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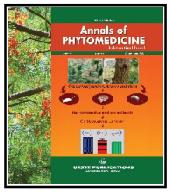

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Phytochemical profiling and bioactive potential of *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph., a thalloid liverwort

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Abstract

The SARS-C0V-2 pandemic is causing mayhem on people all over the world. Although, immunization is progressing quickly, its effectiveness against new variants is unknown. The virus has proven to be exceedingly resistant to treatments, and no drugs have been demonstrated to be totally effective against SARS-COV-2 antiviral. However, a few vaccines have been produced, but best option for now is to adopt preventive steps for now and future as well. In general, the use of herbs is emerging as the best ploy among all preventive measures to enhance the immunity as they have great antiviral potential and antioxidant properties. Bryophytes especially (liverworts) are well known to contain a variety of potentially beneficial compounds such as terpenoids, quinones, phenylpropanoids, flavonoids, *etc*. In recent past, different liverwort extracts and isolated chemicals have demonstrated antibacterial, antiviral, and cytotoxic effects; highlighting the potential of liverworts as herbal treatments and chemical manufacture for application in a variety of goods. However, due to their small size, difficulties in collection, and identification, the bulk of liverworts remain unknown, particularly in India. In this light, an attempt has been made to screen a commonly growing liverwort, *Plagiochasma rupestre* (J.R. Forst. and G. Forst.) Steph. has been evaluated for its phytochemical profile and bioactivity.

1. Introduction

After Angiosperms, the oldest and the second most diverse category of terrestrial plants is “Bryophytes” (Asakawa *et al*., 2017). Though, the Bryophytes hold the most integral part of our biodiversity yet, these terrestrial plant group has received much lesser attention on the status of their conservation (Dulger *et al*., 2005; Ilhan *et al*., 2006; Ojo *et al*., 2007; Alam, 2021) have got equal importances in ICUN system (Cleavitt *et al*., 2005). Ecologically bryophytes perform as a cushion for other vascular plants by conserving water. In humid environments, these morphologically simpler creatures are almost ubiquitous and proliferate in colonies, generating mats. Bryophytes are also referred to as amphibians as they prefer to grow, where water availability is ample (Whitehead *et al*., 2018). They are classified as cryptogams due to the absence of blooms and seeds (Harris *et al*., 2008). Their beneficial qualities are ignored and go unnoticed due to high level of ignorance. However, they can be used as radioactivity indicators, bioindicators of air pollution due to heavy metals, pesticides, erosion control and genetic engineering (Glime *et al*., 2007; Mishra *et al*., 2014). Ethnotherapeutically, this group has been a blessing in the medicinal world, supplying information about clinically active medications derived from indigenous lower plants that are valuable to humans (Shirsat *et al*., 2008).

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However, till now under 10% bryophyte species have been evaluated for their phytochemical profiles (Asakawa *et al*., 2004; Saini *et al*., 2021). Based on the study conducted on these plants, it is established that they contain incalculable value of bioactive chemicals. These valuable bioactive secondary metabolites have exhibited a wide spectrum of biological actions, encompassing antibacterial, and antitumor, cytotoxic, cardioprotective, allergy provoking, antiviral and so forth (Asakawa *et al*., 2017). The SARS-CO-2 pandemic is wreaking havoc around the world. Although, vaccination is progressing at a breakneck pace, its effectiveness against emerging variations is unclear. The virus has turned out be exceptionally robust to therapies, and no medications have been shown to be completely effective against antivirals against SARS-COV-2. Beside, vaccinations have the preferred choice for present and in the future is to adopt preventive precautions. In general, the usage of herbs is emerging as the best suited and viable strategy among all preventive methods to boost immunity due to their high antiviral and antioxidant capabilities (Saini *et al*., 2021).

There are reports on bryophytes, in particular (liverworts), have a high concentration of potentially beneficial compounds such as, phenolic byproducts, flavonoids, acetogenins, lipids and few of the nitrogen containing aromatic compounds and alkaloids and terpenoids (Noda *et al*., 1997; Asakawa *et al*., 2017; Marques *et al*., 2021). It has also been discovered that the antioxidant properties of some liverworts are like that of vascular plants (Sharma *et al*., 2015). Researchers from pharmaceutical and nutraceuticals industries are interested in the antioxidant and free radical scavenging activities of plants. Free radicals are thought to be important in the etiology of many illnesses (Campian *et al*., 2007). Drug and food stability may be reduced as a result of oxidation process. Under both abiotic and

biotic stress ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species) have been identified as critical indications of stress signal cascades (Wojtaszek *et al*., 1997; Haddad *et al*., 2002). Increasing diseases incidences as a result of mounting pollution on the planet need the usage of natural therapeutic antioxidants as consistent dietetic supplements in order to provide better and more effective healthcare (Gechev *et al*., 2006).

Numerous plants, specifically bryophytes, have been reported to have antiviral actions. Remarkably, there is no proof available till date regarding virus infectivity to any bryophytes, revealing that bryophytes have a tight defense reply in the form of their chemical defense (Hirata *et al*., 2000). In this light, an attempt was undertaken to screen the phytochemical compositions and bioactivity of *Plagiochasma rupestre* (J.R. Forst. & G. Forst.) Steph., a routinely growing liverwort as a possible source of useful pytocompounds for future.

2. Materials and Methods

2.1 Collection and identification of plant materials

The fresh thalli of *P. rupestre* were collected from Mount Abu, western Rajasthan at an altitude of 1600 m, 24º31´to 24º43´N and 72º38´ to 72º53´E in September 2021. The taxonomic and ethnobotanical data of the reference specimen were placed in the Banasthali University Rajasthan India (BURI) Herbarium.

2.2 Washing and extraction of sample

Samples were scrutinized to ensure that contaminants such as soil and other plant matter were not present. Plant thalli were then properly washed with running tap water, air dried at room temperature to eliminate any remaining water, and then ground to a fine powder. Extraction was done using the method of Velioglu *et al*. (1998). Different solvents were used for extraction of sample (N-hexane, diethyl ether, methanol and ethyl acetate) (Asakawa *et al*., 2013; 2017) Using a mortar and pestle, the mixture was then placed in an orbital shaker (Metrex-100C-37º C; 120 rpm) for 48 h. Following that, each extract was centrifuged for 30 min at 10,000 rpm, and the supernatant was collected and stored at 4ºC for future use.

2.3 Phytochemical screening

Several chemical assays were performed on all solvent extracts to spot the occurrence of various phytochemicals, *viz*., glycosides, terpenes, alkaloids, polysaccharides, saponins, flavonoids, phenolics, steroids, and lipids. Chemical assays were performed on *P. rupestre* extracts in methanol, diethyl ether, ethyl acetate, and N-hexane to identify different chemicals using standard techniques with minor changes specified by Mitra *et al*. (2019).

2.4 Phenolic content in total

Slightly modified methods described by Adebiyl *et al*. (2012) and Bakar *et al*. (2015) were used to calculate the total phenolic content (TPC). Gallic acid is used as a standard to calculate the total phenols present. The extracts were combined with 5 ml of distilled water and 0.5 ml of 50% Folin Ciocalteu reagent, and after 10 m, 1 ml of 5% sodium carbonate was added. After 120 min of incubation, the absorbance at 720 nm of the test tubes was measured. The outcome was given in milligrams of gallic acid equivalents (GAE) per gram of dry sample weight.

2.5 Flavonoid content in total

The aluminium chloride procedure was used to calculate the total flavonoid content (Adebiyl *et al*., 2012; Vats *et al*., 2016). In that order, the extract was treated with ethanol (95%), $AlCl_3$ (10%), potassium acetate (1 M), and distilled water. The absorbance at 415 nm was measured after 35 min of incubation at room temperature. The results were expressed in terms of quercetin equivalents. (QE milligrams per gram dry weight of sample).

3. Evaluation of antioxidant activity

3.1 Free radical scavenging activity assay

The plant extract's antioxidant activity was quantified in terms of their ability to scavenge, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Blois *et al*., 1958; Chandra *et al*., 2014). 3 ml volume of DPPH solution was mixed with 0.2 ml of each solvent extract and the mixture was incubated at room temperature for 30 min. At 517 nm absorbance was measured. Without any plant extract, DPPH was considered as control. Propyl gallate and ascorbic acid functioned as reference. DPPH scavenging activity percentage was calculated using the following formula:

%DPPH scavenging activity = [(Control absorbance-Sample absorbance) x 100] / Control absorbance

The inhibitory values for various crude extract concentrations were computed. The radical scavenging activity was expressed as an IC_{50} value, which is the concentration of sample extract required to scavenge 50% of the DPPH radical.

4. Nitric oxide scavenging assay (NOSA)

2 ml of sodium nitroprusside in 0.5 ml of phosphate buffer saline was combined with 0.5 ml of extract and incubated for 160 min at 25ºC. 0.5 ml of the incubated mixture was taken and mixed with 1ml of sulfanilic acid. Finally, 1 ml of napthylethylenediamine dihydrochloride was mixed and allowed to stand for 15 min before measuring absorbance at 540 nm. NOSA was assessed in triplicate using (Chobot *et al*., 2006) method and expressed as IC_{50} (µg/ml).

5. Statistical analysis

The results are shown as means (n=3) of three replicates. The IBM SPSS Statistics 20 software was used to analyze all the collected data. Three-way interactions between the variables were achieved. Multiple comparisons are performed for every output variable. To compare data variances, Turkey's $p \leq 0.05$ posttest was used. All data is presented as mean ± standard error.

6. Results

6.1 Phytochemical profiling

Preliminary phytochemical profiling of numerous extracts, including diethyl ether, methanol, ethyl acetate, and N-hexane, was performed to analyze several phytoconstituents, including saponins, alkaloids, flavonoids, polysaccharides, glycosides, steroids, terpenes, phenolics, and lipids. Methanolic extract of *P. rupestre* (Table 1) revealed the presence of flavonoids, alkaloids, carbohydrate, phenols, terpenes, steroid, glycosides, and lipids, but not saponins. N-hexane extract, on the other hand, revealed the presence of phenols, carbohydrates, lipids, and terpenes, but not alkaloids, flavonoids, saponins, steroids,

or glycosides. Furthermore, flavonoids, glucose, phenol, terpenes, and lipids were found in a diethyl ether extract of the same plant, but no alkaloids, saponins, glycosides, or steroids were found. However, the ethyl acetate extract of the examined bryophyte, on the other hand, contained only carbohydrate terpenes and lipids.

Table 1: Qualitative analysis of *P. rupestre* four different solvents

Phytochemicals	Diethyl ether	Methanol	N-hexane	Ethyl acetate
Flavonoids				
NaOH test	+	+	-	-
Shinoda test	-	+	-	_
H_2SO_4 test	+	++	-	-
Carbohydrate				
Fehling solution test	+	+	+	+
Saponins				
Foam test	-	-	-	-
Alkaloids				
Dragendroff's test	-	+	-	-
Mayer's test	-	+	-	-
Hager test	-	+	-	-
Terpenes				
Salkowski's test	+	++	+	+
Steroids				
Libermann-Bruchard test	-	+	-	-
Phenolics				
$FeCl_3$ test	+	++	+	-
Lead acetate test	-	++	+	-
Glycosides				
Borntragor's test	-	-	-	-
Lipids				
AACC approved method	+	+	+	+

Table 2: Total phenolic content of *P. rupestre* in different solvents

Diethyl ether	Methanol	Ethyl acetate	N-hexane
43.04 ± 0.36^b	59.4 ± 0.81^c	50.02 ± 0.62^b	22.02 ± 0.20^a
mg/g/GAE	mg/g/GAE	mg/g/GAE	mg/g/GAE

The data are the means and standard deviations of n=3 separate experiments.

Table 3: Total flavonoid content of *P. rupestre* in different solvents

Diethyl ether	Methanol	Ethyl acetate	N-hexane
35.02 ± 0.29^b	50.04 ± 0.48^c	41.02 ± 0.36^b	18.04 ± 0.19^a
mg/g/QE	mg/gQE	mg/g/QE	mg/g/QE

The data are the means and standard deviations of n=3 separate experiments.

Table 4: Antioxidant potential of *P. rupestre* in different solvents

Solvents	DPPH	NOSA
Diethyl ether	47.08 ± 0.24 µg/ml	50.06 ± 0.26 µg/ml
Methanol	37.04 ± 0.19 µg/ml	39.01 ± 0.21 µg/ml
Ethyl acetate	41.04 ± 0.21 µg/ml	45.04 ± 0.23 µg/ml
N-hexane	52.01 ± 0.37 µg/ml	56.02 ± 0.39 µg/ml

Values are expressed as mean ± SD

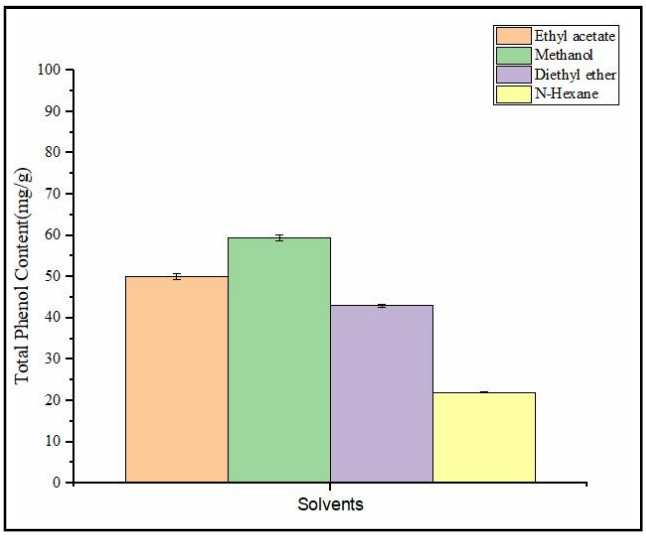

Figure 1: Total phenolic content of *P. rupestre* extract in different solvents.

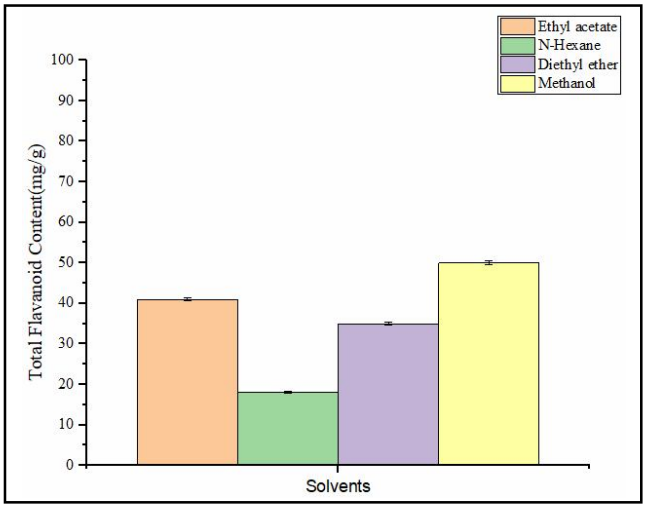

Figure 2: Total flavonoid content of *P. rupestre* extract in different solvents.

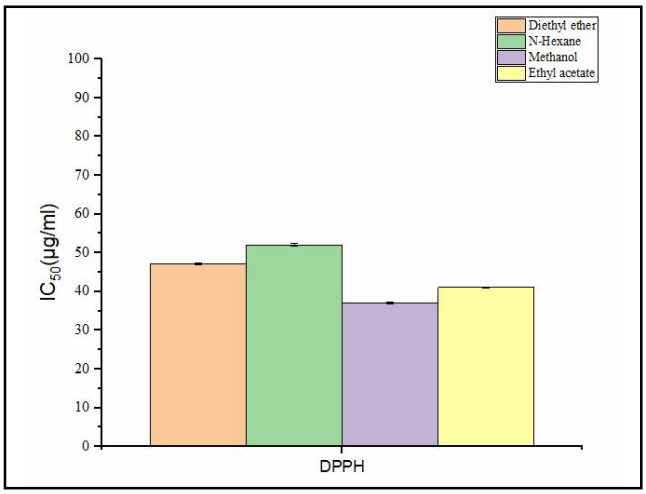

Figure 3: Free radical scavenging activity assay (DPPH) of *P. rupestre* extract in different solvents.

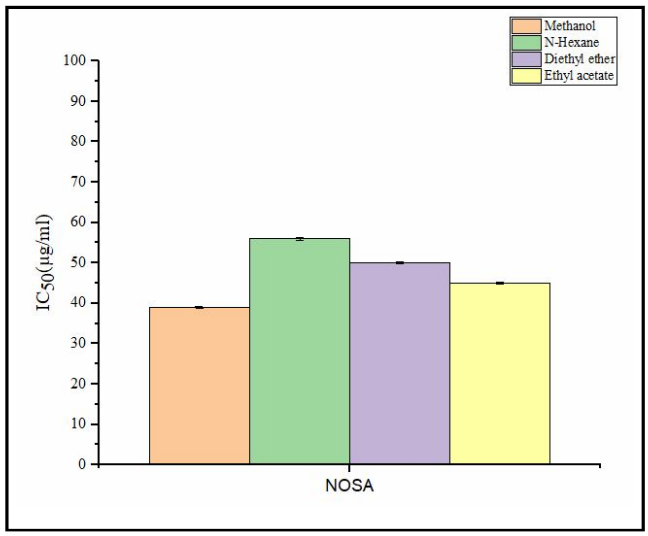

Figure 4: Nitric oxide scavenging assay of *P. rupestre* extract in different solvents.

7. Discussion

Total phenolic content and total flavonoid content of *P. rupestre* was expressed as gallic acid and quercetin, respectively. TPC and TFC of extract in different solvents is shown the highest TPC and TFC of *P. rupestre* were measured in the polar solvent methanol, which was then followed by ethyl acetate. However, the TPC and TFC of ethyl acetate were significantly lower than those of methanol extract (Tables 2, 3; Figures. 1, 2). Due to the polarity of the solvents, the N-hexane extract produced minimal results. As a result, methanolic extract was preferred for further processing. Methanol, on the other hand is an excellent solvent for the extraction of polar antioxidants. The greater total phenolic content levels indicate that it has good antioxidant potential. Plant phenolic compounds are assumed to be responsible for significant free radical scavenging activities; this free radical scavenging activity is linked to their redox properties, which allow them to operate as antioxidants.

DPPH is a free radical and its absorbance reduces as the color changes from purple to yellow due to the antioxidant's radical scavenging activity. NO is a signaling molecule that serves as a vasodilator, neural messenger and so forth. Over production of this free radical has a negative impact on metabolism and may lead to inflammation, cancer and other health problems. NO can cause the formation of hydroxyl radicals and nitric oxide (Halliwell *et al*., 1997). In diffusion limited process, OH radical damages biomolecules such as protein, nucleic acid and polysaccharides. Stress generates hydroxyl radicals, which aggravate a variety of illness (Chen *et al*., 1999). In present study, the methanolic extract of *P. rupestre* demonstrated more antioxidant potential than the other solvents for both the assays, *i.e*., DPPH and NOSA as shown in (Table 4 and Figures 3 and 4) which is justified by greater levels of phenols and flavonoids in the methanolic extract.

8. Conclusion

Though, the angiosperms is most diversified group of plant which have so many herbs that have medicinal and healing properties (Alam, 2019), the liverworts have also more or less similar potential to be used in effective medicines due to their distinctive thallus organization

and phytochemical profile. Because they live everywhere and can develop without any special requirements, they are easily available at a minimal cost and can be used without causing any harm to the human body. As a result, they can be leveraged as a reservoir of beneficial bioactive compounds particularly (phenols and flavonoids) which are found in all plants and compounds with antioxidant potential, to create natural medicines and nutraceuticals with antiviral properties. Antioxidants found naturally in liverworts, have the ability to improve human immunity against a variety of viruses, including Covid-19, by extinguishing free radicals and protecting cell health. The increasing prevalence of viral diseases necessitates the use of natural therapeutic antioxidants dependable salubrious complex for providing improved and effective healthcare, and the extracts of *P. rupestre* in the current study demonstrated significant potential as an antioxidants agent with high phytoconstituents and can benefit the pharmaceutical industries.

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Authors' contribution

RS, SS, 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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