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Research article

Preliminary phytochemical analysis and antibacterial study of crude extract from *Hamelia patens* stems

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ABSTRACT

The present study aimed to analyse the phytochemical and antibacterial activity of different extracts of the tropical plant *Hamelia patens* Jacq. (Rubiaceae). The antimicrobial activity was carried out using agar well diffusion method. Hexane, petroleum ether, ethanol and chloroform stem extracts (100 µL of 50 mg/mL each) were introduced into the wells separately and allowed it to undergo incubation. After the incubation time was over, the plates were observed for zones of inhibition and compared with positive control ciprofloxacin at a concentration of 30 µM. These results provided evidence that the tested plant extracts possess antimicrobial properties which can be tested further in the development of novel antimicrobial agents.

INTRODUCTION

Medicinal plants are alleged to be with healing powers, and people used it for many centuries. Most of the indigenous uses of plant resources started off from the Asian region. Now, almost 80% of the world's total population depends on traditional medicines for its primary health care needs (Ullah et al., 2010). Plants based remedies are mostly used as a substitute for allopathic medicines (Sandya et al., 2006). Local peoples in communities have unearthed the therapeutic activity of medicinal plants against certain types of diseases through their native experiences which got transferred to them from their forefathers. From the ancient times onwards, quite a large number of plants are being used in medicine for a therapeutic or prophylactic reason. The therapeutic properties of medicinal plants are due to the presence of bioactive substances like vitamins, alkaloids, flavonoids, glycosides, tannins and coumarins (Shagal et al., 2012).

All over the world, herbal medicines were used as one of the most important areas of age-old traditional medicine. The study on the medicinal plants is essential to promote the proper use of herbal medicine to determine their potential as a source for the new drugs (Parekh and Chanda, 2007). According to the WHO, the plant-based traditional medicine systems continues to play an essential role in health care, with about 80% of the world's inhabitants relying mainly on traditional medicines for their primary health care (WHO, 2000). Having a view to modern drug discovery, the traditional medicinal plants have been studied, analysed and developed which followed the ethnobotanical lead of indigenous healing used by our traditional medical systems (Pei, 2007).

Indian herbal (plant-based) industries with considerable research in the field of pharmacognosy, phytochemistry, pharmacology and clinical therapeutics have investigated or explored these ayurvedic herbs, which are now designed into various kinds of numerous herbal formulations, which have set foot in the international pharmacopoeia through the study of ethnopharmacology and traditional medicine (Samy and Gopalakrishnakone, 2007).

MATERIALS AND METHODS

Sample collection

The fresh, disease-free stems of *Hamelia patens* were collected in a pre-sterilized polythene bag from the Manamai area, located in Kanchipuram district of Tamil Nadu. The plant material was identified from the department of Life Sciences of the institute. The stems were shade dried at room temperature for 10 days.

Preparation of extracts

The stems were thoroughly washed with clean tap water to remove the presence of any soil and other dirt. The air-dried stems were powdered using a blender and made into a fine powder. The powdered sample was successfully extracted with hexane, petroleum ether, ethanol and chloroform in a soxhlet apparatus. The extracts obtained were dried using vacuum rotary evaporator to get the concentrated extracts. The extracts were subjected to qualitative analysis of various phytochemical constituents present in the extract. All the tests were carried out as per standard procedures (Brindha et al., 19981; Lala, 1993).

Phytochemical analysis

Each extract of *H. patens* was taken apart in 5 mL of 1.5% v/v hydrochloric acid and it is strained out using the Whatman filter paper No 1. After that these filtrates were put through various tests to find out the presence of phytochemicals. To investigate the presence of alkaloids, glycosides, sterols, flavonoids, tannins, carbohydrates, proteins and carotenoids the following standard conventional protocols of qualitative analysis were performed and results were tabulated (Kokate, 1997; Khandelwal, 2006).

Saponins

Two mL of distilled water was mixed with a small quantity of the crude extract in a test tube and shaken vigorously till it froths. The froth thus obtained was mixed with few drops of olive oil and again shaken vigorously. The formation of foam showed the presence of saponins.

Phenolics

A small quantity of the extract was taken in a test tube and 3 mL of 10% lead acetate solution was added to it. A bulky white precipitate indicated the presence of phenolic compounds (Saxena and Saxena, 2012).

Naphthoquinones

The extract (2 mg) was mixed with 2 mL of ether and 2 mL of ammonia solution. The appearance of a reddish-pink colour in the ammonical layer showed the presence of naphthoquinones.

Anthraquinones

Two mg of the extract was boiled with 2 mL of dilute HCl. After cooling at room temperature, 2 mL of ether was added to it. The ethereal layer was taken away and an ammonia solution (2 mL) was added to it. The appearance of pink colour in the ammonical layer showed the presence of anthraquinones.

Coumarins

The extract solution was added drop by drop on the paper soaked with ammonia solution. The formation of fluorescence showed the presence of coumarins.

Triterpenoids

A small amount of the extract was dissolved in chloroform and few drops of acetic anhydride were added to it followed by concentrated sulphuric acid from the side of the test tube. Development of the reddish-brown ring at the junction indicated the presence of triterpenoids.

Carbohydrates

Two mg of the extract was shaken with the few drops of Molisch's reagent and few drops of the sulphuric acid were added to it through the side of the test tube. The appearance of the violet ring at the junction point of two liquids indicated the presence of carbohydrates (Aquino et al., 1990).

Proteins

The extract (5 mg) mixed with 2 mL of alcohol and 2 mL of the Biuret reagent was added to it. The appearance of a violet colour indicated the presence of proteins.

Anti-bacterial activity

Preparation of inoculums

Bacterial pathogens such as gram-positive *Staphylococcus aureus* and gram-negative *E.coli* were used as test organisms. Cultures of bacteria were grown for 12 h in nutrient broth at 37 °C.

Antibacterial activity

The antimicrobial activity of the extracts of *H. patens* was determined by agar well diffusion method. The test strains were first cultured in nutrient broth for 24 h before use. The standardized cell suspension (100 μL) was spread on a Mueller Hinton agar. Sterile cork borer of 4 mm diameter was used to bore wells into the Mueller Hinton agar plates. All four extracts (100 μL each of 50 mg/mL prepared in DMSO) were introduced into the wells separately. The whole setup was allowed to stand at room temperature for about 2 h and then the loaded plates were incubated at 37 °C for 24 h for the bacteria to grow and occupy the entire plate. After the incubation time was over, the plates were observed for zones of inhibition and compared with positive control ciprofloxacin at a concentration of 30 μM. DMSO was used as negative control (Kaminidevi et al., 2015). A clear zone around each well corresponds to the antimicrobial activity of stem extract. The clear zone area was measured using the zone measuring scale.

RESULTS AND DISCUSSION

Phytochemical analysis

The analysis was done to screen the phytochemicals using various methods based on the specific classes. *Hamelia patens* stem extracts showed the presence of anthraquinones, saponins, naphthoquinones, triterpenoids, carbohydrates phenolics, coumarins and proteins (Table 1). The results suggested the presence of many bioactive phytochemicals present in the plant which might be responsible for the antimicrobial activity (Bhasin et al., 2012).

Table1. Phytochemical analysis of different extracts

Phytochemical class	Extracts			
	Petroleum ether	Chloroform	Ethanol	Hexane
Saponins	-	-	+	-
Phenolic compound	-	-	+	-
Naphthoquinones	-	-	+	-
Anthraquinones	-	-	+	-
Coumarins	-	-	+	-
Triterpenoids	+	+	+	+
Carbohydrates	-	-	+	-
Proteins	-	-	+	-

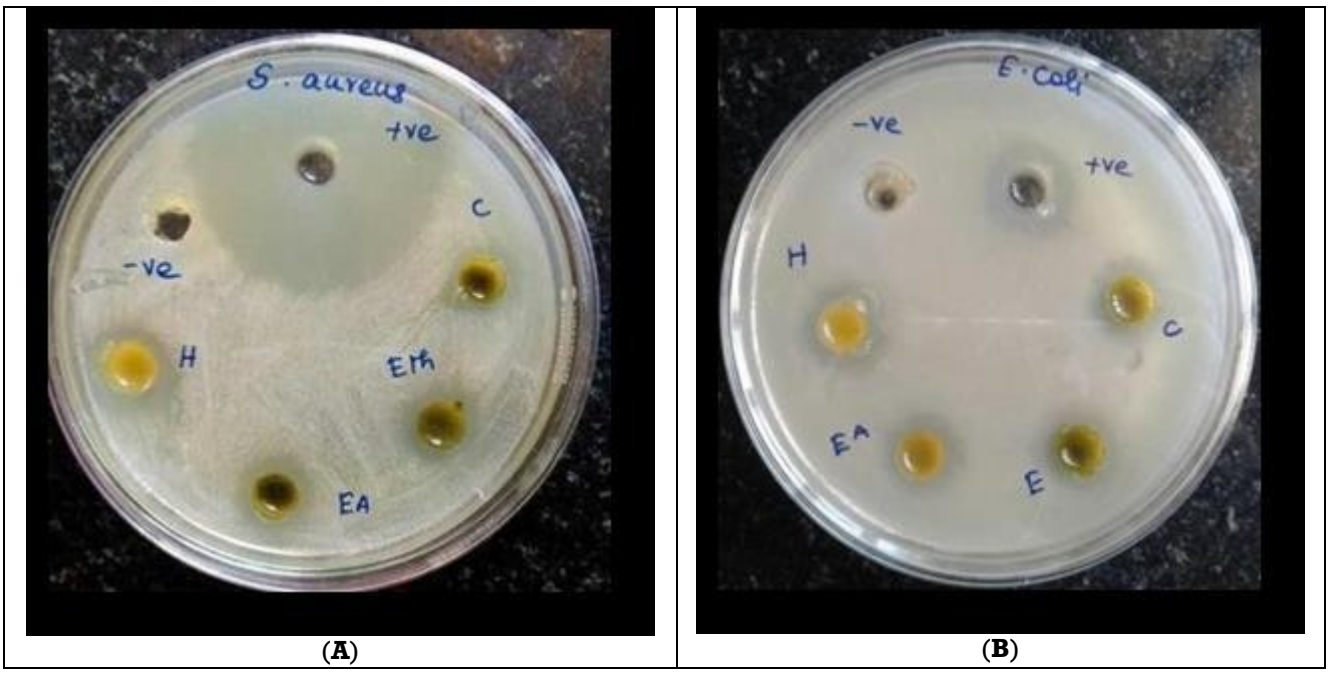
(+) shows presence and (-) shows absence of the phytochemicals

Antibacterial activity

In the present study, the antibacterial activity was analysed with the stem extract of *Hamelia patens* against *E. coli* and *S. aureus*. According to the well plate assay, the chloroform extract showed the minimum inhibition zone by 5.1 and 5.2 mm, respectively against *S. aureus* and *E. coli*. The ethanol extract showed a maximum inhibition zone by 9.8 mm against *S. aureus* whereas the petroleum ether extract exhibited by 9.2 mm against *S. aureus*. Similarly, the hexane extract showed activity by 9.0 mm against *S. aureus* and 7.1 mm against *E. coli*. The zones of inhibition were measured by HiMedia scale and the results are summarized in Table 2. The Petri dishes showing zone of inhibition against both the pathogens are depicted in Fig. 1.

Table 2. Zone of inhibition of stem extracts from *Hamelia patens*

Pathogens	Zone of inhibition (mm)				
	Petroleum ether	Chloroform	Ethanol	Hexane	Ciprofloxacin
E. coli	6.2	5.2	5.3	7.1	8.3
S. aureus	9.2	5.1	9.8	9.0	25.0



(**A**) (**B**)

Fig. 1. Zone of inhibition of the stem extracts against *S. aureus* (**A**) and *E.coli* (**B**). (H = Hexane, C = Chloroform, Eth/E = Pet. ether, EA = Ethyl alcohol, -ve = Negative control, +ve = Positive control)

DISCUSSION

The phytochemicals which are present in plants are the ones behind in preventing diseases and enhancing health. These phytochemicals may lower or reduce the risk of coronary heart disease by averting the oxidation of Low-Density Lipoprotein namely (LDL) cholesterol, by reducing the formation and absorption of cholesterol, and also by systemizing pressure and blood clotting (Olowosulu and Ibrahim, 2006).

From these results, it is understood that the *Hamelia patens* stems were more resistant against gram-positive bacteria compared to the gram-negative bacteria. Further, the detailed study was needed to confirm the resistant against the gram-positive bacteria through testing more bacterial strains.

Previously, a comparative study was done on *Viola odorata* against selected respiratory tract pathogens i.e. *Haemophilus influenza*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Streptococcus pyogenes* (Shanker et al., 2017). The methanol extract exhibited a higher degree of antibacterial activity as compared to aqueous, acetone and petroleum ether extracts. A similar study was done on antimicrobial activity of the methanolic stem extract of locally available *Mentha piperita* which showed activity against clinical isolates of *Escherichia coli*, *Acinetobacter*, *Staphylococcus aureus* and two fungi such as *Candida albicans*, *Candida glabrata* (Pramila et al., 2012). A comparison was also carried out on antimicrobial property of *Tinospora cordifolia* against *Escherichia coli*. It was seen that the ethanolic and methanolic extracts of the stem showed good zone of inhibition (Kumar et al., 2017). Another study on the methanolic extract of the stem callus of *Bacopa monnieri* at the concentrations 0.25, 0.05 and 0.03 mg/disc was investigated for its antimicrobial activity by modified Kirby-Bauer diffusion method. The outcome of this study proved that the extract from the stem callus of *Bacopa monnieri* showed a dose-dependent antimicrobial action against all the tested bacterial as well as fungal species indicated by the zones of inhibition of the microbial growth (Alam et al., 2011). Murugan et al. (2012) and Okoye (2016) also reported similar analysis based on Phytochemistry and antibacterial activity. Camporese et al. (2003) evaluated the antibacterial activity of hexane, chloroform, petroleum ether and ethanol extracts of *H. patens* stems against *E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis,* amongst which only hexane extract was found effective against *E. coli*. The antimicrobial activity of the stem extracts (ethanol, methanol, petroleum ether and aqueous extracts) against *E. coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *S. aureus* and antifungal activity against *C. albicans* and *A. niger* were also studied (Murugan et al., 2012).

CONCLUSION

The phytochemical analysis of *Hamelia patens* showed the presence of naphthoquinones, coumarin glycosides, phenolic compounds, anthraquinones, triterpenoids, carbohydrates and proteins. The ethanolic extract of the plant showed appreciable antibacterial property when compared with the other extracts. Moreover, the extracts exhibited divergence in the antimicrobial activity might be due to variations in their composition of bioactive compounds. Hence, a detailed screening may be done to isolate the active phytochemical constituent so that it may be scientifically proved to access the phyto-pharmacological response of the plant to establish its traditional use.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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