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Phytochemical Screening and Antibacterial Activity of *Plagiochasma intermedium* Lindenb et Gottsche – A Liverwort

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Liverwort *Plagiochasma intermedium* was screened for its active metabolites using standard phytochemical tests. Different concentrations of its aqueous extract were subjected to antimicrobial activity assay using plant pathogenic bacterium *Pseudomonas syringae* as test organism, in the presence of the positive control drugs streptomycin and amoxicillin. Inhibition of bacterial growth was measured in terms of number of colonies and diameter of inhibition zone appeared after 24 hours of incubation at 35 ± 2°C. Results showed that all the concentrations of extract were active in inhibiting bacterial growth. Lower extract concentrations were less effective but higher concentrations showed pronounced effect to check bacterial growth. Among all concentrations 100 per cent extract concentration was found to be most potent.

Key Words: Phytochemical, *Plagiochasma intermedium, Pseudomonas syringae,* Antimicrobial activity, Liverwort.

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but because such information may be of value in disclosing new sources of such economic materials as terpenoides, flavonoides, steroids, tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of *folkloric remedies* (Farnsworth, 1966).

Bryophytes frequently grow in an unfavorable environment as they inevitably biosynthesize secondary metabolites against biotic or abiotic stress. They not only defend against the plant competition, microbial attack, and insect or animal predation, but also function in UV protection, drought tolerance, and freezing survival (*Xie and Lou*, 2009).

Recent advances have shown that bryophytes are the store house of various active secondary metabolites giving them extraordinary potential to fight against pathogens. One of the feature that helped bryophytes to survive and maintain their place in today's flora is their content of biologically active compounds. Although Bryophytes are very familiar, their medicinal importance is not exploited completely (Banerjee and Sen 1979).

Liverworts have fungicide, bactericide and weak biocide (stomach poison) activity against animal pests. The antimicrobial activity of liverworts, seen in extracts of Lunularia, Reboulia and Pallavicinia, is possibly due to lunularic acid. Similarly, petroleum-ether extracts of Barbula and

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Timmiella species have antibacterial activity against both gram-positive and gram-negative bacteria. The active ingredients responsible for antimicrobial effects have been isolated and identified from several bryophytes, such as Polygodial from Porella, Norpiguisone from Conocephalum conicum, and Lunularin from Lunularia cruciata. Some liverworts also have some anti-leukemia activity. Diplophyllin was isolated from Diplophyllum albicans and D. taxifolium, which have significant activity against human epidermoid carcinoma. During the First World War, the Germans used Sphagnum extensively for dressing wounds. Sphagnum pads are better than cotton for dressing wounds as they absorb liquids 3-4 times as much as cotton, have anti-microbial properties, and do not require as frequent changes of dressing (*Asakawa*, 2007). In the present work *Plagiochasma intermedium*- a liverwort was analyzed to detect the presence of antimicrobial secondary metabolites in its aqueous extract.

MATERIALS AND METHODS

Collection of Plant Material

The plant material was collected in rainy season from Mount-Abu, Distt. Sirohi, (Raj) from different localities in both vegetative and sporophytic phases.

Extract Preparation

The collected plant material was washed with tap water till the debris and soil particles were removed. Then it was washed with distilled water repeatedly. To remove extra moisture, the material was dried by pressuring in between blotting papers. For aqueous extract preparation, weighted plant material was grinded in mortar and pestle with equal amount of double distilled water till the formation of fine paste. The smooth paste of extract was filtered through blotting paper and was centrifuged at 6000 rpm for 20 minutes. After centrifugation the supernatant was filtered using Whatman filter paper No.1 and stored in volumetric flasks. This filtrate was used as crude extract (100 per cent concentration). The crude extract was then serially diluted using double distilled water to prepare various concentrations from 10-100 per cent.

Preparation of Antibiotic Solutions of Different Concentrations

Standard solutions were made using the pure drug samples of antibiotic streptomycin and amoxicillin for a positive control. The standard solutions (10 ?g/ml) were diluted using double distilled water to prepare solutions of different strengths from 10 - 100 per cent.

Test Organism

Pure culture of Pseudomonas syringae (MTCC No. 1604) was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh (India).

Bioassay of Antibacterial Activity

All the experiments were set in complete aseptic environment and in triplicates. For each set of experiment a control was also set. For colony count assay one milliliter of 24 hours old bacterial broth culture was serially diluted up to 10-2 dilution. 10 Lof this dilution was then spreaded evenly over the surface of solidified agar medium containing plant extracts / antibiotic solutions of different concentrations in each petri plate, in a 1:1 ratio. All the Petri plates were sealed with Parafilm and incubated at 35 ± 2°C for 24 hours after which bacterial colonies were counted.

For Agar-well diffusion assay medium was poured in petri plates and then was inoculated with the 100 µL of 24 hours old broth culture of test microorganism. Once the agar was solidified, it was punched in the centre of petri plate to make a well of three millimeters diameter using a sterile cork borer. The well was filled with 1mL of the plants extract / antibiotic solutions of different concentrations. The plates were incubated at 35 ± 2°C for 24 hours. The antimicrobial activity was calculated by measuring the diameter of inhibition zone appeared after the incubation period.

Phytochemical Screening

The aqueous extract of *Plagiochasma intermedium* was subjected to various phytochemical tests. The methods of Evans (1997) and Raman (2006) were used to detect the presence or absence of certain bioactive compounds.

Tests for Flavonoids

Ferric-Chloride Test

Plant extract was taken in a test tube and added few drops of freshly prepared neutralized ferric chloride solution. Intense green colour of

the solution indicated the presence of flavonoids. Phenols form a complex with Fe (III), which gives an intensely colored iron-phenol complex.

Lead Acetate Test

Plant extract was taken in a test tube and added few drops of 10 per cent lead acetate solution was added to it. The flavonoids from plant extract get precipitated in the presence of lead acetate giving a yellow bulky appearance.

Alkaline Reagent Test

Extract was treated with 10 per cent ammonium hydroxide solution. In presence of ammonia flavonoids emits yellow fluorescence.

Sodium Hydroxide Test

5ml of 20per cent NaOH is added to equal volume of plant extract. A yellow solution indicated the presence of flavonoids. The reactive group present in flavonoids is Phenol. It is acidic in nature while sodium hydroxide is a base, so this acid base reaction forms a salt sodium phenoxide and water.

Test for Terpenoids

Salkowaski's Test

To find the presence of terpenoids in the extract, plant extract was taken in a test tube and added few drops of concentrated sulphuric acid was added. After shaking well it was allowed to stand. The lower layer turned yellow indicating the presence of terpenoids.

Liebermann-Burchardt Test

Plant extract was taken in test tube and added acetic anhydride, mixed well and then added concentrated sulphuric acid was added from the sides of the test tube. Deep red colour indicated the presence of terpenoids.

Test for Sterols

Salkowaski's Test

The plant extract was taken in a test tube and few drops of sulphuric acid were added. After shaking well, it was allowed to stand. The lower layer turned red indicating the presence of sterols. The sterols get oxidized to give a reddish brown derivative.

Liebermann-Burchardt Test

Plant extract was taken in a test tube and few drops of acetic anhydride were added and mixed well. When concentrated sulphuric acid was added from the sides of the test tube, it showed a brown ring at the junction of two layers and the upper layer turned green, indicated the presence of sterols. The colour is due to the hydroxyl group (-OH) of sterols reacting with the reagents and increasing the conjugation of the un-saturation in the adjacent fused ring.

Test for Alkaloids

Mayer's Test

In a few ml of extract, few drops of Mayer's reagent were added by the side of the test tube. A white or creamy precipitate was not observed; hence presence of alkaloids was not confirmed.

Hager's Test

One or two ml of Hager's reagent was added in a few ml of extract. A prominent yellow

Table 1. The phytochemical profile of *Plagiochasma intermedium* Aqueous Extract

Active Component	Phytochemical Test	Observation	Result
Alkaloids	Mayer's test	No Precipitate	-
Hager's test	No Precipitate		-
Anthroquinon	Borntrager's test	No Layer Formation	-
Cardiac Glycoside	Keller killeni test	Brown Ring	+
Flavonoids	Ferric Chloride Test	Green Colour	+
Lead Acetate Test	Yellow Precipitate		+
Alkaline Reagent Test	Yellow Fluorescence		+
Sodium Hydroxide Test	Yellow Colour		+
Saponins	Froth Test	No Froth Formation	-
Sterols	Salkowaski test	Reddish Brow Colour	+
Liebermann-Burchardt test	Brown Ring		+
Terpenoids	Salkowaski test	Lower layer turned yellow	+
Liebermann-Burchardt test	Deep Red Colour		+

Note: (+) shows Presence and (-) shows absent of the phytochemical

precipitate was not found, hence alkaloids were found to be absent.

Test for Anthroquinons

Borntrager's Test

About 0.5ml of extract was added with 5ml chloroform and shaken for 5 minutes. The extract was filtered and the filtrate shaken with an equal volume of 100 per cent ammonia. No layer formation indicated the absence of anthroquinons.

Test for Cardiac Glycoside

Keller Killeni Test

Few ml of extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution then added 1ml of concentrate sulphuric acid, brown ring obtained at the interface indicated the presence of de-oxysugar characteristic of cardiac glycoside.

Test for Saponins

Froth Test

The extract was diluted with distilled water and made upto 20 ml. The suspension was shaken in a graduated cylinder for 15 minutes. No froth formation indicated the absence of saponins.

RESULTS AND DISCUSSION

The results showed in table 1 suggest that various phytochemicals such as terpenoides, flavonoids, sterols and cardiac glycosides are present in P. intermedium. Tests for alkaloids,

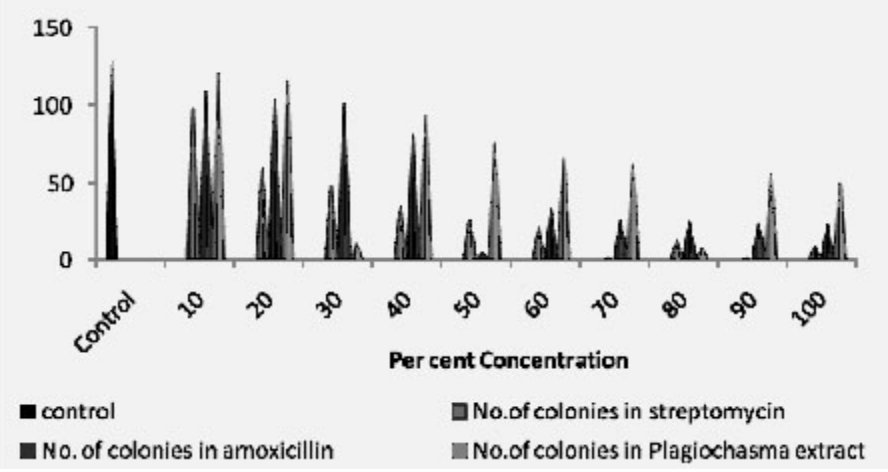

Fig. 1. Number of colonies of P. syringae in different concentrations of antibiotics and plant extract

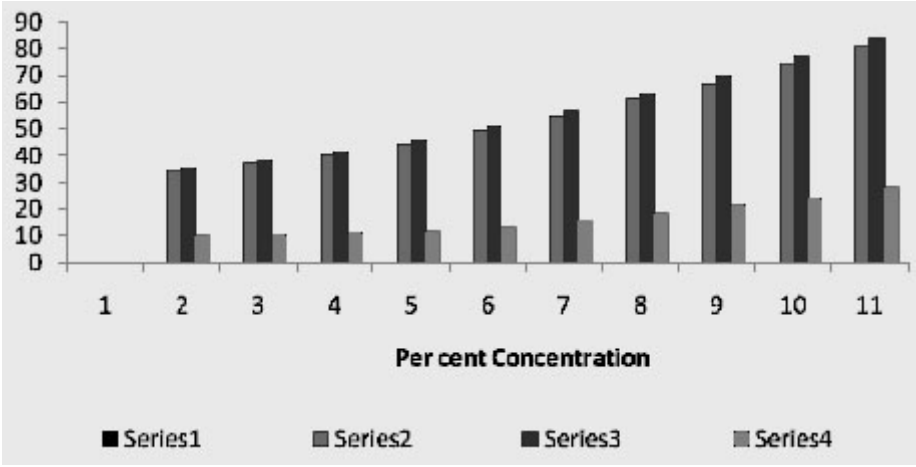

Fig. 2. Zone of inhibition (in mm) of *P. syringae* in different concentrations of antibiotics and plant extract

saponins and anthroquinones gave negative results showing absence of these metabolites. Results of antibacterial analysis showed that the aqueous extract of P. intermedium posses potent activity to inhibit bacterial growth both in terms of number of colonies and zone of inhibition appeared after incubation time. The mean values of zone of inhibition and number of colonies after 24 hours of incubation for different concentrations of both synthetic antibiotics and the plant extracts are shown in figure 1 and 2, respectively. As indicated streptomycin was proved to be strongest to inhibit bacterial growth with an inhibition zone of 90 mm in 100 per cent concentration followed by 100 per cent concentration of amoxicillin with an inhibition zone of 76 mm. Among the different concentrations of aqueous extracts of P. intermedium the maximum zone of inhibition (34.5 mm) was reported in 100 per cent concentration of extract. Minimum zone of inhibition (10.2 mm) was recorded in 10 per cent concentration of extract. No zone of inhibition was observed in the control. Number of colonies in the control was counted to be 130 which decreased to 120 in 10 per cent extract concentration. Continued fall in number of colonies was recorded with increasing extract concentrations. Maximum inhibition of colony growth was reached at 100 per cent extract concentration with number of colonies being 49. All concentrations of antibiotics inhibited bacterial growth effectively, with streptomycin being more effective. Only 8 tiny colonies were seen in 100 per cent streptomycin solution. Number of colonies in 100 per cent amoxicillin was 22 only.

Results of the present experiment are in consistency with all earlier studies. Seven pure flavonoids from five moss species were isolated and identified by Basil and coworkers (1999). Some of these shown pronounced antibacterial effects. Toyota and Asakawa (1999) screened the extract of Plagiochasma appendiculatum and evaluated that it possesses antimicrobial activity which was due to the presence of terpenoids in it. Iwasshina (2003) found that flavonoid compounds are distributed widely in vascular plants and bryophytes, and 5,000 kinds have been reported as naturally occurring substances. Subhisha and Subramoniam (2005) studied in vitro antifungal activity of Pallavicinia lyellii against four fingi viz, Aspergillus niger, Aspergillus fumigatus. Fusarium oxysporum and Candida albicans. A sterodial fraction with remarkable in vitro antifungal activity was isolated. Ludwiczuk and Asakawa (2008) described the chemical constituents of selected liverworts collected in New Zealand, Malaysia, Madagascar, Argentina, Ecuador, and other southern hemispheric countries and stated that bryophytes contain a large number of terpenoids and aromatic compounds. According to them many of compounds isolated from these liverworts have novel carbon skeletons and also have interesting biological activity.

Bodade *et. al.*, (2008) studied antimicrobial effect of some bryophytes using agar diffusion method against different pathogenic microorganisms. The result was then compared with the standard antibiotics ampicillin and nystatin (10 ug/ml). Results indicated that the bryophyte extracts were found to be active against at least one of the test organisms except Racomitrium crispulum.

The present study revealed that P. intermedium contain various secondary metabolites like flavonoids, steroids, terpenoids and glycoside compounds. This confirm the earlier studies of bryophytes and suggest that P. intermedium is also a rich store house of antimicrobial substances. Further it was observed that amoxicillin and streptomycin were very effective against bacterial growth. However, P. syringae is very sensitive for streptomycin. Although all concentrations of P. intermedium extract showed varying levels of activity against the test bacteria, 100 per cent extract was found to be more active than the other concentrations. The antimicrobial activity of extract was might be due to presence of flavonoids, steroids, terpenoids and other polyphenolic compounds. With an increase in extract concentration the zone of inhibition was also increased in all cases. It was observed that size of colonies was smaller in antibiotic solutions in comparison to the plant extracts. It can be concluded that P. intermedium can provide promising source for herbal antibacterial substances along with other bryophytes which were studied earlier by Iwasshina (2003), Asakawa (2008) and Bodade *et. al.*, (2008).

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