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Phytochemical Profiling of a Common Moss *Hyophila involuta* Jaeger. for its Bioactive and Antioxidant Potential Against Viral Infections

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ABSTRACT

Among terrestrial plants, bryophytes constitute a major part of biodiversity and designated as the second largest taxonomic group after angiosperms. It is estimated about 20,000 to 25,000 species of mosses, liverworts and hornworts are existing worldwide. Like angiosperms they also have many ecological and economic impending, especially medicinal value. However, due to natural attraction and practical ease usually angiosperms have been a preferred choice as reservoirs of medicinally important phytoconstituents, and this group of plants has been neglected for a long time despite having many important biologically active compounds. Mosses, the largest taxonomical group of bryophytes, traditionally known for their therapeutic values and their extracts have found various applications in ethnopharmacology. Many of the species of mosses have shown high biological activities and, in several studies, it has been confirmed that mosses have remarkable antibacterial, antiviral and antifungal activities. There have not been any valid reports of viruses infecting bryophytes, and thus it seems possible that bryophytes contain a chemical defense against viruses including COVID-19 due to the presence of secondary metabolites, *viz*., terpenoids, flavonoids and bis-bibenzyls, etc., and some of the bryophytes species have been identified as exhibiting considerable antiviral activity. But, due to their small size, difficulty in collection and identification majority of mosses is remain unexplored for the therapeutic purposes, especially in India. Hence, this preliminary attempt has been made to screen a commonly growing moss species *Hyophila involuta* Jaeger. for its phytochemical profile and bioactive potential.

Keyword: Antioxidant, Bioactive, Bryophytes, Mosses, Secondary metabolites.

INTRODUCTION

Viruses are obligatory intracellular organisms that rely on the host cell system for replication and spread due to their parasitic nature (Helms et al., 2015). Many pandemics, such as Spanish flu (1918 to 1920), Ebola viral disease (2014 to 2016), Chikungunya and dengue fever have been documented in various countries, that is resulting in thousands of deaths and it is still threatening (https://www.worldometers.info/coronavirus/rretrieved on 10.2.2021).

The corona virus pandemic is rapidly spreading over the world as a serious global challenge (Walls et al., 2020). SARS-CoV-2 is a third extremely infectious coronavirus to be discovered in human population, following the severe acute respiratory syndrome corona virus in (2002) and (MERS-CoV) Middle East respiratory syndrome coronavirus in 2012 (Zhou et al., 2015; Chan et al., 2015). SARS-CoV-2 infections span from the mild symptoms and sometimes asymptomatic, infections limited to the superior respiratory tract to acute respiratory syndrome exhibited by dissemination to lower air-way, leading to an inflammation and pneumonia, particularly in the patients having hypertension, diabetes, or heart disease (Li et al., 2020). Many plants have been tested for their anti-viral potential, with a variety of fascinating outcomes (Mugisha et al., 2014; Lamorde et al., 2010; Nyamukuru et al., 2017; Mehrotra, 2020). Bryophytes have been somewhat overlooked and angiosperms are usual preferred group of plants to be favored for such studies.

Bryophytes are reported to be the second oldest group of terrestrial plants in the terms of diversity after the angiosperms (Asakawa et al., 2013). Among three classes of this group, the maximum species diversity is found in the mosses (Gangulee, 1969-1980). Due to minute size and difficulty in collection/identification, these plants are neglected as a source of phytochemical and bioactive compounds. However, they have shown high biological activities in several phytochemicals that have been isolated from many species (Crum, 2001, Krzaczkowski et al., 2009 and Üçüncü et al., 2010). In many species of mosses distinct bioactive substances have been isolated in recent time and it was found that they contain benzoic, pthalic, cinnamic, terpenoids and a few nitrogen containing aromatic compounds, sometimes these are found to be structurally alike in vascular plants (Asakawa et al., 2012). There have been reports of flavonoids, terpenoids, alkaloids and phenolic compounds being present (Asakawa et al. 2013; Marques et al. 2021; Martinez-Abaigar and Nunez-Olivera 2021). Consequently antifungal, antibacterial, antiviral, antioxidants, anti- inflammatory and anti-cancerous efficacies with therapeutic potential have been also observes in mosses (Dey and Mukherjee, 2015).

Antioxidants have been found in angiosperms, gymnosperms, and pteridophytes to combat oxidative damage (Bernaert et al., 2011; Hort et al., 2008). Antioxidants are used extensively in the pharmaceutical industries as they have endogenous defense mechanism against (ROS) Reactive Oxygen Species (Frahm, 2004). There are few reports that provide detail about the unique bioactive principles and antioxidant activity to mosses, and there are still much to learn (Chobot et al., 2008). In the present study *Hyophila involuta* was obtained from the Rajasthan region of India and was further tested for total flavonoid and phenolic content as well as antioxidant activity.

Figure 1: A population of *Hyophila involuta* Jaeger. (x15)

MATERIAL AND METHODS

The moss *Hyophila involuta* Jaeger. (Pottiaceae Schimp.) (Fig. 1) was collected from the Rajasthan (India) during August 2019. Voucher specimen has been deposited in Banasthali University Rajasthan India, BURI-7860318/2019.

Extract preparation

The plant samples were washed carefully and subsequently air dried and grounded using mortar- pestle (Velioglu et al., 1998). Powdered sample of *H. involuta* (1g) was extracted using methanol, n-hexane, ethyl acetate and diethyl ether (Asakawa and Ludwiczuk, 2013; 2017).

Total phenolic content (TPC)

TPC was determined using the method (Vats, 2012). Briefly, the reaction mixture was prepared by mixing 0.5ml of water and 0.125ml of methanolic extract in test tube. Folin-ciocalteu reagent (0.125ml), Sodium carbonate solution (1.25ml), and water (3ml) were added and left to stand for 45 min. The absorbance was steady at 760nm. A result was expressed as mg of gallic acid equivalent/g of dry material. Values were expressed as mean ±S.D (Adedeji et al., 2012).

Total flavonoid content (TFC)

TFC was determined following the method (Vats, 2016). Aluminum chloride (10%), ethanol, potassium acetate of 1M and lastly distilled water was added to the extract of plant. The reaction mixture was then left to stand at room temp for 30 min. The absorbance was steady at 415nm. The results of the extracts were then expressed as mg of quercetin equivalent per gram of dry weight (mgQE/g) (Adedeji et al., 2012).

Table 1: Phytochemical screening of *Hyophila involuta*

Phytochemicals	Tests	Presence and absence of *Hyophila involuta* in different extracts			
		n- hexane	di-ethyl ether	Ethyl acetate	methanol
Alkaloids	Dragendorff's reagent test	+	++	+++	++++
Cardio glycosides	Kellar- Killani test	-	-	-	-
Saponin glycosides	Froth formation 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	+	++	+++	++++

DPPH (2,2- diphenyl -1- picrylhydrazyl) radical scavenging activity.

1ml plant extract was mixed with 1ml of 0.3mM DPPH reagent and left for 30 minute at room temperature in the dark. The absorbance was steady at 517nm. Radical scavenging activity was than expressed as IC_{50} Mean ± SD value (Vats et al., 2012; Pejin et al., 2013).

Nitric oxide scavenging assay (NOSA)

2ml Sodium nitroprusside (10mM) in 0.5ml of phosphate buffer saline (1M; pH 7.4) was mixed with 0.5ml of extract and was left to stand at 25°C for 150 min. Then from the incubated mixture 0.5ml was taken and mixed with 1.0ml of sulfanilic acid reagent. Finally, 1.0ml of 0.1% napthylethylenediamine dihydrochloride was mixed and again kept for incubation for 30 min at room temperature. The absorbance was steady at 540nm. NOSA was than expressed as IC_{50} (µg/ml) from method of (Badami et al., 2003).

Statistical analysis

The results were obtained are given as mean (n=3) in triplicates. The date which are obtained were done by software IBM SPSS Statistics 20. The three way interaction was executed between the chosen variable. Turkey's $p \leq 0.05$ post test was carry out to compare the variance of data. Every data obtained in this study was offered as means ± standard error.

RESULTS

Preliminary phytochemical tests were performed using different tests (Table 1), the presence of alkaloids, flavonoids, carbohydrates, steroids and many more phytochemicals using different solvents. In which the higher yield was obtained in methanol whereas lower yield was obtained in *n*-Hexane.

DISCUSSION

TPC and TFC

Phenols are the usual phytoconstituents in plants, and their constituents have antioxidant properties. Antioxidant capacity of the phenolic compounds have been studied extensively in the treatment of a variety of ailments, including diabetes, inflammation, neurogenerative disease, cardiac disease and cancer (Soobrattee et al.,2005). In this present study the maximum TPC was obtained using n-hexane, methanol, ethyl acetate and diethyl ether (Table 2). The higher yield (24.06 ± 0.05^{c}mg/GAE/g) was obtained with methanol and lower yield in *n*-Hexane. Whereas maximum TFC yield (42.04 ± 0.16^{c}mg/QE/g) was also obtained with methanol and lower yield in *n*-Hexane was obtained (Table 2). Hence, the current study on *H. involuta* showed more or less similar outcomes as reported earlier (Karim et al., 2014).

Antioxidant assays

DPPH is a free radical whose absorbance reduces due to a color change caused by antioxidants radical scavenging activity. NO is a signalling molecule, and excessive amounts of this free radical can disrupt metabolism, resulting in cancer, inflammation and other problems (Halliwell, 1997). In a diffusion-limited reaction, OH radical attacks biomolecules like protein, nucleic acids and polysaccharides (Chen and Schopfer, 1999).

The antioxidant activity of the plant extract as IC_{50} value was determined to be high potential in methanol 38.86±0.19 and 36.54±0.17 (µg/ml; Mean ± SD, n=3) against DPPH and NOSA, respectively (Table 3).

Table: 2 Quantitative analysis of *Hyophila involuta* extract in four solvents

Solvents Variable	Methanol	Ethyl-acetate	Di-ethyl ether	n-Hexane
TPC	24.06±0.05[c]mg/GAE/g	20.04±0.03[b]mg/GAE/g	13.06±0.02[b]mg/GAE/g	07.02±0.01[a]mg/GAE/g
TFC	42.04 ±0.16[c]mg/QE/g	39.05 ±0.10[b]mg/QE/g	21.02 ±0.04[b]mg/QE/g	10.04 ±0.01[a]mg/QE/g

Table: 3 Antioxidant activity of *Hyophila involuta* extract in different solvents

Tests Solvents	DPPH(µg/ml)	NOSA(µg/ml)
Methanol	38.86±0.19	36.54 ±0.17
Ethyl acetate	46.04 ±0.24	41.06±0.21
Di-ethyl ether	50.26±0.29	48.04 ±0.25
n-Hexane	54.04 ±0.36	51.03 ±0.30

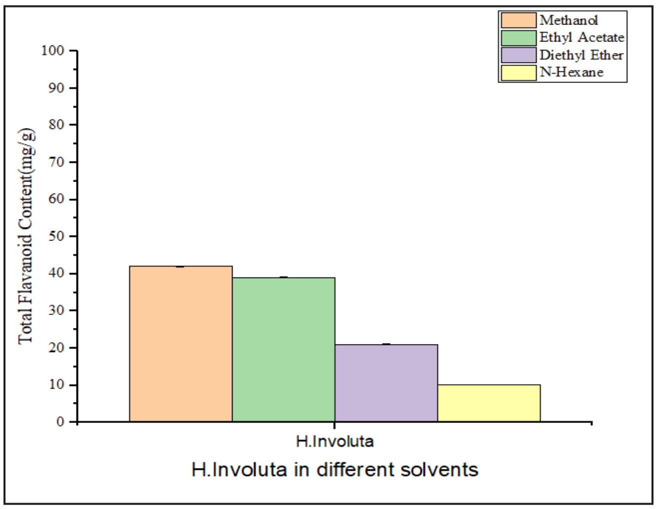

Figure 1: Total flavonoid content in different solvent

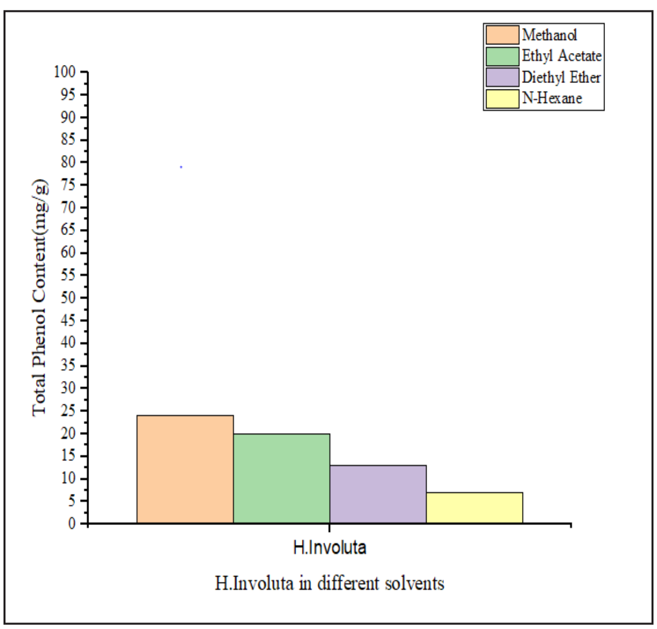

Figure 2: Total phenolic content in different solvent

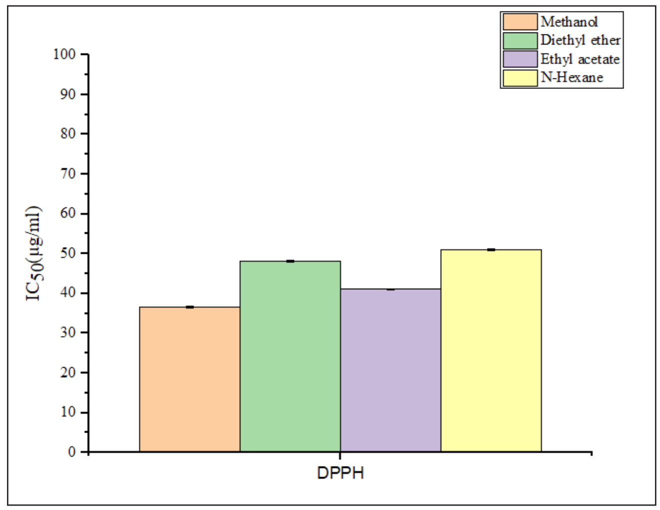

Figure 3: DPPH assay of *H.involuta* extract in different solvents

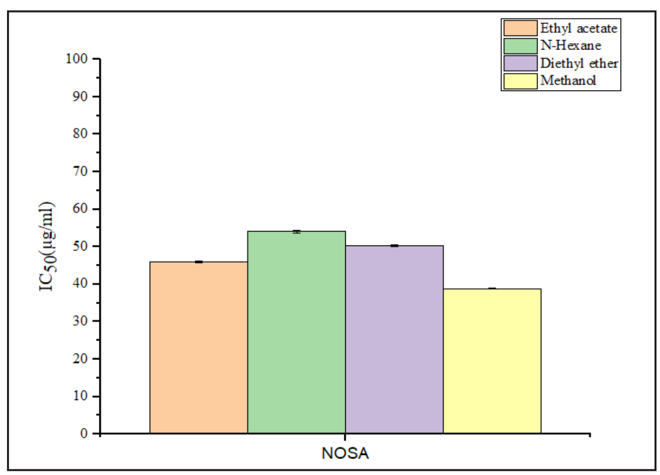

Figure 4: NOSA assay of *H.involuta* extract in different solvents

CONCLUSION

The present study reported the preliminary phytochemistry of *H. involuta* by qualitative and quantitative analysis whereas antioxidant activity was also tested by DPPH and NOSA. Considering the phytochemical profile of *Hyophila involuta* it is evident that the moss would be as a potential source for natural antioxidants and can be used in future herbal formulations to enhance the immunity to prevent the various viral infections attacks including SRAS-Covid-19. In present scenario there is a need that the huge bryo-diversity should be evaluated more and more for their phytochemistry to be used in future.

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Authors' contribution

SS, RS, SJ have contributed equally in performing experiments and preparation of first draft. GPN, SV and AA conceptualized the research and steered the work done. All the authors have finally read the manuscript and approved.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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