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ORIGINAL ARTICLE **OPEN ACCESS**

Pharmacognostical and antioxidant evaluation of liverwort *Marchantia paleacea* from Kumaun region of Uttarakhand

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ABSTRACT
The present study aims to conduct a comprehensive pharmacognostical evaluation of Marchantia paleacea, a liverwort species obtained from the Kumaun region of Uttarakhand. Marchantia paleacea is known for its traditional medicinal uses in the local communities all over the world, Marchantia paleacea is a well-established hepatoprotective, antimicrobial and anti-oxidant plant Marchantia paleacea serves as source of natural antioxidant like phenols and flavonoids. These anti-oxidant phytochemicals which have demonstrated their ability to minimise some acute & chronic ailments like cancer, heart disease & stroke. This study provides valuable insights into its, macroscopic, microscopic, and physicochemical characteristics. The evaluation involved the collection and identification of Marchantia paleacea sample. Special attention was given to the identification and characterization of diagnostic features, such as cell types, oil glands, etc. Physicochemical analyses were performed to determine the foaming index, ash value, extractive values, etc. Preliminary phytochemical screening was conducted to identify the presence of various secondary metabolites, including alkaloids, flavonoids, phenols, terpenoids, and saponins. This article also focuses on the antioxidant properties of Marchantia paleacea. In-vitro antioxidant activity was studied using DPPH, Nitric Oxide, and Hydrogen peroxide methods.Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defence system, plays a crucial role in the development and progression of cardiovascular diseases. The findings of this study contribute to the understanding of Marchantia paleacea's pharmacognostical profile and its antioxidant potential it also provides a foundation for further research on its therapeutic potential.
Keywords: *Marchantiapaleacea, pharmacognosy, physicochemical analysis.antioxidant activity.*

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INTRODUCTION

Marchantia a common liverwort found all over the world. Extensive work has been carried out on genus *Marchantia* over the past few years. Species related to *Marchantia* have been described in ancient Greek texts stating it as a useful plant for application on open wounds as it prevented inflammation and infection *Marchantia* is a representative of an ancient lineage of land plants that colonized our planet millions of years ago hence becoming an important plant in the field of genetics **(**1, 2**).** *Marchantia paleacea* is a well-established hepato-protective, antimicrobial and anti-oxidant plant. *Marchantia paleacea* serves as source of natural antioxidant like phenols and flavonoids **(**3, 4) These anti-oxidant phytochemicals which have demonstrated their ability to minimise some acute & chronic ailments like cancer, heart disease & stroke (5).

Fig 1- *Marchantia paleacea*

Plant Description

Marchantiales are hepaticae in which the thallus is composed of several distinct layer of tissue, of which the upper most, the chlorophyll – bearing layer, nearly always enclosed air chambers, which have communication with the exterior through pores. The rhizoids are of two kinds, smooth and tuberculated. The sex organs are generally united in replicates often borne on long stalked archegoniophores respectively. (6) The genus marchantia is distributed all over the world including about 65 species and out of these 11 species have been reported to be present in India. (7) These species include *M. emarginata*, *M. assamica*, *M. paleacea*, *M. subintegra*, *M. polymorpha*, *M. robusta*, *M. linearis*, *M. pandei*, *M. papillata* subsp. *grossibarba*, *M. hartlessiana* and *M. gemminata.*(8).

Aim and Objective

The aim of the study was to evaluate the pharmacognostical parameters and antioxidant activity of *Marchantiapaleacea* collected from the Kumaun region of Uttarakhand.

MATERIAL AND METHODS

Sample collection and Identification

Marchantia was collected during the month of July-August from Dholchena and adjoining area of district Almora Uttarakhand and Identified as *Marchantiapaleacea* by Dr. A.K. Asthana Principal Scientist at Bryology lab National Botanical Research Institute (CSIR-NBRI), Lucknow Uttar Pradesh.

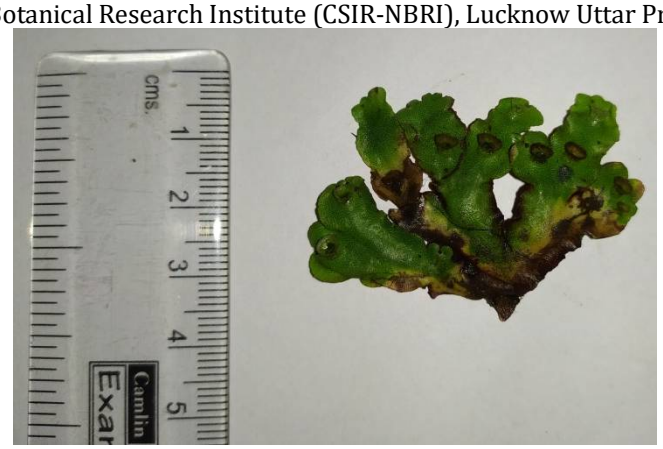


Figure 2- *Marchantia paleacea thallus*

Macroscopy and Microscopy: Visual inspection of sample is a rapid method for identification of the sample. Macroscopic evaluation is based on shape, size, colour, odour /taste, and other surface characters. (9)For microscopic evaluation of plant, studies were conducted using Leica Microscope. Leica ICX50E Microscope with attached camera was used for the studies.

Plant Processing : The collected plant material was cleared of adhered soil and foreign matter, washed and dried in shade. The dried material was powdered using a grinder and stored in an airtight container. The powdered drug was macerated with 80% ethanol at room temperature and then filtered. The filtrate was collected and evaporated using a rotavap. The remaining extract was dried over water-bath.

Physiochemical and Preliminary Phytochemical studies

Physiochemical studies were conducted by official methods described according to the WHO guidelines(WHO 1998) on quality control methods for medicinal plants materials. Preliminary Phytochemical screening was carried out using standard procedures described by Kokate. (10)Various tests for carbohydrates, alkaloid, tannins, phenols saponins, proteins and amino acids were performed.

Estimation of Antioxidant activity

The antioxidant activity of the sample was determined using 3 different methods

a. Nitric oxide method
b. Hydrogen peroxide method and
c. DPPH Method

Nitric oxide method: Nitric oxide scavenging activity was estimated by the using Griess Ilosvay reaction. Sodium nitroprusside decomposes at pH 7.2 producing nitric oxide (NO). NO reacts with oxygen to produce nitrate and nitrite. The quantities of which can be determined using Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitrite ions.

Procedure : Sodium nitroprusside (10mM) in phosphate-buffered saline was mixed with different concentrations (5 - 200µg/ml) of methanol extract of the plant and was dissolved in methanol and incubated at 30 ºC for 2 hours. Same reaction mixture without the extract but the equivalent amount of ethanol served as control.

After the incubation period, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3P04, and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) was added. The absorbance was immediately read at 550 nm. Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid were calculated relative to the control. (11)

Hydrogen peroxide method: The scavenging activity of extract for hydrogen peroxide (H_2O_2) radicals was determined by the modified method of Dehpour. H_2O_2 solution (40 Mm) was prepared in phosphate buffer pH 7.4 and its concentration was determined by measuring the absorbance at 560 nm using a UV spectrophotometer. 0.1mg/ml of the extract was added to the hydrogen peroxide solution and absorbance was measured at 560 nm using a UV spectrophotometer against a blank solution containing phosphate buffer without H_2O_2. (12) The percentage of H_2O_2 scavenging by the extract and standard compound (ascorbic acid) was calculated using the given formula:

$$\textbf{Percentage scavenged } [H_2O_2] = 1\text{-}Abs_{(std)}/Abs_{(cont)} \times 100$$

Where

Abs $_{(cont)}$- Absorbance of the control (without extract) at 560nm;

Abs $_{(std)}$ - Absorbance in the presence of the extract at 560nm.

DPPH Method

Preparation of control: A solution of 0.1 mM DPPH was freshly prepared in methanol. Then 5 ml of freshly prepared solution was added to 5 ml of methanol and incubated in dark for 30 min at room temperature. Then After 30 min, the absorbance was recorded at 517 nm using a UV/VIS spectrophotometer against the blank, i.e., methanol.

Preparation of Standard /Test solution: 5 mg of Rutin was dissolved in 5 ml of methanol to make a stock solution of 1000 µg/ml. A similar procedure was carried out for the test sample. Using serial dilution different concentrations (5, 10, 20, 40, 80, and 160 µg/ml) were prepared with methanol. Equal volumes from these different concentrations of standard/test were taken, added to a methanolic solution of DPPH, and incubated in dark for 30 min at ambient room temperature. Then after 30 min, the absorbance was taken at 517 nm using a UV/VIS spectrophotometer against blank.

Percentage scavenging activity was calculated using the formula:

$$\textbf{\% Radical Scavenging Power} = [A_C\text{-}(A_S\text{-}A_O)]/A_C \times 100$$

Where

A_C = Absorbance of control (DPPH);

A_S = Absorbance of sample / standard + DPPH

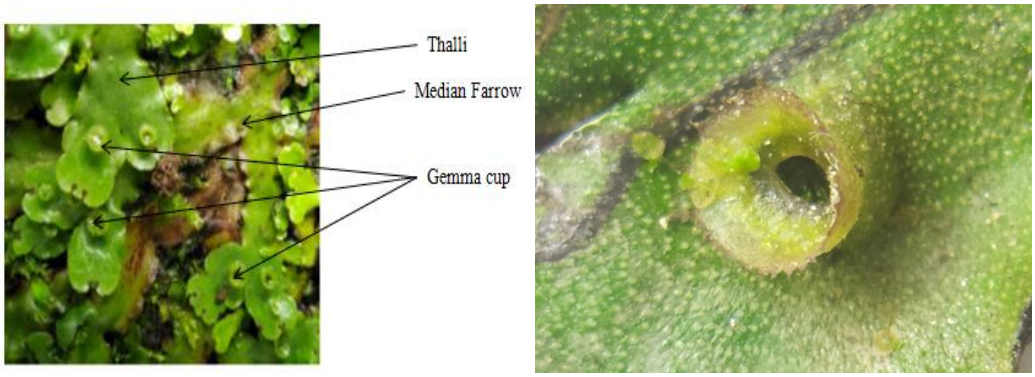
A_O = Absorbance of sample/standard without DPPH interaction.

The scavenging effect was calculated based on the percentage of DPPH scavenged. IC_{50} values of the samples for antioxidant activity were calculated using a standard curve of rutin. (13)

RESULTS

Macroscopy and Microscopy

On macroscopic examination of the drug thallus appeared to be dark green and was dichotomously branched. It was 4-6 cm long, flattned and ribbon like, the thalli had a distinct midrib represented by a furrow (Median furrow). There are cup like structures present on the dorsal surface called the “Gemma Cups”. These gemma cups contain vegetative propagules of marchantia called “gemmae”. The ventral surface contains rhizoids at the midrib region.



a **b.**

Figure No. 3. a- Macroscopy of *Marchantiapaleacea,* b- close-up of a gemma cup

Study of the transverse section showed that the section can be broadly divided in two regions, the upper photosynthetic region and; the lower storage region. The photosynthetic region divided into distinct air chambers (photosynthetic chambers) each containing photosynthetic filament. The upper layer of the chamber comprises of the upper epidermis with barrel shaped air-pore present in it, the storage region is composed of thin-walled cells containing starch grains. The lower epidermis forms the boundary for the storage region. The lower portions of the epidermis contain the rhizoids near the midrib region of the thallus.

On visual inspection of the powder, it was observed that the powder was dark brownish-green with a characteristic odour. On microscopic examination of the powder- starch grains, calcium crystals were observed.

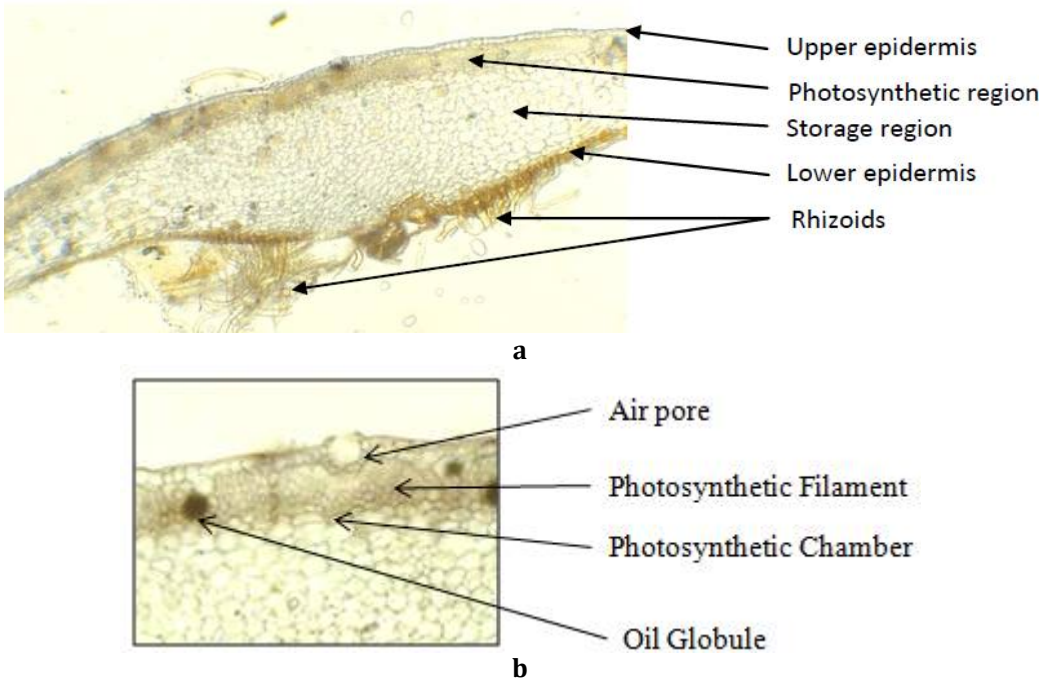


Figure No 4. a - TS of *Marchantiapaleacea* showing various regions. **b**- Close up of photosynthetic chamber showing an air-pore and oil globule.

Figure no.5 Powder Microscopy- a Calcium Crystals, **b-** Starch grain, c- Smooth walled rhizoid

Preliminary Phytochemical Screening

Preliminary Phytochemical Screening of the hydro-alcoholic extract reviled the presence of Carbohydrates, Starch, Proteins, Tannins and Phenolic compounds.

Table No 1. Preliminary Phytochemicals of *Marchantiapaleacea*

Phytochemical	**Result**
Carbohydrate	Positive
Protein	Positive
Alkaloids	Negative
Tannins	Positive
Phenolics	Positive
Saponins	Negative
Starch	Positive

Physicochemical Constants

The Physicochemical parameters are presented in Table No. 2, such as water soluble extractive value was 7mg/g (0.7 %) and alcohol soluble extractive value was 15 mg/g (1.5 %). Total ash was 19.5%, water soluble ash 6.83%, acid insoluble ash 7.66%. (Table No.2,)

Table No 2. Physicochemical parameters of *Marchantiapaleacea*

Parameter	Value
Water Soluble Ext.	10.7%
Alcohol Soluble Ext.	15.52%
Total Ash	19.5%
Acid Insoluble Ash	7.66%
Water Soluble Ash	6.83%
Foaming Index	Below 100

Determination of Antioxidant Activity

Nitric oxide method

- The scavenging activity of *Marchantia* extract for Nitric Oxideradicals was determined for different concentrations of the *Marchantia paleacea* extract (test) using ascorbic acid as standard. The results are expressed as percentage inhibition (%) in Table No. 3. represents the Percentage inhibition of NOradicals scavengingactivity of *Marchantia paleacea* extract and ascorbic acid. The IC_{50} value of the extract was calculated to be 45.51µg/ml.

Table No: 3 Antioxidant Activity of *Marchantia paleacea* extract Nitric Oxide Method

Conc (mg/ml)	% Inhibition Std ±SD	% Inhibition Test ±SD
10	43.25±0.321	39.41±0.119
20	46.5±0.145	40.34±0.101
40	50.12±.0201	44.21±0.214
80	57.69±0.125	48.65±0.108
160	71.01±0.114	58.42±0.224
320	95.64±0.214	75.64±0.785

Hydrogen Peroxide method

The scavenging activity of extract for H_2O_2 radicals was determined for different concentrations of the *Marchantia paleacea* extract using ascorbic acid as standard. The results are expressed as percentage inhibition (%) in Table No. 4 and represents the Percentage inhibition of H_2O_2 radicals scavenging activity of *Marchantia paleacea* extract and ascorbic acid. The IC_{50} value of the extract was calculated to be 1.41µg/ml.

Table No: 4 Antioxidant Activity of *Marchantia paleacea* extract Hydrogen peroxide Method

Conc (mg/ml)	% Inhibition Std ±SD	% Inhibition Test ±SD
0.0625	18.64 ± 0.215	11.99 ± 0.214
0.125	21.20 ± 0.112	13.54 ± 0.235
0.25	28.65 ± 0.135	18.60 ± 0.128
0.5	38.10 ± 0.254	25.60 ± 0.325
1	58.25 ± 0.425	40.24 ± 0.240
2	93.44 ± 0.128	65.58 ± 0.458

DPPH method

The free radical scavenging activity of different concentrations of *Marchantia paleacea* extract on the DPPH free radical was compared with the standard anti-oxidant, Rutin. The results were expressed as percentage inhibition (%) in Table No. 5. And represents the Percentage inhibition of DPPH free radical scavengingactivity of *Marchantia paleacea* extract and Rutin at 517nm. The extract showed a dose-dependent scavenging activity. The IC_{50} value of the extract was calculated to be 57.92µg/ml.

Table No: 5 Antioxidant Activity of *Marchantia paleacea* extract DPPH Method

Conc (mg/ml)	% Inhibition Std ±SD	% Inhibition Test ±SD
5	27.50 ± 0.214	26.45 ± 0.128
10	40.25 ± 0.110	28.98 ± 0.245
20	55.64 ± 0.214	35.46 ± 0.325
40	69.40 ± 0.115	42.58 ± 0.458
80	80.45 ± 0.119	60.33 ± 0.125
160	91.64 ± 0.321	92.45 ± 0.654

DISCUSSION

Standardization plays a crucial role in ensuring the identity, purity, safety, and quality of herbal products. It serves as an essential tool in assessing and maintaining the consistency of herbs and their preparations. Various techniques, including both macroscopic and microscopic methods, are employed in the standardization process (14, 15). The macroscopic examination of *Marchantia paleacea* provided important insights into the external features and overall morphology of the plant. The observed characteristics, such as the dichotomously branched thallus, flattened and ribbon-like shape, and the presence of a distinct midrib represented by a furrow, are consistent with the known macroscopic features of *Marchantia paleacea*. Additionally, the cup-like structures known as gemma cups, containing gemmae, were observed on the dorsal surface, and the presence of rhizoids on the ventral surface at the midrib region was noted. These macroscopic features play a crucial role in the accurate identification and authentication of *Marchantia paleacea*, ensuring its proper classification and differentiation from other plant species.

The study of the transverse section provided further insights into the internal structure of *Marchantia paleacea*. The section displayed two distinct regions: the upper photosynthetic region and the lower storage region. The photosynthetic region consisted of air chambers, each housing photosynthetic filaments, while the upper epidermis exhibited air-pores. On the other hand, the storage region was composed of thin-walled cells containing starch grains. The lower epidermis acted as the boundary for the storage region and was found to contain rhizoids near the midrib region of the thallus. The microscopic examination of the powder confirmed the presence of starch grains and calcium crystals, which are integral components contributing to the overall composition of the plant material and may have impact on its medicinal and therapeutic properties. These microscopic features provide valuable information about the anatomical structure and organization of *Marchantia paleacea*, furthering our understanding of its pharmacognostical characteristics.

Heart disease remains a leading cause of mortality worldwide. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defence system, plays a crucial role in the development and progression of cardiovascular diseases. In the quest to prevent heart disease, research community has turned their attention to polyphenols and flavonoids, two classes of antioxidants found in various plant-based foods. Polyphenols and flavonoids exhibit potent antioxidant properties, primarily attributed to their ability to scavenge free radicals and reduce oxidative stress. These compounds also possess anti-inflammatory, anti-platelet, and vasodilatory effects, which contribute to their cardiovascular benefits (16).

Studies have consistently demonstrated the beneficial effects of polyphenols and flavonoids on heart health. In a study it was found that higher dietary intake of flavonoids was associated with a reduced risk of coronary heart disease and mortality. (17) In another study conducted in 2008 found that increased consumption of flavonoid-rich foods was associated with a significant reduction in the risk of cardiovascular events (18). *Marchantia paleacea* serves as source of natural antioxidant like phenols and flavonoids. These anti-oxidant phytochemicals which have demonstrated their ability to minimise some acute & chronic ailments like cancer, heart disease & stroke (5).Polyphenols and flavonoids present in various plant-based foods have emerged as powerful agents for heart disease prevention. Their antioxidant and anti-inflammatory properties, along with other cardiovascular benefits, contribute to their overall positive impact.

CONCLUSION

In conclusion, this pharmacognostical evaluation of *Marchantia paleacea* provides valuable information on its pharmacognostical, microscopic, and physicochemical characteristics. However, further research is required to explore the specific effects of *Marchantia paleacea* and its bioactive constituents on cardiovascular health, paving the way for the development of novel cardio protective drugs. The study

highlights the medicinal potential of this liverwort species and lays the groundwork for future studies focused on its therapeutic applications.

ACKNOWLEDGEMENT
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CONFLICT OF INTEREST
None

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