



Antimicrobial and antioxidant phytochemicals in thallus extract of *Marchantia paleacea*: An amphibian herb of medicinal value

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ABSTRACT

Phenols and flavonoids present in *Marchantia paleacea* extract have been analysed for their involvement in antimicrobial activities associated to the plant. Literature provides ample leads of the potential utilisation of phenols and flavonoids as potent antimicrobials. Many researchers have deciphered the role of quercetin as an antimicrobial agent. Extract was prepared in 80 % ethanol by soxhlet extraction. The antimicrobial activity was tested using disc diffusion assay against *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* along with the determination of minimum inhibitory concentration (MIC). The study reflects that *Marchantia paleacea* extract shows promising antimicrobial activity against *Salmonella typhimurium* and *Staphylococcus aureus*.

Keywords: *Marchantia paleacea*, phytochemicals; antimicrobials; flavonoid, phenol antioxidants.

INTRODUCTION

Natural products derived from plants and their derivatives are recognized for their significance in pharmaceutical industry. Discovery of new antimicrobials agents from plant origin, as dependable source of antibiotics, has been attracting the attention of the scientific community over the past few decades. Medicinal plant extracts have been recognised for pharmacological activities due to their bioactive phytochemicals, and such phytochemicals require the attention and systematic investigation for product development [1,2,3].

Marchantia Sp. also known as an amphibian of the plant world is a common liverwort that is found in moist shady places all over the world. Extensive work has been carried out on the genus *Marchantia* over the past few years. [4,5] 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. *Marchantia paleacea* is a common representative of genus *Marchantia* and is found all over the world [6,7].

MATERIAL AND METHODS

The dried plant sample was extracted using soxhlet extraction process and 80% hydro-alcoholic extract was prepared (MPE). The prepared extract was then used for further studies. Flavonoid and phenol content of MPE was carried out using method prescribed by Kumar et.al 2014 [8].

Flavonoid estimation: 20 mg of quercetin was weighed accurately and dissolved in 20 ml methanol, and volume was made upto 100 ml with methanol to obtain stock solution (200 µg/ml concentration). Different dilutions of quercetin (30, 60, 90, 120, 150 or 180 µg/ml) were prepared of stock soln. using serially dilution method. To 0.5 ml aliquot of each dilution, 1.5 ml of 95% methanol, 0.1 ml of aluminum chloride (10%), 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water were added and then incubated at room temperature for 30 mins. The absorbance of the reaction mixture was measured at 415 nm at UV/VIS spectrophotometer. Test solution was prepared in similar manner. To prepare blank AlCl₃ was substituted with the same amount of distilled water [8].

Estimation of total phenol content: 20 mg of gallic acid was accurately weighed and placed in a 100 ml volumetric flask. It was then dissolved in a 20 ml of 50% v/v methanol and the final volume was made up to 100 ml with 50% methanol to obtain a stock solution of gallic acid with concentration 200 µg/ml. Different dilutions of gallic acid (20, 40, 60, 80, 100 or 120 µg/ml) were prepared of stock soln. using serially dilution method. 1 ml aliquot of each dilution was further diluted to 10 ml with 50% methanol in test tube. 1.5 ml Folin-Ciocalteu's reagent was added to each test tube. The test tubes were then incubated at room temperature for 5 min. to each test tube we added 4 ml of 20% w/w aqueous sodium carbonate then the final volume was adjusted to 25 ml. the test tubes were incubated at room temperature for 30

mins. Absorbance was measured at 765 nm using UV/VIS spectrophotometer against blank i.e 50% methanol. Test solution was prepared in similar manner [8].

Extimation of Antibacterial activity: Antimicrobial activity of *Marchantia paleacea* extract (MPE) was carried out at Sir J. C. Bose Technical Campus, Bhimtal, Kumaun University. The extract was tested against four species of microorganisms namely *Salmonella typhimurium* (3224), *Escherichia coli* (724), *Staphylococcus aureus* (737) and *Pseudomonas aeruginosa* (424). The assay was carried out using disk diffusion method using ciprofloxacin as the standard drug. To determine the minimum inhibitory concentration (MIC), the micro broth dilution method as described by Basri and Fan in 2005 was used. [9] Stock solutions of the extracts were prepared using acetone which ranged from 2 to 0.1 mg/mL. The stock was then diluted solutions to create test solutions, which was added to well microtiter plate containing MH broth. 100 μ L of inoculum suspension (1×10^8 CFU/mL) was added prepared in sterilized water to each well. Cultures without extract were used as negative controls. MIC is considered as the lowest concentration of the extract that inhibits the visible bacterial growth. To ensure accuracy the experiment was performed in triplicate [10].

RESULTS

Antioxidant Phytochemicals

The study revealed that the sample had high total phenol content (14.62 % w/w) and a total flavonoid content of 4.14 % w/w. Phenols and flavonoids are both classes of bioactive compounds that have been shown to have various health benefits, including antioxidant and anti-inflammatory properties. The high levels of both phenols and flavonoids in the sample suggest that it may have significant potential for use in developing natural products with therapeutic properties.

Antimicrobial Activity

The disk diffusion method is a widely used assay for testing the antimicrobial activity of a substance against various bacterial strains. The activity of MPE, was tested against four bacterial strains using disk diffusion method. The results revealed that MPE exhibited activity against all four bacterial strains (given in table no.2.) The test sample showed similar level of activity against *S. typhimurium* and *S. aureus*. MIC Value of the sample was found to be 200 μ g /ml for *S. typhimurium* 700 μ g /ml for, *E. coli*, 500 μ g/ml for *S. aureus*, and 200 μ g /ml for *P. aeruginosa*.

Table No. 1. Total Flavonoid and Total Phenol Content in *Marchantia paleacea*

Test sample	Total phenols content (% w/w) Mean ⁿ ± S.D.	Total flavonoids content (% w/w) Mean ⁿ ± S.D.
MPE	14.62 ± 0.08	4.14 ± 0.04

Table No. 2. *M. paleacea* extract showing antibacterial activity against pathogenic strains

S.N.	Bacterial Strains	Zone of Inhibition (mm ± SD)		MIC (µg/mL)
		Test	Standard	AJMR
1	<i>Salmonella typhimurium</i>	25 ± 0.3	32 ± 0.6	200
2	<i>Escherichia coli</i>	12 ± 0.3	34 ± 0.6	700
3	<i>Staphylococcus aureus</i>	25 ± 0.3	36 ± 0.6	500
4	<i>Pseudomonas aeruginosa</i>	12 ± 0.3	20 ± 0.3	200

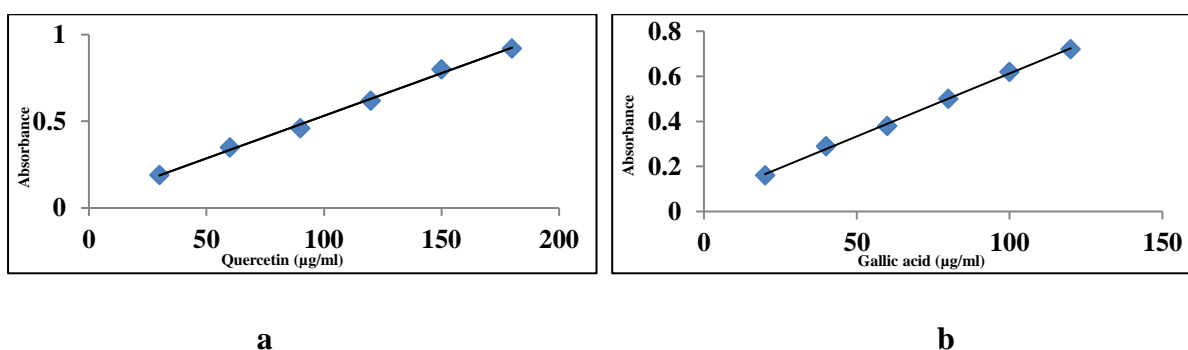


Fig 1. (a) Total Flavonoid Content, (b) Total Phenol Content

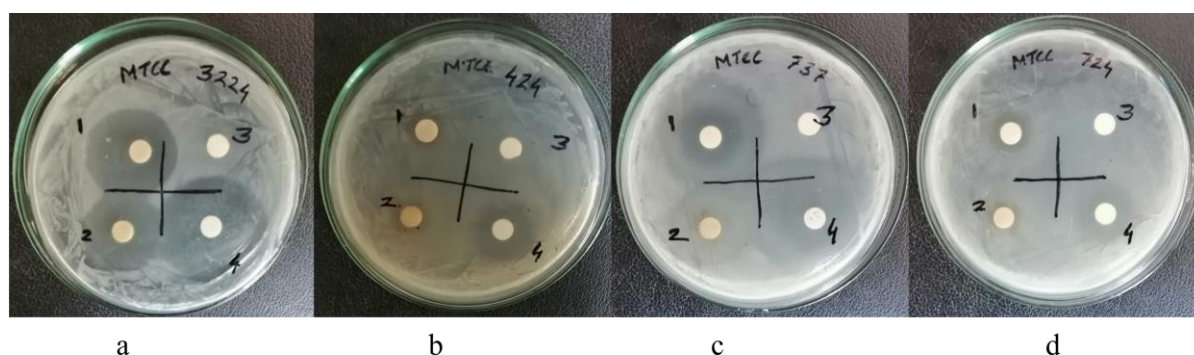


Fig. 2 *M. paleacea* extracts antibacterial activity against pathogenic strains (a *Salmonella typhimurium*, b *Escherichia coli*, c *Staphylococcus aureus* & d *Pseudomonas aeruginosa*)

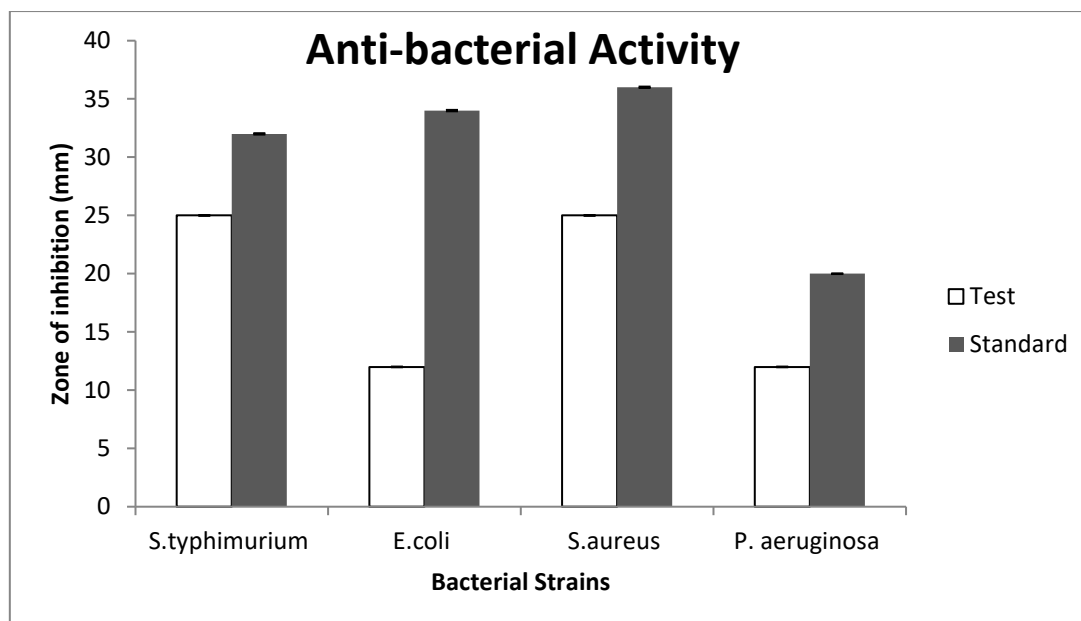


Fig. 3. *Marchantia paleacea* extract showing antibacterial activity against pathogenic strains

DISCUSSION

High levels of phenols and flavonoids in the sample suggest that it may have significant potential for use in developing natural products with therapeutic properties. Phenolic compounds and flavonoids are known to have potent antioxidant and anti-inflammatory effects, which are crucial in the prevention and management of several diseases. [11]. The role of these phytochemicals in providing potential antimicrobial action is also a key feature in many recent investigations. The antimicrobial screening revealed that the extract exhibited activity against all four bacterial strains. The antimicrobial activity of the sample may be attributed to the high levels of phenols and flavonoids, which have been shown to possess antimicrobial properties. [12,13] Quercetin which is a major flavonoid of *Marchantia* sp. [5] may be responsible for this antimicrobial property [14].

The findings of this study suggest that *Marchantia paleacea* has potential for use in the development of natural products with antimicrobial activity and can be used in the treatment of acute gastroenteritis and skin ailments (like abscesses, furuncles & cellulitis) and other diseases that are caused by *S. typhimurium* and *S. aureus* infections.

FUNDING

Nil

CONFLICT OF INTEREST

None

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