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Phytochemical Profiling, Antioxidant and Anti-Cancerous Activity of *Hydrogonium arcuatum* (Griff.) Wijk. & Marg. (Bryophyta: Pottiaceae)

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ABSTRACT

Background and Objective: Bryophytes, the second-largest category of plants make up a considerable portion of biodiversity found in arid regions. This investigation aims to reveal the antioxidant capacity and phytochemical makeup of *Hydrogonium arcuatum* thereby enhancing understanding of its biological attributes. **Materials and Methods:** After the gametophytic thalli were removed from their natural habitats and thoroughly cleaned, extracts were made using normal procedures. Using well-established protocols linked to GC-MS, TLC and FTIR techniques, phytochemical profiling, antioxidant activity and anti-cancerous activities were evaluated and results were observed. **Results:** Methanolic extract contained tannins, alkaloids, flavonoids and phenols, according to phytochemical screening. In contrast, a confirmatory test using the TLC method revealed the presence of flavonoids and alkaloids. Methanolic extract yielded the greatest results in the antioxidant experiment, followed by ethyl acetate extract. Caryophyllene, which has been demonstrated to have antiviral, anti-inflammatory and cytotoxic qualities, is confirmed to be present by the GC-MS approach. At 1000 µg/mL, *H. arcuatum* extract showed the greatest cytotoxicity against HT-29, but not against HCT-116. At dosages of 50 and 100 µg/mL, it did not affect either of the two cell lines. **Conclusion:** The *H. arcuatum* has been shown to exhibit a highly significant increase in cytotoxicity against cell lines, because of its strong cytotoxic action. This suggests that it may be a valuable treatment option for colorectal cancer.

KEYWORDS

Anti-cancerous, bryophyte, *Hydrogonium arcuatum* moss, phytochemicals, pottiaceae

INTRODUCTION

Bryophytes, the second-largest category of plants after angiosperms, make up a considerable portion of biodiversity, with 16,600 species worldwide and 850 species in India[1,2]. These plants are classified into three main groups: Hornworts (Anthocerotopsida), liverworts (Marchantiopsida) and mosses (Bryopsida). They are phylogenetically located between algae and pteridophytes[3-5].

Bryophytes are distributed around the world in a variety of biological environments, from the tropics to the polar and alpine regions. In our country, they are primarily found in the Eastern and Western

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Himalayas, South India and Central India[6,7]. They are divided into 21 orders that belong to 66 families, which contain 328 genera and 1578 species[8-10].

They are used in the manufacture of furniture and household items, gardening, the pharmaceutical industry (e.g., for surgical dressings, medications, antibiotics, etc.) and as packing material[11,12]. The second-most specious category after angiosperms, mosses make up a sizeable portion of the diversity (ca. 13,000 species) of terrestrial plants[13]. They can even be found in many harsh settings where vascular plants are few or nonexistent.

Since it is well known that, in contrast to higher vascular plants, bryophytes lack any type of mechanical or physical protection, they have an excellent metabolism and remarkable defense mechanisms to withstand a variety of biotic and abiotic challenges. Due to this, the majority of bryophytes create a variety of secondary metabolites with distinctive flavors or aromas; these secondary metabolites also have antibacterial properties that are effective against bacteria and fungi[14].

Phytochemicals are plant-derived secondary metabolites. Several bryophytes have been examined for the existence of diverse phytochemicals including flavonoids, organic acids, sterols, phenolic acids, triterpenes, lipids and aldehydes. These substances are created synthetically and stored in very high concentrations[12,15]. Many terpenoids with about 1400 structures have been isolated from bryophytes as secondary metabolites, viz., pinguisanes, myltaylanes and ventricosanes[16-19]. Apart from this, a lot of different types of secondary metabolites seemed to be isolated from bryophytes. These secondary metabolites have been analyzed for their respective functions. All terpenoid classes, such as monoterpenoids, diterpenoids, sesquiterpenoids and etheric oils, are isolated from mosses and liverworts.

Among all secondary metabolites, flavonoids are considered the most interesting polyphenols in the defense system of bryophytes, as they help to combat various environmental stresses[20]. Bryophytes are a good source of tetraterpenoid carotenoids; many of them have been isolated from species of mosses and liverwort. Previously, it was investigated that bryophytes possess an excessive amount of secondary metabolites such as terpenoids, phenolics (flavonoids and bibenzyle derivatives), glycosides, fatty acids, as well as some aromatic compounds[21,22]. Whereas in few tropical moss plants, the presence of alkaloids, flavonoids, phenols, saponins and steroids was investigated, which could be a possible source of beneficial drugs in the treatment of chronic diseases[23].

In the human body, the production of reactive oxygen species (ROS), superoxide and hydrogen peroxide is the most common physiological process[24]. A high level of ROS can be produced by exogenous chemicals and endogenous metabolic processes, which are directly connected to cardiovascular diseases such as hypertension[25], diabetes and atherosclerosis[26]. Cirrhosis, emphysema, genotoxicity, inflammation and cancer have also been correlated with ROS effects[27]. Presently, it is widely acknowledged that many plant extracts and phytochemicals have specific antibacterial properties and can be useful in therapeutic treatments[9]. In order to show its effectiveness, many studies have been carried out recently in many countries, including India[28]. Mosses have shown antioxidant properties which were assayed by the DPPH method[29].

Since mosses have the potential to be a source of a wide range of secondary metabolites, they are successfully incorporated into a range of pharmaceutical goods, such as antibiotics, surgical dressings and herbal treatments. Studies based on the phytochemistry of bryophytes show that a variety of biologically active substances, including organic acids, aliphatic compounds, carbohydrates, proteins, steroids, lipids, terpenoids, polyphenols, fatty acids, sugar alcohol, acetogenins, phenyl-quinines, phenolics and aromatic

compounds, are present in bryophytes and are responsible for their many bioactivities[15,30]. In India, burned ash of mosses is mixed with honey and used as an ointment for cuts, wounds and burns[12]. These pharmacological effects include antimicrobial, antifungal, cytotoxic, antitumor, vasopressin antagonist, cardiotonic, allergy-causing, insecticidal and piscicidal[22].

The main objective of this study was to assess the phytochemical constituents of the moss, *Hydrogonium arcuatum* from Mount Abu, Rajasthan. Qualitative assays determined the presence of phenol, flavonoid and alkaloids in the methanolic extracts of *H. arcuatum* and then subsequent quantitative assays were performed to determine the total phenolic content (TPC), total flavonoid content (TFC) and total alkaloid content (TAC) in the methanolic extracts. Further GC- MS and TLC analyses were performed to ascertain active compounds in these extracts. The DPPH assay and nitric oxide scavenging activity assay were performed for its anti- oxidant potential. The functional groups of phytoconstituents found in the plant extract were determined by FTIR assay. Cytotoxic effect of the methanolic extract of *H. arcuatum* was studied against two human colorectal cancer cell lines HT-29 and HCT-116.

MATERIALS AND METHODS

Collection of plant: The plant material, i.e., selected moss species of the family Pottiaceae, was collected from Mount Abu, western Rajasthan, at an altitude of 1600 m, 24°31′ to 24°43′N and 72°38′ to 72°53′E; during the months of August 2022, subsequently in September 2022 some samples were procured from the Banasthali University Rajasthan, India (BURI) Herbarium. The collected materials were kept in brown paper packets directly in the case of dried specimens and for wet specimens, blotting paper was used to soak up excess moisture and dry the specimens. For the morpho-taxonomic study, the collected plant specimens were air-dried at room temperature and kept in brown paper herbarium packets (size, "6×4'' inches). The morpho-taxonomic work was completed in the first week of October 2022, followed by the initiation of phytochemical work in the second week of October, which was completed in November 2022.

Identification and screening of plant material: In order to identify the samples, morphological study of plants was done in October 2022 using the Olympus Japan SZ-PT Stereoscopic Zoom Binocular Microscope to examine the plants' external morphology and the Olympus OIC HC 10068 Compound Microscope and Olympus CH 20i Binocular Compound Microscope were used to examine the cellular details and microscopic structures (No. 76026946 for the Nikon Coolpix L21). Identifications were made following careful examination of the specimens, with the assistance of the insightful contributions of former bryologists, current literature and herbarium deposited with pertinent data in the Banasthali University, Rajasthan, India (BURI) herbarium (BURI-1717/2023).

The samples were first stretched by soaking them in lukewarm water for at least half an hour at room temperature before being used to study. Samples were studied microscopically for gametophytes like leaves, stems and rhizoids. The identification of these species was done with the help of previously available specimens and literature.

Preparation of plant extracts: For the preparation of plant extract, standard procedures were followed[31]. Prior to extraction, all the identified samples were thoroughly washed with running tap water to free them from any adhering soil and debris and then they were finally rinsed with distilled water and air-dried in the shade until the water content became almost nil. Washed and air-dried plant samples were further ground into a fine powder with the help of a mortar and pestle using solvents (methanol, ethyl-acetate, di-ethyl ether and n- hexane) and subjected to 48 hrs of 37°C shaking at 120 rpm in an orbital shaker, the Metrex-100C. After centrifuging each extract for 30 minutes at 10,000 rpm, the supernatant was collected and stored at 4°C for further use[32].

Phytochemical assay

Preliminary qualitative analysis: Using established techniques, the preliminary phytochemical analysis of methanol, ethyl acetate, diethyl ether and hexane was checked for the presence of several phytochemicals in a number of bryophytes[33,34].

Quantitative analysis

Estimation of total phenolic content (TPC): Total phenolics were quantified colorimetrically using the Folin-Ciocalteu method[35]. The 0.5 mL of water and 0.125 mL of the methanolic extract were put into a test tube. In that sequence, 0.125 mL of the Folin-Ciocalteu reagent, 1.25 mL of sodium carbonate solution and 3 mL of water were added. The mixture was then allowed to stand for 90 min. The absorbance was recorded at 760 nm. Gallic acid equivalents (GAE) based on dry material were used to calculate the total phenol concentration (mg GAE/g dry weight of sample). These tests were conducted in triplicate.

Estimation of total flavonoid content (TFC): The approach of Adebiyi *et al.*[23] (2016) was used to determine the total flavonoid content. The plant extract was mixed with 10% aluminum chloride, 95% ethanol, 1M potassium acetate and distilled water. The reaction mixture was allowed to stand at room temperature for thirty minutes. At 415 nm, the absorbance was recorded spectrophotometrically. The extracts' dry weight in milligrams of quercetin equivalent (mgQE/g) was used to express the results.

Estimation of total alkaloid content (TAC): The total alkaloid content (TAC) was calculated using a spectrophotometric method[36]. This process relates to the reaction between alkaloids and bromocresol green (BCG). The plant sample was filtered after being individually dissolved in 1 mg/mL and 2 N-HCl solvents. The pH of the phosphate buffer mixture was neutralized with 0.1 N NaOH. As 5 mL of BCG (bacillus Calmette-Guérin) solution and 5 mL of phosphate buffer were each added to 1 mL of this mixture in a separating funnel. Chloroform was used to extract the resulting complex after the mixture had been vigorously stirred. Chloroform was further used to dilute the sample to volume in a 10 mL volumetric flask. The complex's chloroform absorbance was recorded at 470 nm.

Gas Chromatography-Mass Spectroscopy (GC–MS) analysis: The GC-MS analysis was performed as per the protocol described by Abu Bakar *et al.*[37]. A Thermo Scientific Triple Quadruple (trace 1300, Tsq 8000 triple quadruple MS) GC-MS instrument was used for the analysis. Initially, the column temperature was set to 50°C for 4 min followed by an increase to 320°C for 20 min at a rate of 7°C per min. The sample injection volume was kept at 0.11, split mode was 20:1 and the temperature of the injector was then set at 280°C. Helium was used as carrier gas and the flow rate was maintained at 1 mL/min and the run time was 60 min. The method of obtaining mass spectra in the m/z 40-700 range involved electron ionization at a potential of 70 eV. The sample's chromatogram was identified by comparing the mass spectra to the NIST (National Institute of Standards and Technology) library data and the GC retention time to the recognized standards.

Thin layer chromatography (TLC) analysis of secondary metabolites: On the basis of the findings of the qualitative phytochemical analysis, TLC of the samples was performed using known standards. A precisely measured volume of extract was dissolved in methanol solvent to obtain a known concentration. The standard silica gel 60F254 aluminum sheet (310 cm^{-1}) was used to separate the extract into an appropriate mobile phase. A microcapillary tube was used to spot the sample on the aluminum sheet. Various combinations of solvent systems were used to separate the compounds and their varied polarity was examined as a result. With a solvent system that contained an ethyl acetate: methanol volume ratio of (85:15), a chromatogram was created. The TLC sheet was lowered into the chosen solvent system's chamber and the solvent was allowed to soak through to the bottom third of the sheet. It was then removed from the chamber and air-dried. To see the spots, the TLC sheet was then kept in the UV room.

After that, a particular kind of compound was finished, with one compound erectly separated spots being the result. The amount of space shifted over the entire amount of space covered by the solvent is comparable to the retention factor (Rf) for each point[38].

Antioxidant assay

DPPH (2, 2-Diphenyl-1-Picrylhydrazy) radical scavenging activity: The procedure outlined by Pejin *et al.*[39] was utilized to ascertain the impact of the crude methanolic extract on free radicals. As 4 g of DPPH, were dissolved in 100 mL of methanol. The extract was combined with 2 mL of DPPH solution. Using an ELICO double beam SL 210 UV Vis Spectrophotometer at 517 nm, the decrease in the DPPH free radical was assessed after the 30 min incubation period. The IC_{50} value was used to calculate the scavenging capacity of the methanolic extract. It is described as a sample concentration that causes a 50% decrease in oxidative radicals. The strength of the antioxidant scavenging activity increases with decreasing IC_{50} values. The extract's scavenging activity was evaluated using the percentage of decolorization. The sample's scavenging activity was calculated using the sample's percentage of decolorization.

Nitric oxide scavenging activity: The 2 mL Sodium nitroprusside (10 mM) in 0.5 mL phosphate buffer saline (1M; pH 7.4) was mixed with 0.5 mL extract and incubated for 150 min at 25°C. The 0.5 mL of the incubated mixture was taken and mixed with 1.0 mL of sulfanilic acid reagent. Finally, 1.0 mL of 0.1% naphthylethylenediamine dihydrochloride was mixed and incubated for 30 min at room temperature. The absorbance was recorded at λ540 nm. The NOSA was calculated and expressed as IC_{50} (µg/mL) from the method of Badami *et al.*[40]

Fourier Transform Infrared Spectrophotometer analysis (FTIR): Four species of moss extracts were directly loaded into the FTIR spectrum and a spectrometer was obtained from Shamran *et al.*[41] The FTIR spectrometer (Thermo Scientific) had a wavelength range of 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1} and this wavelength range was used to determine the functional groups of phytoconstituents found in the plant extract.

Assessment of cytotoxicity: For the cytotoxicity assessment, two human colorectal cancer cell lines namely, HT-29 and HCT-116 were procured from ATCC, Manassas, VA. Routine propagation of cell lines was done as per the standard protocol[42]. Cell viability was assessed by the XTT assay. Absorbance was recorded by a microplate reader at λ660 nm and λ475 nm.

Statistical analysis: The mean (n = 3) of three replicates has been used to present the data. The collected data was analyzed using IBM SPSS Statistical 20 software. The data were compared using one-way ANOVA followed by the Tukey's test, with a significance level of less than 0.05. The data presented is expressed as a standard deviation.

RESULTS

Phytochemical screening: In qualitative analysis alkaloids, phenol, flavonoid, tannins and terpenoids were present in the methanolic extract of *H. arcuatum*. Gallic acid, quercetin and atropine were used to express the total content of phenol, flavonoid and alkaloid of *H.* arcuatum (Table 1-2; Fig. 1). Large amounts of phenol, flavonoid and alkaloids were produced from *H. arcuatum* using methanol as a solvent; however, much lower amounts of these contents were produced using n-hexane as a solvent.

Antioxidant assay: The antioxidant activity of the plant extract as IC_{50} value was determined to be high potential in methanol followed by ethyl acetate (µg/mL; Mean±SD, n = 3) against DPPH and NOSA, respectively (Table 3; Fig. 2).

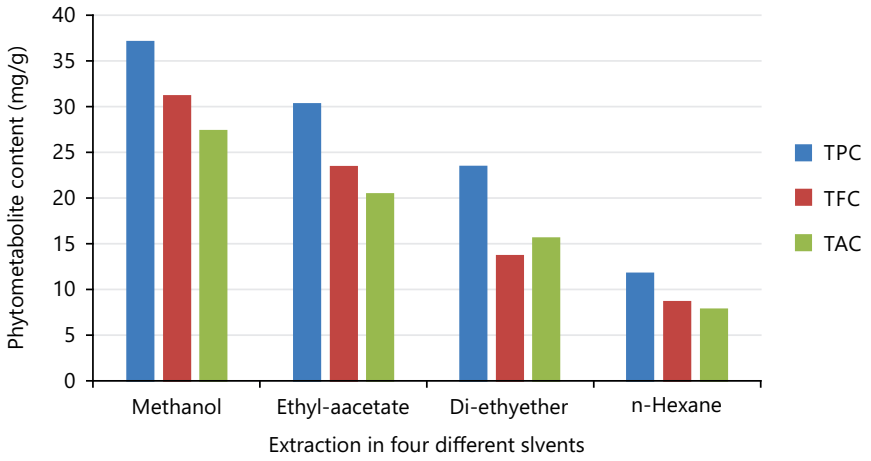

Fig. 1: *Hydrogonium arcuatum's* phytometabolie content on four different solvents

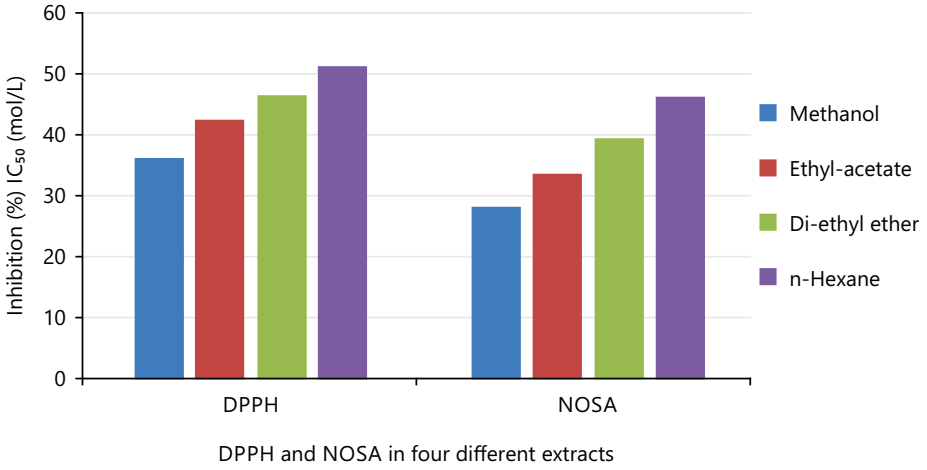

Fig. 2: *Hydrogonium arcuatum's* antioxidant content in various solvents

Table 1: Qualitative analysis of *Hydrogonium arcuatum* in different extracts

		Presence and absence of *Hydrogonium arcuatum* in different extracts			
Phytochemicals	Test	n- hexane	Diethyl ether	Ethyl acetate	Methanol
Alkaloids	Dragendorff's reagent test	-	+	++	+++
Phenols	Ferric chloride test	+	+	++	+++
Saponin glycosides	Froth formation test	-	-	-	-
Cardiac glycosides	Kellar- Killani test	-	-	-	-
Tannins	Ferric chloride test	-	+	++	+++
Proteins	Xanthoprotein test	-	-	+	+++
Steroids	Salkowski test	-	-	-	-
Terpenoids	Salkowski test	+	+	++	+++
Amino acids	Millon's test	-	+	+	+++
Carbohydrate	Molisch's test	-	+	++	+++
Fats	Saponification test	-	-	+	++
Flavonoids	Shinoda test	+	+	++	+++
Anthraquinone	Borntrager's test	-	-	+	++

+++: High presence, ++: Moderate presence, +: Low presence and -: Absent

Thin layer chromatography: Thin layer chromatography was used to separate different chemicals from a methanolic extract of *H. arcuatum* and the findings show four spots with distinct colors and Rf values calculated by comparing the solute and solvent travel distances. The spots that appeared on sheet were

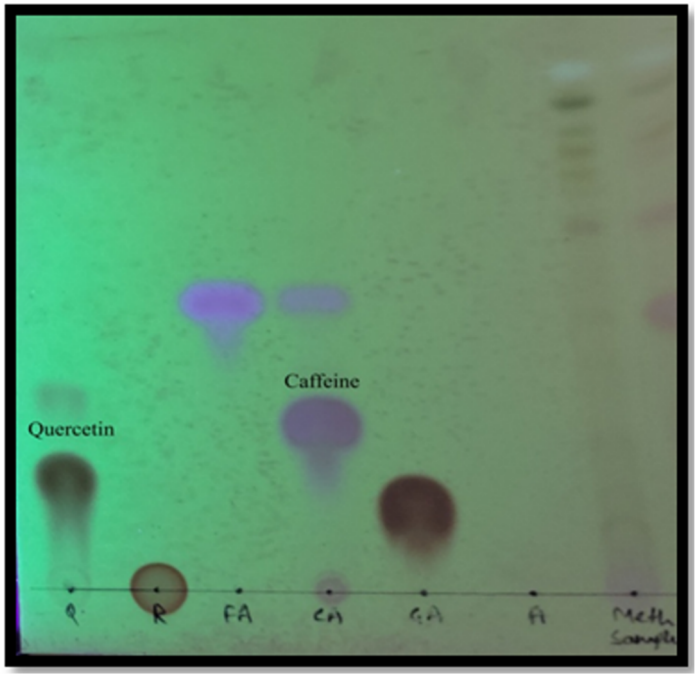

Fig. 3: Bioactive compounds of *Hydrogonium arcuatum* in TLC

Table 2: Quantitative analysis of *Hydrogonium arcuatum* extract in four solvents

Solvents variable	Methanol	Ethyl-acetate	Di-ethyl ether	n-Hexane
TPC	38.05±0.29^{c} mg/GAE/g	31.02±0.17^{b} mg/GAE/g	24.02±0.11^{b} mg/GAE/g	12.04±0.04^{a} mg/GAE/g
TFC	32.02 ±0.23^{c} mg/QE/g	24.06 ±0.19^{c} mg/QE/g	14.04 ±0.08^{b} mg/QE/g	09.02 ±0.01^{a} mg/QE/g
TAC	28.08±0.16^{c} mg/g	21.02±0.05^{b} mg/g	16.04±0.04^{b} mg/g	08.04±0.02^{a} mg/g

TPC: Total phenol content, TFC: Total flavonoid content and TAC: Total Alkaloid content

Table: 3 Antioxidant activity of *Hydrogonium arcuatum* extract in different solvents

Tests solvent	Methanol	Ethyl-acetate	Di-ethyl ether	n-Hexane
DPPH (µg/mL)	36.74±0.17	43.06±0.24	47.03±0.32	52.02±0.39
NOSA (µg/mL)	28.76±0.17	34.04±0.22	40.03±0.36	47.02±0.32

DPPH: 2,2-diphenyl-1-picrylhydrazyl and NOSA: Nitric oxide scavenging assay

Table 4: Thin layer chromatography analysis of bioactive compounds of *Hydrogonium arcuatum*

	RF value		
Compound	Standard	Sample	Secondary metabolites present in moss extract
Quercetin	0.44	0.42	Flavonoids
Rutin	0.35	_	_
Gallic acid	0.15	_	_
Caffeine	0.53	0.53	Alkaloid
Ferulic acid	0.42	_	_
Atropine	_	_	_

calculated with Rf values of 0.42 and 0.53, respectively, which confirms the existence of quercetin and caffeine as per standard values (Table 4; Fig. 3).

GC-MS analysis: The result shows that the *H. arcuatum* methanolic extract revealed the 7 different bioactive compounds like caryophyllene, diethyl phthalate, carotene and many more (Table 5; Fig. 4).

FTIR spectrum: The IR spectra were used to evaluate the existence of different chemical compounds in *H. arcuatum* based on peak values in the infra-red region. The methanol extract contained the groups like May 10, 2024C=C, C-H, C-O, O-H and N-H (Table 6; Fig. 5).

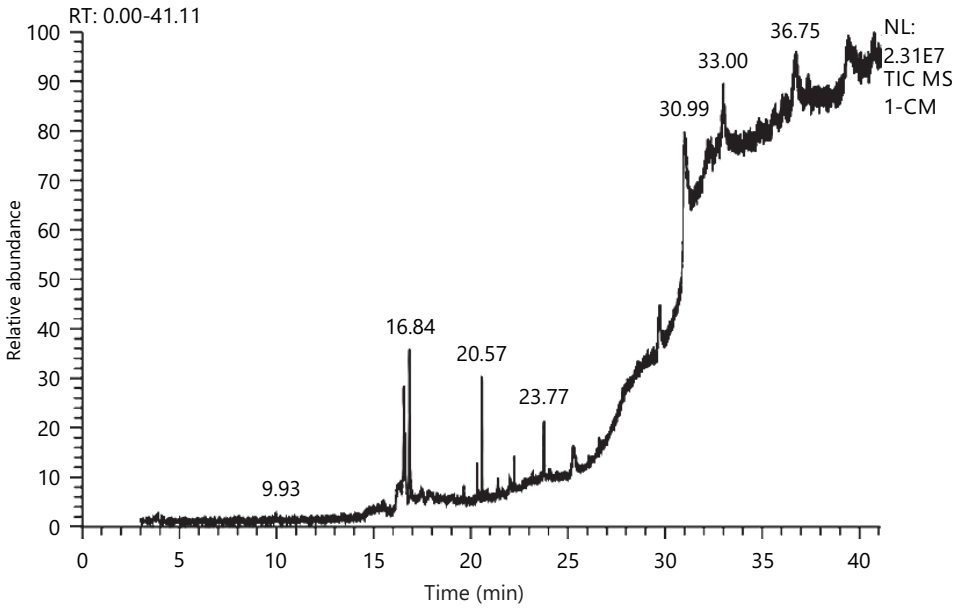

Fig. 4: GC-MS analysis of extract *Hydrogonium arcuatum*

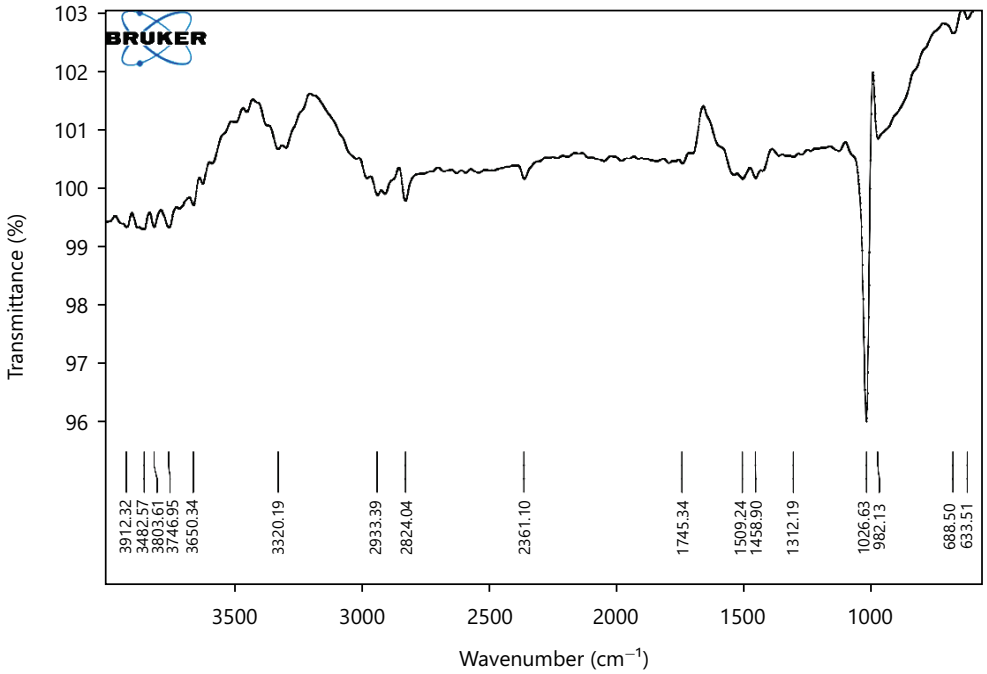

Fig. 5: FTIR of *Hydrogonium arcuatum*

Table 5: GC-MS analysis of bioactive compounds of methanolic extracts of *Hydrogonium arcuatum*

RT	Area	Names of the compound	Molecular Formula
9.93	13.23	Caryophyllene	$C_{15}H_{24}$
16.84	62.48	Diethyl phthalate	$C_{12}H_{14}O_4$
20.57	12.00	Benzenepropanoic acid,3,5-bis(1,1-dimethylethyl)-4-	$C_{18}H_{28}O3$
23.77	5.07	hydroxyl-methyl ester	$C_{41}H_{58}O$
		Carotene,3,4-didehydro-1,2-dihydro-1-methoxy-	
30.99	24.24	Glycine, N-[(3a,5a)-24-oxo-3-[(trimethylsilyl)oxy]cholan-24-yl]-,methyl ester	$C_{30}H_{53}NO_4SI$
33.00	8.72	7,7,9,9,11,11-Hexamethyl-3,6,8,10,12,15-hexaoxa-	$C_{14}H_{36}O_6S_{I3}$
36.75	5.17	7,9,11-trisilaheptadecane	$C_{18}H_{36}O$
		6,10,14-trimethyl-2-pentadecanone	

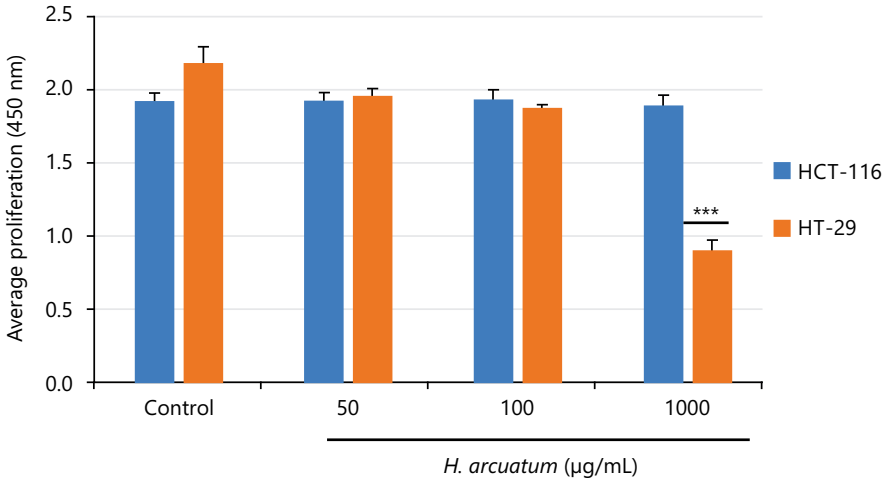

Fig. 6: Effect of *Hydrogonium arcuatum,* methanolic extract against
Proliferation of CRC cell lines. Where stars (*p<0.05; **p<0.01 and ***p<0.001) indicates significant differences from control and values represents Mean±SD

Table 6: Detection of functional group in *Hydrogonium arcuatum* by FTIR

Frequency	Group	Appearance	Compound
633.51	C-Br stretching	Strong	Halo compound
688.50	C = C bending	Strong	Alkene
983.13	C = C bending	Strong	Alkene
1026.63	C-N stretching	Medium	Amine
1312.19	S = O stretching	Strong	Sulfone
1458.90	C-H bending	Medium	Alkene
1509.24	N-O stretching	Strong	Nitro-compound
1745.34	C = O stretching	Strong	Cyclopentanone
2824.04	C-H stretching	Medium	Aldehyde
2933.39	C-H stretching	Medium	Alkane
3320.19	N-H stretching	Medium	Secondary amine
3650.34	O-H stretching	Sharp	Alcohol

Cytotoxicity assessment: *Hydrogonium arcuatum* extract exhibited the maximum cytotoxicity against HT- 29 but not against HCT-116 at a concentration of 1000 µg/mL and did not show any effect on both cell lines at doses of 50 and 100 µg/mL significantly ($p<0.001$) when compared to control (Fig. 6).

DISCUSSION

The phytochemical screening of a qualitative analysis of *H. arcuatum* revealed the presence of alkaloids, phenols and flavonoids in the methanolic extract and a moderate amount of carbohydrates, tannins, carbohydrates and terpenoids. The methanolic extract of *H. arcuatum* under investigation contained the highest level of total phenolic, flavonoid and alkaloid content. The over-expression of defense response genes to fight during stress makes it clear that phytoconstituents exhibit a shift in their quantities[43]. The results obtained by Karim *et al.*[44] are in accordance with the current findings, where a high amount of TPC was observed in the methanol extract.

The antioxidant activity of the plant extract as IC_{50} value was determined to be high potential in methanol and low in n-hexane against DPPH and NOSA, respectively. As a result, numerous attempts to evaluate the pharmacological and nutraceutical potential of bryophytes have been made and it has been demonstrated that these amphibious plants are a priceless source of antioxidative, anti-cancerous, antibacterial and antiviral chemicals[45].

The presence of caryophyllene, carotene, etc. was revealed by the GC-MS analysis. Caryophyllene, a phenol derivative is known to possess anti-inflammatory, antiviral, antifungal and anti-cancer properties. Several

essential oils such as pepper and clove include caryophyllene, a naturally occurring bicyclic sesquiterpene in their formulation[46]. Caryophyllene is known to contain a rare cyclobutene ring which is an uncommon occurrence in the natural world.

The FTIR analysis of *H. arcuatum* methanol extract contained the groups like C=C, C-H, C=O, C-N, O-H and N-H. The peak at 1509 cm^{-1} showed the presence of nitro compounds and 1745 indicates the presence of cyclopentanone. Similar results were also reported by other researchers for the methanolic extract of moss[47].

The *H. arcuatum* extract exhibited the maximum cytotoxicity against HT-29 but not against HCT-116 at a concentration of 1000 µg/mL and did not show any effect on both cell lines at doses of 50 and 100 µg/mL. Using the MTT method, the cytotoxicity potential of certain moss essential oils was evaluated in previous research on human immortalized keratinocytes, non-tumor cells and colorectal and breast tumor cells (HCT- 116 and MCF-7). Treatment with the sample essential oil in the different cell lines does not cause any harm in majority of the detected concentrations[48].

Further investigation into the specific phytochemical constituents of *H. arcuatum*, particularly those responsible for its observed biological activities, could provide deeper insights into its potential therapeutic applications. Exploration of the mechanisms underlying the observed cytotoxicity against HT-29 cells could elucidate the pathways through which *H. arcuatum* exerts its anti-cancer effects. Comparative studies with other bryophytes or traditional medicinal plants may help identify unique bioactive compounds and broaden the understanding of *H. arcuatum*'s pharmacological potential.

However, there are a few limitations of this study, such as, the study could benefit from additional experiments to validate the observed biological activities of *H. arcuatum*, such as *in vivo* studies or further cell line investigations. The cytotoxic effects observed against HT-29 cells need to be confirmed through additional assays and exploration of potential mechanisms of action. The extrapolation of the findings to clinical applications may be limited due to the use of cell-based assays and the absence of *in vivo* studies, warranting caution in interpreting the potential therapeutic relevance of *H. arcuatum*.

CONCLUSION

The present study was carried out on *Hydrogonium arcuatum*. Phytochemical screening revealed that the methanolic extract of *H. arcuatum* contains mainly alkaloids, phenols and flavonoids. The non- enzymatic assays DPPH and NOSA were used to evaluate the antioxidant capabilities of *H. arcuatum*. The primary objective of the DPPH assay is to assess the antioxidant potential of plant extracts including methanol and ethyl acetate. The GC-MS, FTIR and TLC were used to estimate the bioactive chemicals in a plant extract. Cytotoxicity assessment was done to investigate the effects on HT-29 and HCT-116 colorectal. It was found that *H. arcuatum* showed a highly significant increase in the cytotoxicity against HT-29 cell lines at 1000 concentration, significantly ($p<0.001$) when compared to control. The use of these bryophytes may be thought of as a useful method for treating colorectal cancer due to their cytotoxic activity, which lowers disease recurrence and can be used to create new drugs for a range of diseases in the pharmaceutical sector. The findings of this study suggest that bryophytes are abundant in phytoconstituents with significant bioactivities, including the potential for anti-proliferative effects. It is possible, however, that these will be useful in the future for other drug therapies for cancer cell lines.

SIGNIFICANCE STATEMENT

This study was aimed at unraveling the anti-oxidant potential, phytochemical profile and anti-cancerous activity of *Hydrogonium arcuatum* to highlight the rich phytochemical constituents of mosses. Scientists

nowadays are looking for natural plant-based treatment options and phytochemicals from bryophytes can provide the much-needed alternative to chemically synthesized medicines. In the present study, extracts of *H. arcuatum* formulated in different solvents were subjected to GC-MS, FTIR and TLC procedures. In the phytochemical screening, methanolic extracts revealed the presence of tannins, alkaloids, flavonoids and phenols. The GC-MS analysis confirmed the presence of Caryophyllene, a potentially anti-viral, anti-inflammatory and cytotoxic compound. The significant cytotoxic activity of methanolic extract of *H. arcuatum* suggested its potential in the treatment of colorectal cancer.

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