Indian Journal of Natural Products and Resources
Vol. 15(4), December 2024, pp. 500-514
DOI: 10.56042/ijnpr.v15i4.10026

Cytotoxicity and antioxidant activities of methanolic extract of *Marchantia polymorpha* and *Dicranum scoparium* using network pharmacology, molecular docking, and experimental approaches

Shivom Singh[1], Gaurav Bhadauriya[1], Anurag Agrawal[2]*, Vipin Bhati[2], Shivani Gore[3] and Kajal S. Rathor[4]*

[1]Department of Environmental Science, [3]Department of Biotechnology, ITM University, Gwalior 474001, Madhya Pradesh, India

[2]Department of Pharmacology, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida 201310, Uttar Pradesh, India

[4]Department of Biotechnology, SMS Govt. Model Science College, Gwalior 474009, Madhya Pradesh, India

Received 31 March 2024; revised received 23 October 2024; accepted 29 October 2024

A comparative study was conducted to assess the antioxidant activity, cytotoxicity, and molecular docking of methanolic extracts from *Marchantia polymorpha* (Marchantiaceae) and *Dicranum scoparium (Dicranaceae)*. The species *M. polymorpha* and *D. scoparium* remain largely unexplored in terms of their biochemical characterization and potential pharmacological activities. Phytoconstituents in the methanolic extracts were identified using spectral analysis. The cytotoxicity study utilized the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay with U-87 human glial cell lines, while antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Additionally, a network pharmacology approach was employed to identify molecular targets in relation to cytotoxicity using the isolated compounds. Molecular docking was performed to evaluate the interactions between the isolated phytoconstituents of *M. polymorpha* and *D. scoparium* with the estrogen receptor ESR1 (Estrogen Receptor 1) (PDB ID: 1GWQ). The DPPH assay results demonstrated that antioxidant activity increased with higher extract concentrations. The percentage scavenging activity ranged from 4.41 to 88.05% for *M. polymorpha* and from 3.69 to 88.46% for *D. scoparium*, with minimum activity observed at 15.62 µg/mL and maximum activity at 1000 µg/mL. The superoxide radical scavenging activity ranged from 0.00 to 48.09% for *M. polymorpha* and from 0.00 to 45.39% for *D. scoparium*. Network pharmacology analysis identified the estrogen receptor (ESR1) as a common target involved in cancer. Molecular docking studies revealed that quercetin exhibited the strongest interactions with ESR1 among all the selected phytoconstituents from *M. polymorpha* and *D. scoparium*.

Keywords: Anticancer, Bioinformatics, Estrogen receptor 1, Mosses, Scavenging

IPC code; Int. cl. (2021.01)− A61K 36/00, A61K 36/10, A61P 35/00, A61P 39/00

Introduction

Bryophytes, encompassing mosses and liverworts, have been recognized as rich repositories of diverse and potentially valuable natural compounds. These encompass polysaccharides, lipids, rare amino acids, terpenoids, phenylpropanoids, quinones, and other specialized metabolites[1]. Moreover, beyond their traditional roles in ethnomedicine, bryophytes exhibit promising potential as sources of innovative therapeutic agents. They have been employed in treating various conditions, such as hepatic disorders, skin ailments, and cardiovascular diseases, and as agents for reducing fever and promoting wound healing. Additionally, certain bryophyte species have demonstrated notable anti-tumor activities against different cancer cell lines[2]. Despite their considerable potential, bryophytes have received comparatively limited research attention due to their small size and the challenges associated with collecting sufficient quantities for routine chemical analysis[3]. Nevertheless, recent studies have illuminated the bioactivities present in methanolic extracts of diverse bryophyte species, encompassing elements like flavonoid and phenolic content, DPP4 inhibition, metal chelation, antioxidant properties, and antiglycation activities[4].

The extracts from bryophytes have been observed to accommodate a combination of primary metabolites, such as fatty acids and amino acids, alongside secondary metabolites like sterols, terpenoids, and polyphenols[5]. These secondary metabolites have exhibited noteworthy antimicrobial effects against fungi, Gram-negative as well as Gram-positive bacteria[6]. Furthermore, the potential of moss extracts

*Correspondent author
Email: anuragagrawal86@hotmail.com; drkajals101@gmail.com
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extends to cytotoxic and antineoplastic attributes, implying their promising role as agents against cancer[5].

Liverworts, a specific category of bryophytes, are notable for containing diverse secondary metabolites with different bioactivities. Among these compounds, bibenzyls and bisbibenzyls are prominent examples, present not only in liverworts but also in mosses and hornworts. Asakawa *et al*. reviewed that different species of *Marchantia* vary in their chemical compositions, e.g., South African species possess different chemicals other than German and Japanese species[7]. The chemical diversity observed in *M. polymorpha* L. and *D. scoparium* reflects their basal phylogenetic position among land plants and highlights their potential applications in pharmacology and agriculture. Investigating their secondary metabolites may lead to the discovery of novel compounds with antimicrobial, antifungal, and potentially antiviral properties. Although limited research has been conducted on *M. polymorpha* and *D. scoparium* regarding molecular docking and chemical characterization, these species chemical diversity reflects their basal phylogenetic position among land plants. The mode of action of bryophytic secondary metabolites can vary depending on the specific compound and target. The multi-target activities of many secondary metabolites can explain the medical application of complex extracts from medicinal plants for various health disorders[8]. For example, docking analyses have revealed high-affinity interactions between secondary metabolites present in bryophytes and important enzyme residues, indicating their potential neuroprotective activities[9].

Further research is needed to explore the full potential of bryophytes as sources of novel therapeutics and to uncover their chemical constituents and biological properties[10]. Although this study focused on a specific bioactive compound and its interaction with a particular receptor, it demonstrates the potential of molecular docking in understanding the binding interactions of bryophyte-derived compounds with target proteins.

Material and Methods

Sample collection and identification

Bryophytes were meticulously gathered from two distinct locations in Uttarakhand, India: Artola (29º23.711'N, 79º28.000'E, Alt. 2069.59 meters) and Jageshwar (29º37.644'N, 79º50.296'E, Alt. 1848.61 meters). These samples were handled with the utmost care, encased in polythene covers, and expediently transported to the laboratory for further examination. The meticulous identification of the collected bryophytes was facilitated by the expertise of knowledgeable professionals, along with references from authoritative literature (Gangulee, Smith, Lang and Stech,) and the comprehensive resources accessible on the Global Biodiversity Information Facility (GBIF) website (https://www.gbif.org/). For both *M. polymorpha* and *D. scoparium*, herbarium specimens were prepared to preserve the collected samples for long-term reference and study. The specimens were carefully cleaned, dried, and mounted on herbarium sheets, following standard bryological techniques. The herbarium accession numbers for *M. polymorpha* and *D. scoparium* are 201609100a and 201609101a.

Preparation of extracts

The extraction methods mostly rely on solvents such as ethanol, methanol, water, and acetone, which are the most common solvents used for *in vitro* antioxidant testing[11]. Extracts, depending on the solvent used and the selected species, contain variable concentrations of phenolics, polyphenols, bibenzyls, and terpenoids, which are responsible for the antioxidant activity[12]. This study focused on the chemical composition of pure methanol extracts of *M. polymorpha* and *D. scoparium*. Methanol was used as the solvent for extraction due to its high polarity and effectiveness in extracting a wide range of bioactive compounds, including alkaloids, phenolics, flavonoids, and terpenoids. Its ability to efficiently penetrate plant tissues enables the extraction of both polar and non-polar compounds. The plant material was meticulously cleansed, air-dried under shaded conditions, finely chopped, and then subjected to sequential extraction. Crude extracts were obtained using a Soxhlet apparatus by performing extraction with a 90% methanol-water system to yield the methanol extract. Simultaneously, approximately 10 g of fresh thalli of *M. polymorpha* and *D. scoparium* were extracted with 100 mL of methanol at room temperature for 24 hours. The resulting extracts were concentrated using a rotavapor at 45°C.

Isolation

TLC separation of the phytochemicals

During the TLC separation, aluminium-precoated silica gel 60 F254 plates with a thickness of 1 mm were utilized. These plates were activated at 110°C

for 1 hour. A variety of solvent systems, including ethyl acetate: butanol: formic acid, chloroform: methanol, and ethyl acetate: toluene: formic acid, were employed to identify distinct secondary metabolites. The secondary metabolites from the methanolic extract were examined using UV light at wavelengths of 254 and 366 nm. Visualization of the separated compounds was achieved through spraying techniques using various reagents, including $AlCl_3$, Folin-Ciocalteu reagent, and $FeCl_3$, which enhanced the detection of specific phytochemicals.

HPLC separation of the phytochemicals

The plant samples were shade-dried and dissolved in HPLC-grade methanol at a concentration of 0.1 mg/µL. The resulting solution was filtered through a 0.22 µ Millipore membrane filter before analysis. The HPLC analysis was conducted on an RPC-18 column with a Luna 5-micron C-18 (2) Phenomenex reverse phase column (250 x 4.6 mm) using two LC-6AD pumps (Shimadzu, Kyoto, Japan) with a CTO-10 AS VP column oven (Shimadzu, Kyoto, Japan), SPD-M20A diode array detector (Prominence, Shimadzu, Kyoto, Japan), and a CBM-20A communications bus module (Prominence, Shimadzu, Kyoto, Japan). The software class VP series version 6.1 (Shimadzu, Kyoto, Japan) was used to control the HPLC, which was equipped with a Rheodyne injector and set to maintain a column temperature of 40°C. The solvent system was set in binary mode with methanol: water (80:20 v/v) at a flow rate of 1 mL/min and UV detection in the range of 190-350 nm at 1000 psi. The HPLC analysis enabled the collection of fractions corresponding to particular maximum peaks with specific retention times using a fraction collector.

FTIR spectroscopy for determination of bryophyte chemical content

The analysis was performed to detect the presence of functional groups associated with the secondary metabolites. The TLC-purified extracts of *M. polymorpha* and *D. scoparium* were dried and powdered to a particle size of approximately 100 µm, with 2 mg of the powdered samples subjected to FTIR spectral analysis using the KBr pellet method. The spectra of the samples were taken using a Perkin Elmer Fourier Transform Infrared Spectrophotometer, measuring absorbance between 400 and 4500 cm^{-1}. At least three spectra were obtained for each sample to ensure consistency and reliability in the results.

NMR spectroscopy for determination of bryophyte chemical composition

The chromatographically purified extracts, prepared as solutions in deuterated chloroform ($CDCl_3$), were subjected to ^1H NMR and ^{13}C NMR analysis to elucidate the structural nature of the major secondary metabolites and related compounds. ^1H NMR (Proton NMR) and ^{13}C NMR (Carbon 13 - NMR) were performed on Bruker 800 Mz NMR, operating at 400 MHz for ^1H and 100.623 MHz for ^{13}C NMR. The two most common standards are TMS (tetramethylsilane, (Si $(CH_3)_4$), which has been assigned a chemical shift of zero, and $CDCl_3$ (deuterochloroform), which has chemical shifts of 7.26 for ^1H NMR and 77 for ^{13}C NMR. The scale is commonly expressed as parts per million (ppm) independent of the spectrometer frequency. The scale is the delta (δ) scale.

Network pharmacology

In this study, network pharmacology methods were employed to identify various genes (targets) that are supposed to be involved in the progression of cancer. Target identification was performed to identify the drug target and disease target. For the identification of disease targets initially, the smiles form of the drug structure was obtained from the chembl database (https://www.ebi.ac.uk/chembl/); this smiles were pasted into the Swisstarget prediction tool (https://www.swisstargetprediction.ch/), and the data report was extracted in excel file. Disease targets were extracted from the genecards (https://www.genecards.org/), a freely available database in the form of an Excel file[13].

Identification of common targets

After the identification of potential targets of drug and disease, we have found common targets from the identified targets. Venn diagrams are the preferred tools for pinpointing shared targets of diseases and compounds[14]. We have used Venny 2.1 for the construction of the Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/).
Simultaneously, the KEGG pathway from the string database (https://string-db.org) was used to find out the interactions among compound targets[15].

Analysis and Visualization of the Network

The extracted data were organized in an Excel sheet, which was imported into Cytoscape 3.10.1. software(https://cytoscape.org/). The networks of gene/protein and drug interaction were constructed.

Molecular docking

For the molecular docking study, the 3D crystalline structure of the target, i.e. Estrogen receptor1 for exhibiting the binding modes and predicting the mechanism of actions of ligands was downloaded from the protein data bank (PDB ID: 1GWQ) and prepared using Schrodinger's Suite 2022-4's Protein Preparation Wizard by removing crystal fluids and adjusting bond order with hydrogen additions. The Epik module was used to complete the protonation and tautomeric states of acidic and basic residues at pH 7.2 by adding missing side chains and loops. OPLS3e (Optimized Potentials for Liquid Simulations) molecular force-field was used to minimize the protein, with the crystallographic heavy atoms root mean square deviation (RMSD) set to 0.30 Å. A grid box with its centre at the active site's centroid.83) was created using a van der Waals scale of 1.0 for the receptor and a partial charge cut-off of 0.25. Using Chemsketch (ACD freeware, 2021.2.0), the structures of all ligands were prepared, and energy was minimized. The binding sites for targets were used to start a virtual workflow in XP (Extra Precision) mode with their default settings. The prediction of the ligand's conformation and pose (its projected placement and orientation inside these sites) is the first step in docking, followed by assessments of the binding affinity. Using the Glide score, glide energy data, and hydrogen bond analysis, the optimal binding pose was determined.

Cell lines and culture medium

HDF (Human dermal fibroblast) and U-87 (Human Glial cell) cell lines were procured from the National Centre for Cell Sciences (NCCS), Pune, India. HDF and U-87 stock cells were cultured in Ham's F12 medium supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100 µg/mL), and amphotericin B (5µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated with TPVG solution (0.2% trypsin, 0.02% EDTA, 0.05% glucose in PBS). The stock cultures were grown in 25 cm^2 culture flasks, and all experiments were carried out in 96 microtiter plates (Tarsons India Pvt. Ltd., Kolkata, India).

Cytotoxicity test -MTT assay

The monolayer cell culture was trypsinized, and the cell count was adjusted to 1.0 x 10^5 cells/mL using Ham's F12 medium, which contained 10% FBS. To each well of the 96-well microtiter plate, 0.1 mL of the diluted cell suspension (approximately 10,000 cells) was added. After 24 h, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium, and 100 µL of different test concentrations of test drugs were added onto the partial monolayer in microtitre plates. The plates were then incubated at 37°C for 3 days in a 5% CO_2 atmosphere, and microscopic examination was carried out, and observations were noted at 24 h intervals. After 72 h, the drug solutions in the wells were discarded, and 50 µL of MTT in PBS was added to each well. The plates were gently shaken and incubated for 3 h at 37° C in a 5% CO_2 atmosphere. The supernatant was removed, 100 µL of propanol was added, and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540 nm[6]. The percentage growth inhibition was calculated using the following formula and the concentration of test drug needed to inhibit cell growth by 50% (CTC_{50}) values was generated from the dose-response curves for each cell line.

Antioxidant activity

The total antioxidant activity was determined by the phosphor-molybdenum method[16,17] and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex, which has the maximal absorption at 695 nm. They weighed accurately 55 mg of each extract and the standard ascorbic acid and dissolved in 5 mL of DMSO. The lower dilutions were made serially with DMSO. An aliquot of 0.1 mL of the sample solution containing a reducing species in DMSO was combined in an Eppendorff tube with 1 mL of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a water bath at 95°C for 90 min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm. The total antioxidant capacity was expressed as mcg equivalent of ascorbic acid.

Scavenging of superoxide radical by alkaline DMSO method

In the alkaline DMSO method, superoxide radical is generated by the addition of Alk-DMSO hydroxide to air-saturated DMSO. The generated superoxide remains stable in the solution, which reduces nitro blue tetrazolium into formazan dye at room temperature, which can be measured at 560 nm. It involves the reaction of the extract with alkaline

DMSO and nitro bluete trazolium (NBT) to form a coloured formazan product. The intensity of the colour is proportional to the scavenging activity of the extract against superoxide radicals[18-21]. Exactly 7 mg of each of the extracts was weighed accurately and separately dissolved in 1.5 mL of DMSO. These solutions were serially diluted with DMSO to obtain lower dilutions. To the reaction mixture containing 1 mL of alkaline DMSO (1 mL, 1% distilled water, 5 mMNaOH) and 0.3 mL of the extracts in DMSO at various concentrations or standards, 0.1 mL of NBT (1 mg/mL) was added to give a final volume of 1.4 mL. The absorbance was measured at 560 nm[22].

In vitro antioxidant activity test-DPPH method

Bryophyte samples tested by this method were dissolved in methanol at specific concentrations to prepare stock solutions. The assay was carried out on a 96-well microtiter plate. About 10 µL of micropipette was prepared from these stock solutions, and 200 µl DPPH (2,2-diphenylpicrylhydrazyl) solution was added to them and left for inhibition. The final concentrations of the test and standard solutions used were 1000, 500, 250, 125, 62.5, 31.25, 15.625, and 7.812 µg/mL. Colour transformations and antioxidant activities were tested by comparison with the control (solvent). After incubation in the dark for 30 min, the absorbance values of the samples were measured at 490 nm, and the inhibition % was calculated using the absorbance values of each sample and blank control test[23].

Results

TLC analysis

Tables 1 and 2 provide the Rf values of different metabolites across various solvent systems. These findings suggest that methanolic extracts encompass a wide spectrum of secondary metabolites. TLC studies revealed that the methanolic extract of bryophytes, specifically *M. polymorpha* and *D. scoparium*, exhibited orange, blue, and yellow fluorescent bands or ribbons, confirming the presence of flavonoids, phenols, etc.

HPLC analysis

The gallic acid, benzoic acid, lupeol, and quercetin were identified as prominent constituents in the methanolic extracts of *M. polymorpha* and *D. scoparium*. The HPLC procedure yielded precise identification and quantification of two established phenolic compounds within *M. polymorpha*. Notably, Benzoic acid was found in substantial abundance alongside Gallic acid, as outlined in Table 3. This tabulated data demonstrates the presence of Gallic acid in both species. However, the concentration was significantly more pronounced in *D. scoparium* when contrasted with *M. polymorpha*, a fact visually conveyed through the specific details depicted in Fig. 1a-b for *M. polymorpha* and for *D. scoparium* respectively.

FT-IR spectral analysis

A total of 4 compounds were determined from the methanol extract of *M. polymorpha* and

Table 1 — Data showing the TLC analysis of methanolic extract of *Marchantia polymorpha*

Phytoconstituents	Solvent System	Spraying reagent	Spot colour	No. of spot	Rf value
Flavonoids	Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5)	$AlCl_3$ reagent	Orange	6	0.28, 0.54, 0.61, 0.75, 0.82, 0.86
Phenols	Chloroform: Methanol (27:0.3)	FolinCiocalteau reagent	Blue	5	0.24, 0.36, 0.46, 0.52, 0.65
	Ethyl acetate : Toluene: Formic acid (2.2:1.1:1.1)	FolinCiocalteau reagent	Green	4	0.28, 0.46, 0.65, 0.71
Terpenoids	Ethyl acetate : Toluene: Formic acid (2.2:1.1:1.1)	$FeCl_3$ reagent	Yellow	5	0.15, 0.19, 0.26, 0.34, 0.48
	Methanol: Water (6:4)	$FeCl_3$ reagent	Brownish Grey	4	0.26, 0.54, 0.64, 0.76

Table 2 — Data showing the TLC analysis of methanolic extract of *Dicranum scorpion*

Phytoconstituents	Solvent System	Spraying reagent	Spot color	No of spot	Rf value
Flavonoids	Ethyl acetate: Butanol: Formic acid (2.5:1.5:0.5)	$AlCl_3$ reagent	Orange	5	0.30, 0.44, 0.55, 0.62, 0.86
Phenols	Chloroform: Methanol (27:0.3)	FolinCiocalteau reagent	Blue	4	0.30, 0.40, 0.46, 0.58
	Ethyl acetate : Toluene: Formic acid (2.2:1.1:1.1)	FolinCiocalteau reagent	Green	3	0.18, 0.24, 0.38
Terpenoids	Ethyl acetate: Toluene: Formic acid (2.2:1.1:1.1)	FeCl3 reagent	Yellow	3	0.28, 0.36, 0.48

Table 3 — HPLC summary report of samples

S. N.	Sample	Gallic Area (RT)	Caffeic Area (RT)	Lupeol Area (RT)	Quercetin Area (RT)	Benzoic acid (RT)	Concentration (µg/g)	Chromato-gram reference
1	MP	33.201 (2.08)	-	-	-	282.00 (3.82)	Gallic acid: 2.23 Benzoic acid: 84.02	Fig. 1
2	DS	3387.76 (2.09)	-	97.51 (3.15)	95.32 (4.55)	-	Gallic acid: 27.97 Lupeol: 89.07 Quercetin: 30.74	Fig. 2

MP: *Marchantia polymorpha*
DS: *Dicranum scoparium*

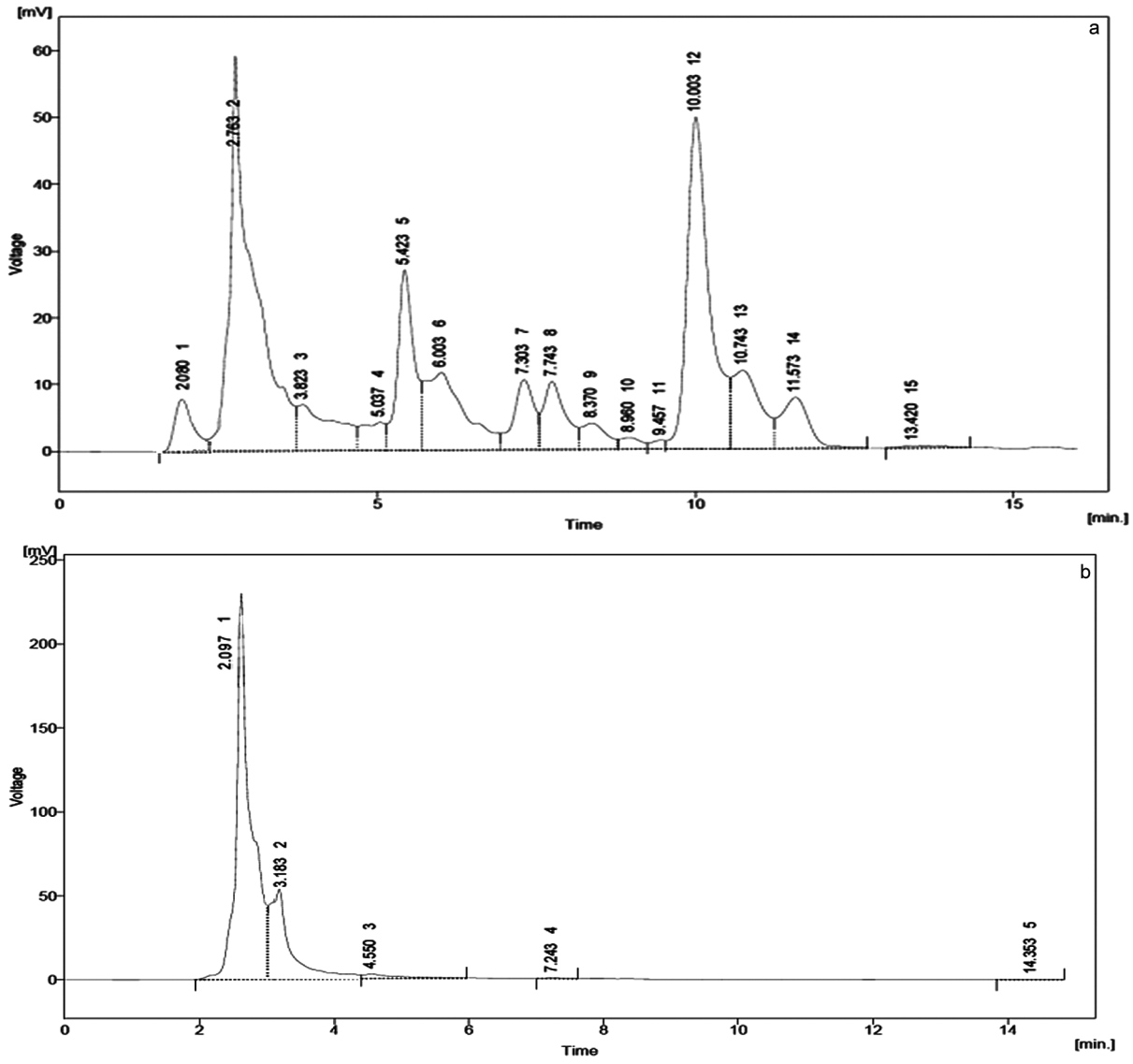

Fig. 1 — HPLC Chromatogram and peak profiles of methanolic extract from a) *M. polymorpha*; and b) *D. Scoparium*.

D. scoparium. Fig. 2a-b illustrate the intricate spectra of *M. polymorpha* and *D. scoparium*, showcasing a multitude of distinct functional groups. In the spectrum of *M. polymorpha* and *D. scoparium*, the most notable peaks observed at different frequencies for these bands have been consolidated in Tables 4 and 5. The relative intensities of the main bands and their positions indicated variations between compounds. Maksimova *et al*., revealed that bryophytes primarily consist of carbohydrates. FT-IR

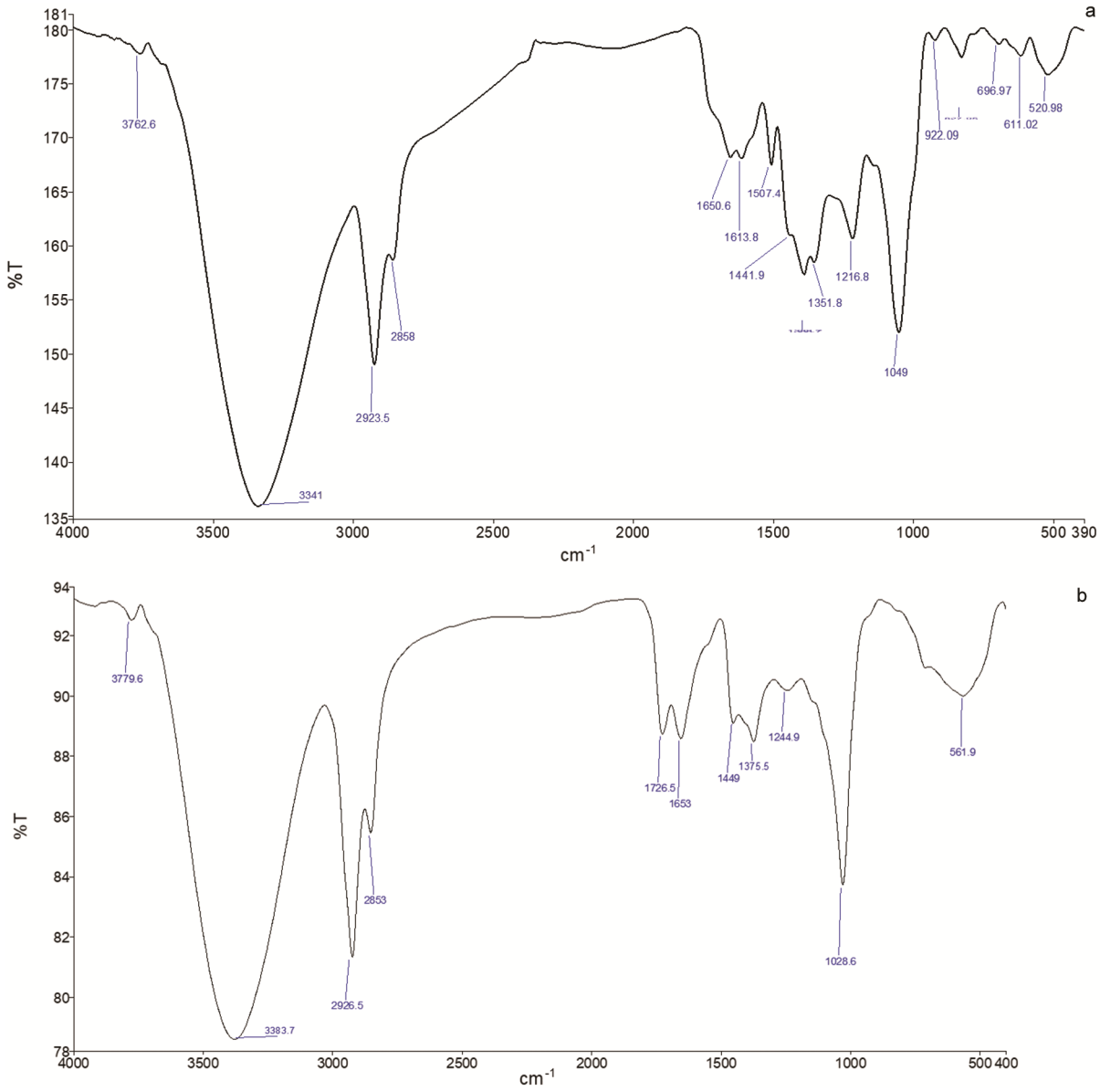

Fig. 2 — Major spectral bands in the Fourier Transform IR spectra obtained from methanol extract of a) *M. polymorpha*; and b) *D. scoparium*.

Table 4 — FTIR analysis results of methanolic extract from *M. polymorpha*- prominent peaks and corresponding functional groups

Frequency Range (cm^{-1})	Absorption (cm^{-1})	Group	Compound Class
4000-3000	3762.6, 3341	O-H stretching	Alcohol
3000-2500	2923.5, 2858	C-H stretching	Alkene, Aldehyde
1670-1600	1650.6, 1613.8	C=C stretching	Conjugated Alkene, α, β-Unsaturated Ketone
1600-1300	1507.4, 1414.9, 1351.8	N-O stretching, O-H bending	Nitro Compound, Alcohol
1400-1000	1216.8, 1049	C-O and CO-O-CO stretching	Vinyl Ether, Anhydride
1000-650	922.09, 696.97	C=C bending	Alkene

spectroscopy provided information on the abundance of hydroxyl (OH) groups in combination with carbonyl (C=O) groups, while ^{13}C-NMR spectroscopy indicated the presence of compounds such as phenolics and lipids. Additionally, the absence of lignin in the studied bryophytes was confirmed.

NMR spectral analysis

The compounds identified in this study were characterized through a detailed comparison of their ^{1}H NMR and ^{13}C NMR data with existing literature, corroborating the proposed structures. Fig. 3a-d illustrate the ^{1}H and ^{13}C NMR absorption peaks for

Table 5 — FTIR analysis results of methanolic extract from *D. scoparium*- prominent peaks and corresponding functional groups

Frequency Range (cm^{-1})	Absorption (cm^{-1})	Group	Compound Class
4000-3000	3779.6, 3383.7	O-H stretching	Alcohol
3000-2500	2926.5, 2853	C-H stretching	Alkene, Aldehyde
2000-1650	1726.5, 1653	C=O stretching, C=C stretching	Aliphatic Ketone, Alkene
1600-1300	1449, 1375.5	C-H bending, O-H bending	Alkane, Phenol
1400-1000	1244.9, 1028.6	C-O and CO-O-CO stretching	Aromatic ester, Anhydride

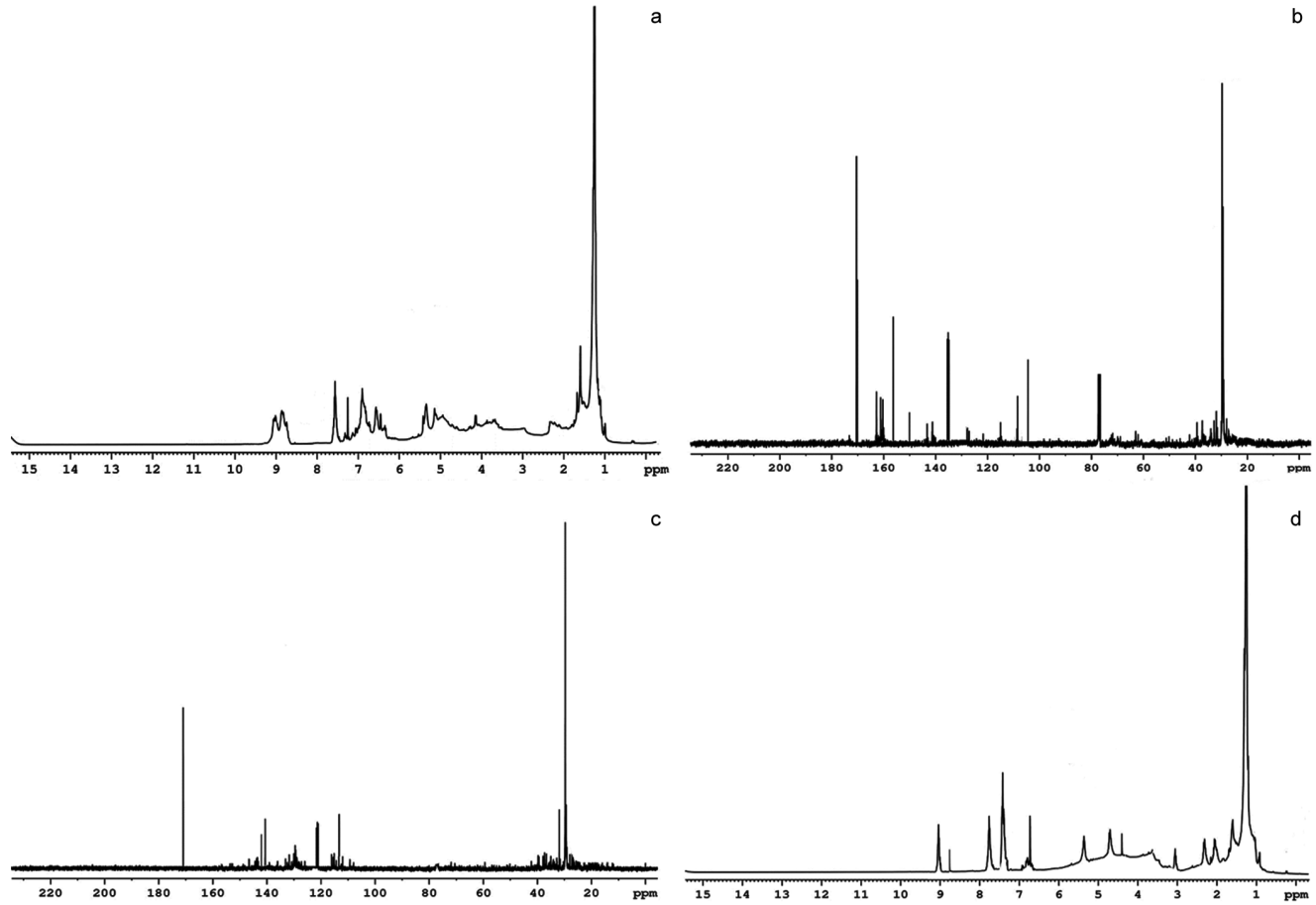

Fig. 3 — a) ^{1}H NMR spectra of *M. polymorpha*; b) ^{1}H NMR spectra of *D. scoparium*; c) ^{13}C NMR spectra of *M. polymorpha*; and d) ^{13}C NMR spectra of *D. scoparium*.

the various compounds analyzed, providing substantial evidence for their structural elucidation.

Compound 1: The ^{1}H NMR spectrum revealed a singlet at δ 7.3 (2H, s, H-3, and H-7), indicative of ortho-coupled aromatic ring protons. The ^{13}C NMR spectrum exhibited quaternary carbon signals at δ 171 (C-1), 144.9 (C-4 and C-6), 141.2 (C-5), 122.6 (C-2), and 114.6 (C-3 and C-7). These findings strongly confirm that gallic acid is the key bioactive compound in *Marchantia polymorpha* and *Dicranum scoparium* (Fig. 3a-d).

Compound 2: The ^{1}H NMR spectrum (400 MHz, $CDCl_3$) displayed signals at δ 11.67 (s, 1H), 8.20 (d, 2H), 7.68 (t, 1H), and 7.68 (t, 2H). The corresponding ^{13}C NMR spectrum (100 MHz, $CDCl_3$) showed peaks at δ 172.60, 133.89, 130.28, 129.39, and 128.65. Based on this spectral data, Compound 2 was identified as benzoic acid in *M. polymorpha* (Fig. 3a-c).

Compound 3: The ^{1}H NMR spectrum (200 MHz, $CDCl_3$) revealed signals at δ 4.62 (s, H-29a), 4.45 (s, H-29b), and 3.15 (dd, H-3), along with additional signals at δ 1.25, 0.99, 0.91, 0.89, 0.81, 0.77, and 0.75, corresponding to a total of seven methyl groups (21-H, 7s). The ^{13}C NMR spectrum displayed 30 signals corresponding to the terpenoid structure of

the lupin skeleton, with the carbon bonded to the hydroxyl group (C-3) resonating at δ 79.9, and the olefinic carbons appearing at δ 151.4 and 109.2 (Fig. 3b-d).

Compound 4: The ^{1}H NMR spectrum revealed signals at δ 6.20 (d, 1H, C-6), 6.40 (d, 1H, C-8), 6.90 (d, 1H, C-5'), 7.55 (dd, 1H, C-6'), 7.67 (d, 1H, C-2'), 8.97 (3H, 3xOH, C-3', C-3, C-4'), 10.25 (1H, OH, C-7), and 12.35 (1H, OH, C-5). The ^{13}C NMR spectrum showed signals at δ 94.2 (C-8), 99.4 (C-6), 103.9 (C-10), 115.2 (C-4'), 116.1 (C-5'), 121.7 (C-6'), 136.6 (C-3), 147.2 (C-3'), 148.7 (C-2), 112.3 (C-2'), 157.05 (C-5), 161.4 (C-7), 164.9 (C-9), and 176.7 (C-4) (Fig. 3b-d).

Network pharmacology

A total of 100 targets by each compound (gallic acid, lupeol, benzoic acid, quercetin) were recognized from the Swiss target prediction tool as well as the disease targets (were also extracted from the genecards database. After that, the common targets between the drug and disease were isolated from Venny 2.1 in the form of a Venn diagram. The number of common targets is shown in supplementary Figs. 1, 3, 5, and 7. Identified common targets from venny 2.1 imported into string database by selecting multiple proteins and organisms as Homo sapiens in the search window. String database provides a network of gene-gene interaction. The data on gene-gene interactions was extracted into an Excel file and imported into the cytoscape. In cytoscape, cytohubba module was used to identify 10 genes from the imported network demonstrated in supplementary Figs. 2, 4, 6, 8, and Table 6. Among the identified top 10 genes of all four compounds, we have found ESR1 common for gallic acid, benzoic acid, and lupeol, and PTGS2 is common for gallic acid, benzoic acid, and lupeol.

Antioxidant analysis

DPPH radical-scavenging activity

The assessment of free radical scavenging activity in *M. polymorpha* and *D. scoparium* was conducted utilizing the DPPH assay method, with ascorbic acid employed as the reference standard for free radical scavenging activity. In the series of extracts derived from the bryophytes under investigation, it was observed that the DPPH activity exhibited a positive correlation with the concentration levels of the extracts, as illustrated in (Table 7). The percentage scavenging activity of *M. polymorpha* ranged from 4.41 to 88.05%, with the lowest observed at a concentration of 15.62 µg/mL and the highest at a concentration of 1000 µg/mL (Fig. 4a). Similarly, the percentage scavenging activity of *D. scoparium* exhibited a range of 3.69 to 88.46%, with the minimum value occurring at a concentration of 15.62 µg/mL and the maximum at a concentration of 1000 µg/mL (Fig. 4b). In the context of these antioxidant assays, the methanolic extracts of *M. polymorpha* and *D. scoparium* demonstrated noteworthy results in the DPPH scavenging assay, with IC50 values of 85.68 and 84.15 µg/mL, respectively. Furthermore, the total antioxidant capacity of *M. polymorpha* and *D. scoparium* was determined to be 779.33 and 756.24 µg/mL, as depicted in Figs. 5a-b.

Superoxide radical scavenging activity

The evaluation of superoxide radical scavenging activity in *M. polymorpha* and *D. scoparium* was conducted using DMSO as the free radical scavenger

Table 7 — Scavenging of DPPH radical and superoxide radical for *Marchantia polymorpha* and *Dicranum scoparium*

Samples	IC_{50} values µg/mL by methods		TAC
	DPPH	SOD	
Marchantia polymorpha	85.68±4.05	>1000	779.337
Dicranum scoparium	84.15±2.13	>1000	756.243
Standards	Quercitin	Rutin	
	20.3±0.64	>1000	

Table 6 — Top 10 gene of lupeol

	lupeol		quercetin		benzoic acid		gallic acid	
Rank	Name	Score	AKT1	23	ALB	44	EGFR	30
1	ESR1	32	EGFR	23	ESR1	26	SRC	28
2	PTGS2	30	SRC	23	PTGS2	23	ESR1	28
3	CYP3A4	25	MMP9	23	GSK3B	18	BCL2	26
4	CYP19A1	23	IGF1R	23	MAOA	15	PTGS2	23
5	HMGCR	22	MET	22	RARA	15	DRD2	18
6	MAPK14	22	PARP1	22	HDAC1	15	MAOA	17
7	GSK3B	20	MMP2	15	PARP1	15	COMT	16
8	MDM2	19	ABCB1	14	HDAC2	13	CYP19A1	16
9	IGF1R	17	KDR	11	HDAC3	13	ESR2	14
10	CYP17A1	17	AKT1	23	ALB	44	EGFR	30

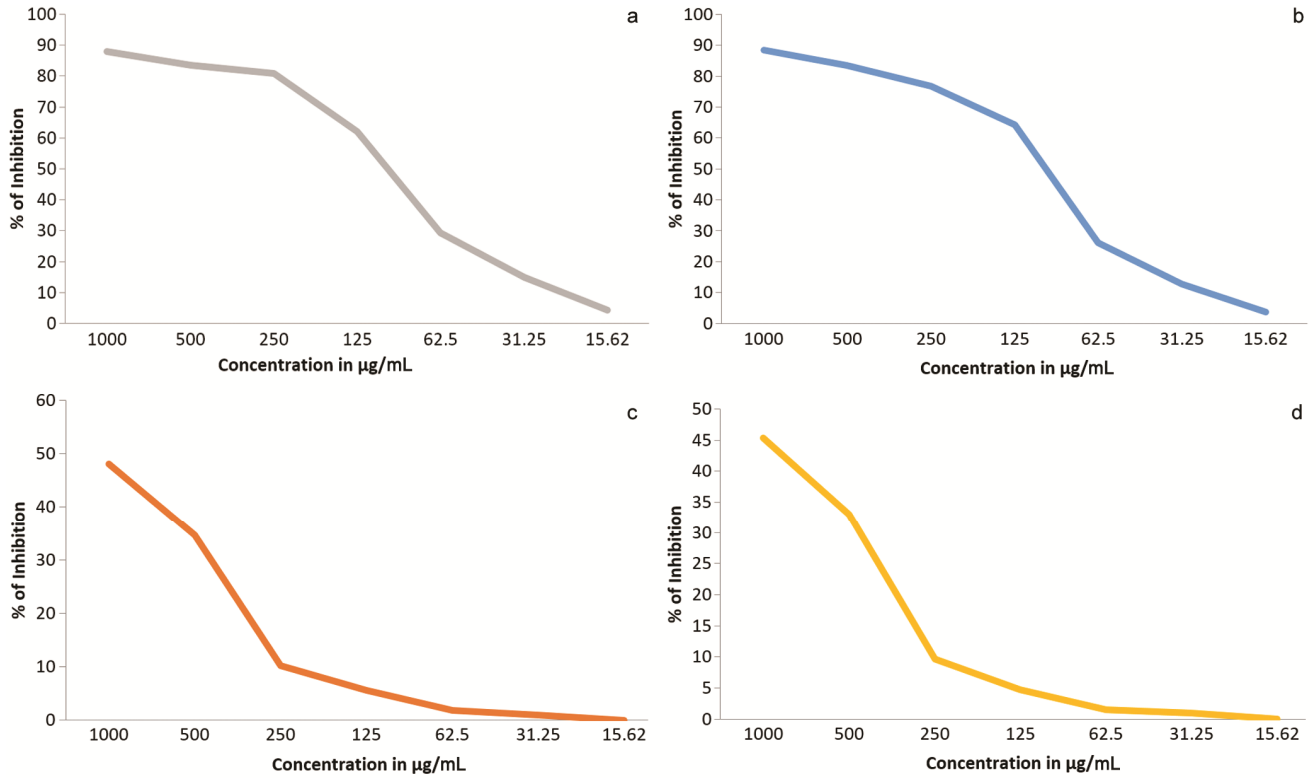

Fig. 4 — Scavenging of a) DPPH radical for *M. polymorpha*; b) DPPH radical for *D. scoparium;* c) superoxide radical for *M. polymorpha*; and d) superoxide radical for *D. scoparium.*

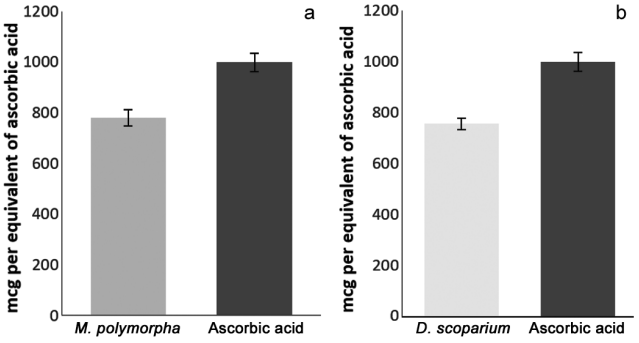

Fig. 5 — Total antioxidant capacity of a) *M. polymorpha*; and b) *D. scoparium.*

and rutin as the reference standard. Among the tested bryophyte extracts, an enhancement in superoxide radical scavenging activity was observed as the concentration of extracts increased, as presented in (Table 7). The percentage scavenging activity of *M. polymorpha* spanned from 0.00 to 48.09%, with the lowest value recorded at a concentration of 15.62 µg/mL and the highest at a concentration of 1000 µg/mL (Fig. 4c). Conversely, the percentage scavenging activity of *D. scoparium* ranged from 0.00 to 45.39%, with the minimum value occurring at a concentration of 15.62 µg/mL and the maximum at a concentration of 1000 µg/mL (Fig. 4d). In the antioxidant assays, methanolic extract of *M. polymorpha* and *D. scoparium* have shown significant results in superoxide radical scavenging assay with IC50. The IC50 value was observed as >1000 in *M. polymorpha*, and in *D. scoparium* IC50 value was observed as >1000. (Table 7).

Cytotoxicity study

The cytotoxicity effects of methanolic extracts of *M. polymorpha* and *D. scoparium* were examined via HDF (Human dermal fibroblast) cells and U-87 (Human Glial cell) cell lines using MTT assay. The enzyme activity (mitochondrial dehydrogenase) was directly proportional to the amount of formazan formed and indicated the percentage of viability of cells in the well. The shape of dose-response curves indicated a significant inhibition of cell growth in a dose-dependent manner from 62.5-1000 µg/mL concentration in the 24 hours of treatments (Table 8 and Fig. 6a).

Cell growth was significantly lower (p <0.05) when extract-treated cells were compared to control

cells. The IC50 was graphically obtained from the dose-response curve. The IC50 was >1000 in *M. polymorpha*, while in *D. scoparium*, the IC50 was 82.10±3.7 µg/mL. Against the HDF cell line, the percentage cytotoxicity of *M. polymorpha* ranged from 1.90±0.5 to 36.18±8.3 with the minimum at 62.5 µg/mL and maximum at 1000 µg/mL. On the other hand, the percentage cytotoxicity of *D. scoparium* ranged from 38.99±3.1 to 88.40±0.8 with a minimum of 62.5 µg/mL and a maximum of 1000 µg/mL.

The percentage cytotoxicity of *M. polymorpha* against the U-87 cell line ranged from 1.51±0.3 to 47.11±1.8 with the minimum at 62.5 µg/mL conc. and maximum at 1000 µg/mL. On the other hand, the percentage cytotoxicity of *D. scoparium* ranged from 47.06±1.0 to 65.46±0.4 with a minimum of 62.5 µg/mL and a maximum of 1000 µg/mL. On the other hand, the IC50 was >1000 in *M. polymorpha*, while in *D. scoparium*, IC50 was 141.99±13.3 µg/mL (Table 9 and Fig. 6b).

Docking study

In the current study, a molecular docking study was carried out to find out the rationale for the biological results, i.e. anticancer activities demonstrated by natural products. In this study, all ligands interacted with estrogen receptor1 in the active pocket and have quite similar interactions. However, some of them could form necessitate interactions with target receptors but demonstrated very appreciable binding energy (Fig. 7 and Table 10).

Discussion

In the search for new biologically active compounds, bryophytes represent a largely underexplored resource. Compared to higher plants, relatively few studies have focused on the composition of secondary metabolites and their biological activities in Bryophytes. This lack of attention leaves significant potential for discovering novel compounds within these ancient plant forms. In our research, two widespread bryophyte species, *M. polymorpha,* and *D. scoparium*, were harvested and screened for their bioactive compounds, cytotoxicity, and antioxidant potential. Notably, gallic acid, benzoic acid, lupeol, and quercetin were identified as prominent constituents in their methanolic extracts. Similarly, Mandia identified

Table 8 — Cytotoxic properties of *Marchantia polymorpha* and *Dicranum scoparium* against HDF cell line

S. No	Name of test compound	Conc. (µg/mL)	% Cytotoxicity	CTC_{50} (µg/mL)
1	*Marchantia polymorpha*	1000	36.18±8.3	>1000
		500	24.40±0.6	
		250	20.29±2.2	
		125	5.31±0.2	
		62.5	1.90±0.5	
2	*Dicranum scoparium*	1000	88.40±0.8	82.10±3.7
		500	83.12±1.4	
		250	80.24±1.5	
		125	73.73±0.6	
		62.5	38.99±3.1	

Table 9 — Cytotoxic properties of *Marchantia polymorpha* and *Dicranum scoparium* against U-87 cell line

S No	Name of test compound	Test Conc. (µg/mL)	% Cytotoxicity	CTC_{50} (µg/mL)
1	*Marchantia polymorpha*	1000	47.11±1.8	>1000
		500	11.83±4.1	
		250	6.91±0.7	
		125	4.71±0.9	
		62.5	1.51±0.3	
2	*Dicranum scoparium*	1000	65.46±0.4	141.99±13.3
		500	58.55±2.9	
		250	52.20±0.8	
		125	49.70±0.2	
		62.5	47.06±1.0	

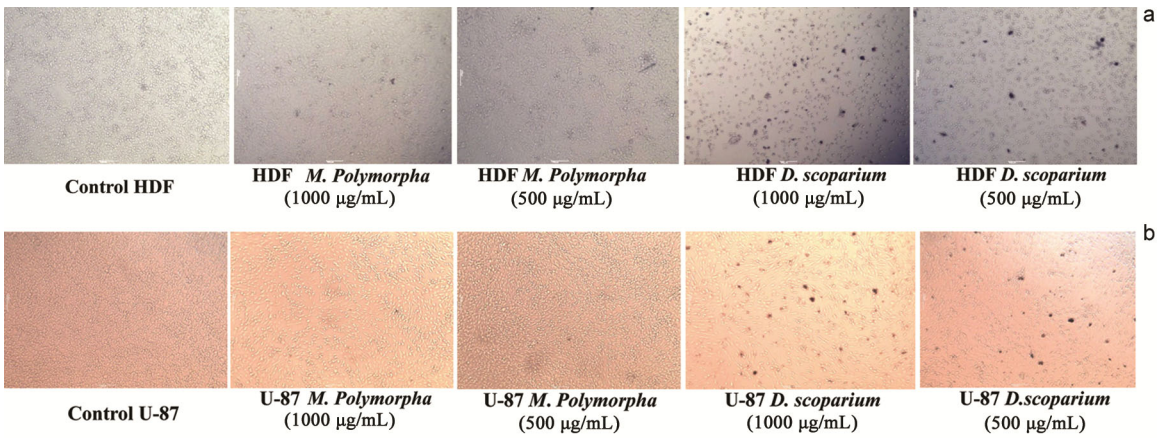

Fig. 6 — Cytotoxic properties of *M. polymorpha* and *D. scoparium* against a) HDF cell line; and b) U 87 cell line.

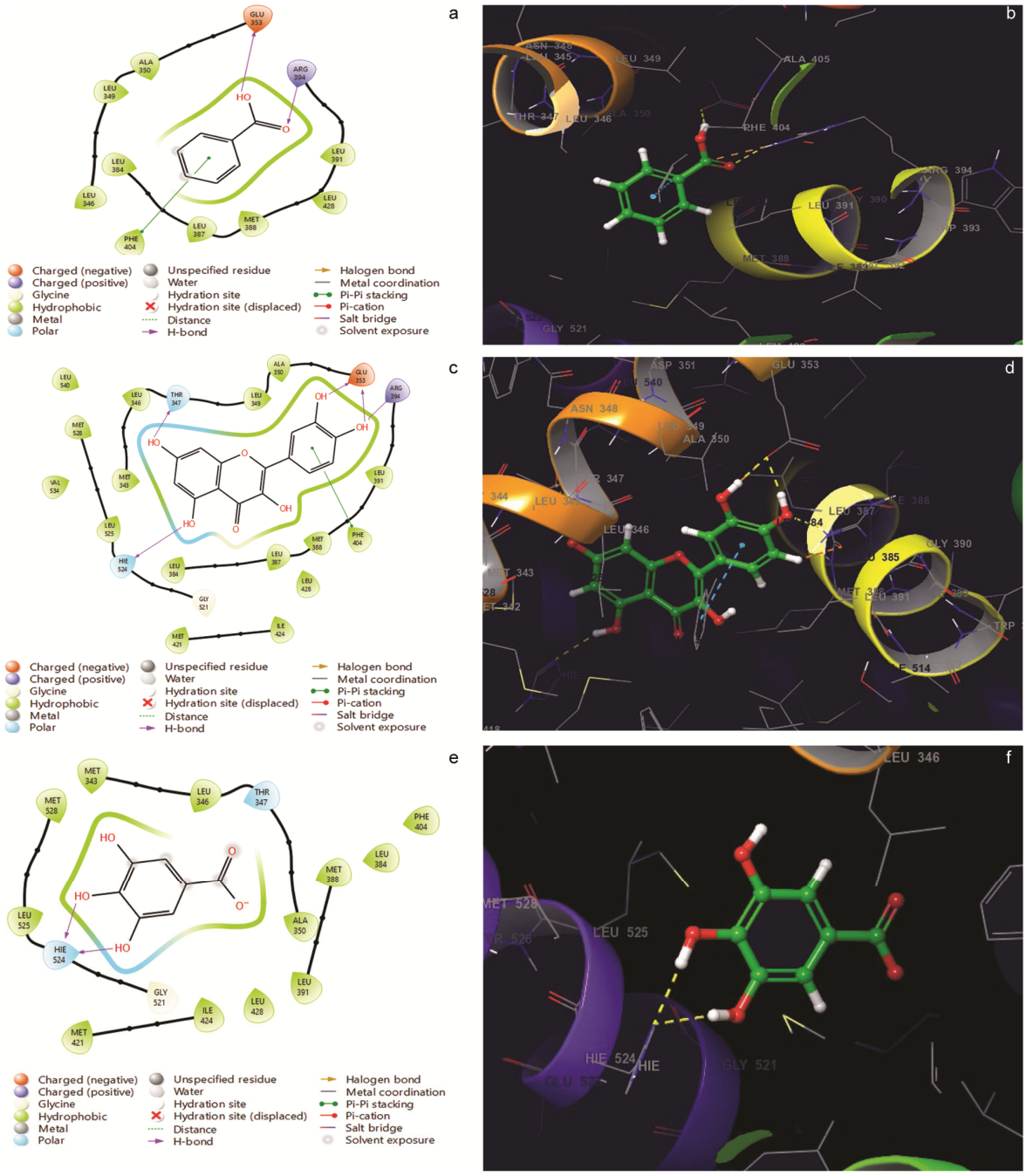

Fig. 7 — 2D and 3D interactions between ligands and Target where a,b) Benzoic acid; c,d) Quercetin; and e,f) Gallic acid.

Table 10 — Binding interactions between ligands and estrogen receptor

S. N.	Ligands	Docking energy	H-bond formation	Other interactions
1	Gallic acid	-6.69763	His524 (2.20Å), (2.07Å)	Met343, Leu346, Thr347, Ala350, Leu384, Met388, Leu391, Phe404, Val418, Met421, Ile424, Leu428, Gly521, His524, Leu525, Met528
2	Quercetin	-11.9752	Thr347 (2.06Å), Glu353(1.67Å, 1.84Å), Arg394 (1.87Å), His524 (2.36Å)	Met343, Leu346, Leu349, Ala350, Leu384, Leu387, Met388, Leu391, Phe404, Met421, Ile424, Leu428, Gly521, His524, Leu525, Met528, Val534, Leu540
3	Benzoic acid	6.1455	Glu353(1.67Å, 1.84Å), Arg394 (1.87Å)	Leu346, Leu349, Ala350, Leu384, Met388, Leu391, Phe404, Leu428

flavonoids, alkaloids, terpenoids, and phenolics in *M. polymorpha*'s methanolic extract, while in our study, undetectable alkaloids might result from environmental and sampling differences[24]. Whereas Das *et al*. linked bisbibenzyls such as marchantin A, marchantin C, and perrottetin E to antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer effects, highlighting the potential of *M. polymorpha*'s secondary metabolites. Additionally, they identified isoprenoids in the ether extract of *M. polymorpha*[25]. Osterdahl, isolated a complex flavonoid-O-triglycoside from *D. scoparium*, revealing intricate flavonoid derivatives[26]. However, Asakawa *et al*. and Tanaka *et al*. identified volatile terpenoids and aromatic compounds in *M. polymorpha*[27,28]. Mathew *et al*. employed chromatographic techniques to identify several phenolic compounds in *Sphagnum magellanicum*, including gallic acid, caffeic acid, vanillic acid, chlorogenic acid, p-coumaric acid, salicylic acid, and 3,4-dihydroxybenzoic acid. Notably, the methanol extract derived from mosses exhibited the most significant antioxidant activity in their study[29]. Additionally, Talukdar *et al*. emphasized the role of specific compounds, distinguished by unique Rf values during TLC separation, in contributing to the antimicrobial activity of bryophytes. The major chemical constituents of *Marchantia* include steroids, triterpenoids, and flavonoids such as luteolin, apigenin, and quercetin[30].

The antioxidant activity of methanol extracts from *M. polymorpha* and *D. scoparium* was assessed using the DPPH free radical scavenging assay. When a free radical scavenger is present, it pairs with the electron, leading to a loss of absorption and resulting in discolouration that matches the number of electrons captured[31]. This bleaching of DPPH absorption indicates the ability of the extracts to scavenge free radicals[32]. In our study, methanol extracts from *M. polymorpha* and *D. scoparium* results showed that the scavenging effect on DPPH radicals increased significantly with higher concentrations of both extracts (ranging from 15.62 to 1000 µg/mL) and the standard (ascorbic acid), indicating a strong concentration-dependent antioxidant activity. Compared to ascorbic acid, the highest total antioxidant activity was observed in the methanol extract of *M. polymorpha*, followed by *D. scoparium*, suggesting that methanol is effective at extracting the phytochemicals responsible for antioxidant capacity. The findings of our study align with those reported by Kaneria *et al*., who observed that the methanol extract from *M. zapota* leaves contained a high phenol content, exhibited effective DPPH scavenging activity, and included cardiac glycosides and triterpenes. These results emphasize the effectiveness of methanol as a solvent for extracting phytochemicals responsible for antioxidant activity[33]. This extract also contained cardiac glycosides and triterpenes. Similarly, Chunhakant and Chaicharoenpong, noted that quercetin and gallic acid, isolated from *M. zapota* fruits, demonstrated significant antioxidant activity[34]. This observation suggests that, in addition to phenolic compounds, other non-phenolic constituents may also significantly contribute to the observed antioxidant effect[33]. Superoxide radicals are highly reactive and can initiate oxidative damage to lipids, proteins, and DNA, as well as participate in redox signaling and regulate cellular processes. Although superoxide radicals have relatively weak chemical reactivity and cannot penetrate lipid membranes, they are rapidly converted into H_2O_2 by superoxide dismutase[35]. In our study, methanol extracts from *M. polymorpha* and *D. scoparium* demonstrated that the methanol extract exhibited the highest scavenging activity against superoxide radicals. Many reports in the literature associate the SO_2 scavenging of plants and their components with strong antioxidant activity.

In the present study, an MTT assay was used to determine the cytotoxicity of the methanol extract of *M. polymorpha* and *D. scoparium* done in five different concentrations (125, 250, 500, and 1000 µg/mL) on HDF (Human dermal fibroblast) and U-87 (Human Glial cell) cell lines at 72 hrs duration. Compared to the Normal Control, the methanol extract of *M. polymorpha* showed no cytotoxicity and possessed cell proliferation activity on both cell lines. Whereas methanol extract of *D. scoparium* showed very slight cytotoxic. From the result, it is concluded that the extracts are not doing any harm to the Human dermal fibroblast cells and human Glial cells up to a concentration of 1000 µg/mL. These observations of morphological changes may be due to the presence of active chemical compounds like flavonoids, phenols, phytosterols, and alkaloids. The docking studies provide insights into the potential interactions and binding affinities between the compounds and the target proteins, which can help in understanding the mechanisms of action and predicting their anticancer

activity. In current research work, molecular docking analysis demonstrated that Quercetin exhibited the maximum binding energy and more hydrogen bond interactions amongst other ligands. However, all three ligands interacted with Estrogen Receptor1 in its active pocket. Therefore, it can be concluded from the molecular docking analysis that quercetin might be a potential lead for further exploration as an ESR1 inhibitor.

Conclusion

In vitro studies carried out using human dermal fibroblast and human glial cell lines demonstrated that methanolic extracts prepared from the bryophytes *M. polymorpha* and *D. scoparium* did not exhibit any toxicity to any cell lines up to the maximum concentration used in *in vitro* studies. A molecular docking in this study indicated that quercetin, present in the extracts of both species, is responsible for their anticancer activity by inhibiting the Estrogen receptor1 (ESR1). These studies offer insights into the interactions between bioactive compounds and target proteins, enhancing the identification and understanding of their anticancer potential.

Conflict of interest

The authors declare that they have no known competing financial or other interests that could have appeared to influence the work reported in the paper.

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