.59%), Longipinene (8.16%), Dotriacontane (6.45%), Tris(2,4-di-tert-butylphenyl) phosphate (6.22%), Phytol (5.82%), Stigmasterol (5.43%), Ethriol (4.20%). Chemical constituents, their Retention time, peak area %, molecular weight and molecular formula are given in Table 2. The liverwort contains a variety of bioactive compounds with pharmacological potential.

Discussion

The present study indicates that the extraction solvent plays a significant role in the phytochemical composition and antioxidant activity of the plant. Both polar (methanol, aqueous and acetone) and non-polar (petroleum ether and hexane) solvents were used in the extraction of bioactive compounds from *Dumortiera hirsuta*. Among all the extracts, polar solvents *i.e.*, methanol and acetone were able to extract higher phenols, flavonoids and terpenoids and showed better antioxidant activity. Literature published by Jayaprakash *et al.* (2001) explains this difference due to the polarities of various compounds present in the plant. The phytochemical contents of *D. hirsuta* were higher than other liverwort species such as *Lepidozia borneensis* (TPC=12.42 mg GAE/g, TFC = 9.36 mg CE/g) and *Plagiochila beddomei* (TPC = 19.3 mg GAE/g, TFC = 16.9mg QE/g) (Abu Bakar *et al.*, 2015; Manoj and Murugan, 2021). Phenols and flavonoids are acknowledged as “radical scavengers” due to their high electron or hydrogen-atom donating ability. This might be the reason for strong antioxidant, anti-inflammatory and anti-carcinogenic potential of phenols. Flavonoids also are believed to reduce the process of oxidation of less dense lipoproteins, hence preventing cardiovascular diseases (Meccariello and D’Angelo, 2021). Terpenoids constitute one of the major classes of secondary metabolites. These are oxygenated alkene derivatives and

are known to have numerous pharmaceutical applications. A study conducted on *Thuidium tamariscellum*, showed significant amount of terpenoid content *i.e.*, 25.95 mg/g (Mohandas and Kumaraswamy, 2018).

Present study demonstrated the antioxidant activity of *D. hirsuta* extracted in different solvents using DPPH radical scavenging assay, FRAP assay, Metal chelating assay and Total antioxidant activity. Methanol extract showed significant DPPH radical scavenging and metal chelating activity. Whereas, the highest reducing power and total antioxidant activity were exhibited by acetone extract. Among all the studied solvents aqueous extract showed minimal scavenging potential. A study reported the antioxidant activity of three liverworts *i.e.*, *Marchantia paleacea*, *M. linearis* and *Conocephalum conicum* (Mukhia *et al.*, 2014). DPPH radical scavenging potential of the liverworts was reported to be 18.82 mg/mL, 44.88 mg/mL and 68.44 mg/mL, respectively. While the metal chelating activity was 47.32 mg/mL for *C. conicum*, 86.52 mg/mL for *M. linearis* and 122.13 mg/mL for *M. paleacea*. The ferric reducing power in methanol extract of moss *Rhodobryum roseum* and liverwort *L. borneensis* was $34.73 \pm 0.44 \mu\text{M}$ and $211.13 \pm 1.29 \text{ mM Fe}^{2+} \text{ g}^{-1}$, respectively (Sabharwal *et al.*, 2023; Abu Bakar *et al.*, 2015).

Results obtained in our study witnessed a linear correlation among phenols and radical scavenging activity. In a similar study, liverworts of Darjeeling Himalaya were investigated for their bioactive compounds and *in-vitro* pharmacological properties, reporting that the polyphenol content and radical scavenging activity of the liverworts had a positive correlation with each other, also both these factors were highly influenced by the polarity of different solvents (Mukhia *et al.*, 2017). In contrast, Chobot *et al.* (2006) found no correlation between the phenolic content and radical scavenging activity of studied bryophytes. Few studies reported the effect of different seasons on the concentration of antioxidants, phenols, and flavonoids in liverworts (Yadav *et al.*, 2022; Thakur and Kapila, 2017). Bryophytes harvested during particular seasons influenced their phytochemical profile and antioxidant potential. Presently also, the population picked during the post-monsoon season (during September to November) was rich in antioxidants and phytochemicals.

FTIR spectra of *D. hirsuta* extract revealed the presence of phenols, alkaloids and alcohols (3339.87 cm^{-1}), alkanes (2924.13 cm^{-1}), ketones (2853.98 cm^{-1}), aliphatic amines (1609.48 cm^{-1}), phenyl groups (1355.17 cm^{-1}) and carboxylic acids ($1053.39\text{-}1216.96 \text{ cm}^{-1}$). The hydroxyl groups, phenols, alcohols, alkanes,

ketones, phenyl groups and carboxylic acid peaks verify the existence of polyphenols in *D. hirsuta*, which is attributed to its radical scavenging activity. Raj *et al.* (2019) reported the presence of polyphenols confirmed by the occurrence of above discussed peaks, suggestive of the strong antioxidant activity of this species. FTIR spectroscopy of *D. hirsuta* clearly revealed the presence of hydrogen donating-hydroxyl group, which accounts for the antioxidant potential of the plant.

GC-MS profiling of methanol fraction of *D. hirsuta* thallus discovered the presence of bioactive molecules with significant medicinal potential. Among all the reported bioactive compounds, Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester had the most prominent peak. This compound has anti-inflammatory, antioxidant and anthelmintic activities (Al-Marzoqi *et al.*, 2015). Longipinene, a sesquiterpene, has been reported to possess antifungal potential (Manoharan *et al.*, 2017). Dotriacontane is known to exhibit anticonvulsant activity (Jordan *et al.*, 2020). Phytol, a diterpenoid has been reported with antioxidant, anticancer, anti-inflammatory, anti-diuretic and antimicrobial activities (Alagammal *et al.*, 2012). Additionally, phytol serves as precursor for synthetic Vitamin K and E. Studies also showed that phytol has unique antibacterial properties against pathogens like *S. aureus* (Inoue *et al.*, 2005). Bakrim *et al.* (2022) reported the antioxidant, antidiabetic, anticancer, anti-inflammatory, antimicrobial and anti-osteoarthritis activities of triterpene stigmaterol. Thirunavukkarasu *et al.* (2016) reported the anticancer and antidiabetic activity of the compound ethriol.

Study conducted by Durant-Archibold *et al.* (2018) reported the volatile composition of *D. hirsuta* from Panama, where sesquiterpenes constituted the major volatile composition of the liverwort with compounds α -gurjunene (37.3%) and β -selinene (33.7%). Komala *et al.* (2010) identified nootkatene, neophytadiene, α -humulene, guaia-6,9-dien-4 β -ol, α -humulene epoxide and a dumortane derivative in samples of this species from Indonesia. An unknown sesquiterpenoid, Germacrene D, ent-1(10)E,5-Egermacradien-11-ol and 4 β -hydroxygermacra-1(10), 5-diene were reported in GC-MS analysis of Japanese *D. hirsuta* (Ludwiczuk *et al.*, 2008). The volatile composition of Brazilian *D. hirsuta* was studied using gas chromatography (Saritas *et al.*, 1998). They identified β -caryophyllene, valencene, α -cubebene, α -guaiene and γ -gurjunene. These variations in volatile composition could be attributed to geographical differences across regions.

Conclusion

Eventhough synthetic antioxidants are frequently used in a variety of industries, there are drawbacks to this practice, including possible health risks, environmental issues, consumer concerns, and the possibility of antioxidant resistance. Hence, there is a need for less toxic and more effective natural antioxidants. The present study provided the phytochemical analysis and antioxidant activity of various solvent fractions of liverwort *D. hirsuta*. The results of studies suggest that methanolic extract of *D. hirsuta* may be useful in defense against the free radicals damage, possibly be due to its high phenolic content and radical quenching potential. Based on the findings, it can be considered that this plant has a great potential of use as natural supplements.

Statements and declarations

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

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References

- Abu Bakar, M.F., Abdul Karim F., Suleiman M., Isha A. and Rahmat A. (2015). Phytochemical constituents, antioxidant and antiproliferative properties of a liverwort, *Lepidozia borneensis* Stephani from Mount Kinabalu, Sabah, Malaysia. *Evidence-based Complementary and Alternative Medicine*, **2015**, 1-9.
- Alagammal, M., Tresina P.S. and Mohan V.R. (2012). GC-MS determination of bioactive components of *Polygala javana* DC. *Int. J. Curr. Pharmaceut. Res.*, **4(2)**, 42-44.
- Alam, A. (2012). Some Indian bryophytes known for their biologically active compounds. *Int. J. Appl. Biol. Pharmaceut. Tech.*, **3(2)**, 239-246.
- Alam, A., Tripathi A., Vats S., Behera KK. and Sharma V. (2011). *In vitro* antifungal efficacies of aqueous extract of *Dumortiera hirsuta* (Swagr.) Nees against sporulation and growth of post-harvest phytopathogenic fungi. *Arch Bryol.*, **103**, 1-9.
- Al-Marzoqi, A.H., Hameed I.H. and Idan S.A. (2015). Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). *Afr. J. Biotech.*, **14(40)**, 2812-2830.
- Asakawa, Y. (1984). Some biologically active substances isolated from hepaticae: terpenoids and lipophilic aromatic compounds. *The J. Hattori Botanical Lab.*, **56**, 215-219.
- Asakawa, Y. (2007). Biologically active compounds from

bryophytes. *Pure Appl. Chem.*, **79(4)**, 557-580.

- Bakrim, S., Benkhaira N., Bourais I., Benali T., Lee L.H., El Omari N. and Bouyahya A. (2022). Health Benefits and Pharmacological Properties of Stigmasterol. *Antioxidants*, **11(10)**, 1912.
- Benzie, I.F. and Strain J.J. (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods in Enzymology*, **299**, 15-27.
- Chobot, V., Kubicová L., Nabbout S., Jahodáč L. and Vytlačilová J. (2006). Antioxidant and free radical scavenging activities of five moss species. *Fitoterapia*, **77(7-8)**, 598-600.
- Daniel, G and Krishnakumari S. (2015). Quantitative analysis of primary and secondary metabolites in aqueous hot extract of *Eugenia uniflora* (L) leaves. *Asian J. Pharmaceut. Clin. Res.*, **8(1)**, 334-338.
- Dinis, T.C., Madeira V.M. and Almeida L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxy radical scavengers. *Arch. Biochem. Biophysics*, **315(1)**, 161-169.
- Doughari, J.H., Human I.S., Bennade S. and Ndademi P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: Possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *J. Medicinal Plants Res.*, **3(11)**, 839-848.
- Durant-Archibold, A.A., Allen N.S., Garrido A., Ledezma J.G and Gupta M.P. (2018). An analysis of volatile components of the Liverworts *Dumortiera hirsuta* subsp. *hirsuta* and *Dumortiera hirsuta* subsp. *nepalensis* (Dumortieraceae) from Panama and taxonomic observations on the species. *Nat. Prod. Commun.*, **13(8)**, 1031-1034.
- Ghasemzadeh, A., Jaafar H.Z.E. and Rahmat A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules*, **15(6)**, 4324-4333.
- Ghorai, N., Chakraborty S., Gucchait S., Saha S.K. and Biswas S. (2012). Estimation of total Terpenoids concentration in plant tissues using a monoterpene, Linalool as standard reagent. *Protocol Exchange*, 1-6.
- Inoue, Y., Hada T., Shiraishi A., Hirose K., Hamashima H. and Kobayashi S. (2005). Biphasic effects of geranylgeraniol, terpenone and phytol on the growth of *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, **49(5)**, 1770-1774.
- Jayaprakasha, G.K., Singh R.P. and Sakariah K.K. (2001). Antioxidant activity of grape seed (*Vitis vinifera*) extracts on peroxidation models in vitro. *Food Chem.*, **73(3)**, 285-290.
- Jordan, K.S., Godfred A.A., James M.A. and Germain S.T. (2020). Anticonvulsant activities of friedelan-3-one and n-dotriacontane both isolated from *Harungana madagascariensis* Lam (Hypericaceae) seeds extracts. *J. Medicinal Plants Res.*, **14(10)**, 509-517.
- Khanam, R., Chaudhary B.L., Khanam S. and Kumar P. (2011). Antibacterial activity of *Marchantia palmata* Nees. *Asian J. Biochem. Pharmaceut. Res.*, **2(1)**, 27-36.
- Komala, I., Ito T., Yagi Y., Nagashima F. and Asakawa Y. (2010). Volatile components of selected liverworts and cytotoxic, radical scavenging and antimicrobial activities of their crude extracts. *Nat. Prod. Commun.*, **5(9)**, 1375-1380.
- Ludwiczuk, A., Nagashima F., Gradstein R.S. and Asakawa Y. (2008). Volatile components from selected Mexican, Ecuadorian, Greek, German and Japanese liverworts. *Nat. Prod. Commun.*, **3(2)**, 133-140.
- Manoharan, R.K., Lee J.H., Kim Y.G., Kim S.I. and Lee J. (2017). Inhibitory effects of the essential oils α -longipinene and linalool on biofilm formation and hyphal growth of *Candida albicans*. *Biofouling*, **33(2)**, 143-155.
- Manoj, G.S. and Murugan K. (2012). Wound healing potential of aqueous and methanolic extracts of *Plagiochila beddomei* steph.- a bryophyte. *Int. J. Pharmacy Pharmaceut. Sci.*, **4(2)**, 222-227.
- Meccariello, R. and D'Angelo S. (2021). Impact of polyphenolic-food on longevity: An elixir of life. An overview. *Antioxidants*, **10(4)**, 507.
- Mohandas, G.G. and Kumaraswamy M. (2018). Antioxidant activities of terpenoids from *Thuidium tamariscellum* (C. Muell.) Bosch. and Sande-Lac. a Moss. *Pharmacognosy J.*, **10(4)**, 645-649.
- Mukhia, S., Mandal P., Singh D.K. and Singh D. (2017). Study of bioactive phytoconstituents and *in-vitro* pharmacological properties of Thallose Liverworts of Darjeeling Himalaya. *J. Pharmacy Res.*, **11(5)**, 490-501.
- Mukhia, S., Mandal P., Singh D.K., Singh D. and Choudhury D. (2014). *In-vitro* free-radical scavenging potential of three liverworts of Darjeeling Himalaya. *Int. J. Pharmaceut. Sci. Res.*, **5(10)**, 4552-4561.
- Nandiyanto, A.B.D., Oktiani R. and Ragadhita R. (2019). How to read and interpret FTIR spectroscopy of organic material. *Indonesian J. Sci. Tech.*, **4(1)**, 97-118.
- Pejin, B. and Bogdanoviæ-Pristov J. (2012). ABTS cation scavenging activity and total phenolic content of three moss species. *Hemijskaindustrija*, **66(5)**, 723-726.
- Prieto, P., Pineda M. and Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry*, **269(2)**, 337-341.
- Rahman, T., Hosen I., Towhidul Islam M.M. and Shekhar H.U. (2012). Oxidative stress and human health. *Adv. Biosci. Biotech.*, **3**, 997-1019.
- Raj, A., Vennila L., Krishnan A., Satheesh A., Asaikumar L., Sivasangari S. and Kanimozhi K. (2019). Antioxidant activity and FT-IR analysis of methanolic extract of *Adhatoda beddomei*. *Res. J. Life Sci., Bioinform.*,

- Pharmaceut. Chem. Sci.*, **5(3)**, 426.
- Sabharwal, H., Shukla G., Kondepudi K.K., Maurya R., Kapila S. and Dogra N. (2023). Phytochemical Analysis and *in vitro* Assessment of Extracts of *Rhodobryum roseum* for Antioxidant, Antibacterial and Anti-Inflammatory Activities. *J. Herbs, Spices & Medicinal Plants*, **29(4)**, 419-437.
- Saritas, Y., Bülow N., Fricke C., König W.A. and Muhle H. (1998). Sesquiterpene hydrocarbons in the liverwort *Dumortiera hirsuta*. *Phytochemistry*, **48(6)**, 1019-1023.
- Sembiring, E.N., Elya B. and Sauriasari R. (2018). Phytochemical screening, total flavonoid and total phenolic content and antioxidant activity of different parts of *Caesalpinia bonduc* (L.) Roxb. *Pharmacog. J.*, **10(1)**, 123-127.
- Thakur, S. and Kapila S. (2017). Seasonal changes in antioxidant enzymes, polyphenol oxidase enzyme, flavonoids and phenolic content in three leafy liverworts. *Lindbergia*, **40(5)**, 39-44.
- Thirunavukkarasu, K., Rajkumar P., Selvaraj S. and Kumaresan S. (2016). GC-MS analysis of *Gymnema sylvestre* leaves methanolic extract for antidiabetic and anticancer drug identification. *J. Chem. Pharmaceut. Sci.*, **9(2)**, 1011-1013.
- Wang, X., Cao J., Wu Y., Wang Q. and Xiao J. (2016). Flavonoids, antioxidant potential and acetylcholinesterase inhibition activity of the extracts from the gametophyte and archegoniophore of *Marchantia polymorpha* L. *Molecules*, **21(3)**, 360.
- Yadav, S., Srivastava A., Biswas S., Basu S., Singh S.K. and Mishra Y. (2022). Seasonal changes in the antioxidative defence system of a liverwort *Dumortiera hirsuta*. *J. Plant Growth Regulation*, **41(3)**, 1265-1275.