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ANTIBACTERIAL ACTIVITY OF *HAMELIA PATENS* AGAINST HUMAN PATHOGENS

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Abstract – *Hamelia patens* Jacq. (Rubiaceae) is a common ornamental plant with many medicinal properties. The present investigation was carried out in order to assess the antibacterial property of the ethanolic and aqueous extracts of *H. patens* Jacq. on the common human pathogens *viz. Escherichia coli*, *Vibrio harveyi, Staphylococcus aureus* and *Bacillus cereus* by Kirby- Bauer disc diffusion assay. The antibacterial activity of the biosynthesized silver nanoparticles was also carried out. The preliminary phytochemical screening revealed the presence of alkaloids, tannins, phenols, glycosides, saponins, flavonoids, protein, carbohydrate and terpenoids in both aqueous and ethanolic extracts. The nanoparticles biosynthesized using *H. patens* were spherical in shape and grayish in color. Both the extracts showed similar activities against all four strains of bacteria. Nanoparticle solution showed considerable activity against *Escherichia coli* with an inhibition zone of 11mm. Maximum zone of inhibition was exhibited by the standard antibiotic drug Amoxycillin.

INTRODUCTION

Plants are one of the important sources of medicines since the time immemorial. Humans have been utilizing plants to control various health problems and to prevent diseases in different countries since the earliest days of mankind.Recently, there has been tremendous increase in the use of plant-based health products in developing as well as developed countries resulting in an exponential growth of herbal products globally (Monokesh and Biplab, 2012). Plants are now being evaluated on the basis of their traditional uses for the discovery and development of new drugs (Monokesh and Biplab, 2012). The important advantages claimed for therapeutic uses of medicinal plants in various ailments are; their safety besides being economical, effective and their easy availability. Because of these advantages, the medicinal plants have been widely used by the traditional medical practitioners (Arshad *et al.,* 2012).

Hamelia patens Jacq. belongs to the family Rubiaceae. It is an ornamental plant with orange-red flowers, hence it is known as firebush. It is a tropical plant that can tolerate drought and many types of soil conditions. *H. patens* Jacq. is a large perennial shrub or small tree, mostly native to American subtropics and tropics. In Mexico, it is used for 42 different medicinal purposes, especially to stop bleeding, healing sores, and in menstrual disorders, pimples, malaria, sores, skin diseases, blisters, eczema, stomach ache, athlete's foot, skin lesions, rash, insect bites, itching, headache, asthma, burn, scurvy, inflammation, rheumatism, nervous shock, post-partum pain, uterine and ovarian afflictions, expel intestinal worms and dysentery (Arshad *et al.,* 2012; Vijay *et al.,* 2016; Tim *et al.,* 1991).

Hamelia patens Jacq. is commonly used for treatment of wounds in many parts of the world. Hence, it is of interest to know if it possesses antimicrobial activity. Plant extracts have great potential as antibacterial compounds against microorganisms (Gislene *et al.,* 2000). Nowadays bio-synthesized metal nanoparticles are also increasingly used to target bacteria as an alternative to antibiotics. Thus, the present study focuses on the preliminary phytochemistry, antibacterial activity and silver nanoparticle assay of *Hamelia patens* Jacq. The antibacterial property of the biosynthesized nanoparticles was also assayed.

MATERIALS AND METHODS

Collection and identification of plant materials

Aerial parts of *Hamelia patens* Jacq. were collected from Panampilly Nagar, Ernakulam, Kerala, India on 2nd May 2019. The plant specimen was identified using standard authentic taxonomic literature (Gamble, 1921).

Preparation of the plant extract

Fresh aerial parts of the plant were collected and washed under tap water to get rid of dust particles, cut into small pieces, shade dried and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work. The aqueous and ethanolic extracts were prepared by weighing 20 g of each of the powdered samples and mixed thoroughly with 200 mL of each solvent. They were allowed to soak in the solvent for 48 hours at room temperature. The extracts were then filtered through Whatman no. 1 filter paper. The ethanolic extract obtained was air dried and later in a water bath. The aqueous extract obtained was evaporated at 50 °C in hot air oven. The extracts were then dissolved in distilled water for further studies.

Preliminary phytochemical screening

The plant extracts were tested for the presence of various phytochemicals by using standard methods (Sahira and Cathrine, 2015; Thulasi and Krishnakumar, 2018).

Silver Nanoparticle Assay

Silver Nanoparticle assay was carried out in order to find out whether the plant biosynthesized silver nanoparticle or not. The powdered samples were used for the assay (Nisha *et al.,* 2016).

Preparation of silver nitrate solution

2 mM Silver nitrate solution was prepared by adding 0.0339g of silver nitrate in 100 mL of double distilled water. The solution was mixed thoroughly and stored in brown colored bottle in order to prevent auto-oxidation of silver.

Preparation of the plant extract

25 g of the powdered sample was taken in 250 mL beaker and boiled along with 100 mL distilled water. After 10 minutes of boiling solution was cooled to room temperature and filtered using Whatman's no. 1 filter paper. The collected extract was used for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles

10 mL of extract was added to 90 mL of 2 Mm aqueous silver nitrate solutions (1:9 ratio) and mixed thoroughly by manual shaking. The beaker was then placed under sun light for reduction into silver nitrate nanoparticle. After 10 minutes color changes were noted. This indicates the preliminary confirmation for the formation of plant mediated silver nanoparticle.

Purification of silver nanoparticles

After 5 hours, grey nanoparticles started to settle at the bottom. The solution was centrifuged at 8000 rpm for 15 minutes, supernatant was discarded and the pellet containing nanoparticles were taken out on a petriplate and kept in hot air oven to dry at 50 °C for 4-5 hours. The silver nanoparticles were then taken out on a glass slide and observed under 40 X resolution of the microscope and photographs were taken.

Antibacterial Assay

Kirby- Bauer disc diffusion method was performed for the antibacterial assay (Woldeyes *et al*., 2012). Four different bacterial strains were used in the present study which belonged to gram-positive (*Staphylococcus aureus, Bacillus cereus*) and gram-negative categories (*Escherichia coli, Vibrio harveyi*). *Bacillus cereus* is a gram positive, rod shaped, facultatively anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are gram positive, facultatively anaerobic, round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are gram negative, facultative anaerobes commonly found in the lower intestine of warm blooded organism which cause food poisoning in their hosts. *Vibrio harveyi* are gram negative, bioluminescent, marine, rod shaped bacterium responsible for Luminous Vibriosis.

The pure cultures maintained in slants were collected from Microbiology laboratory, Post Graduate and Research Department of Botany, Maharaja's College, Ernakulam, Kerala, India for the study. Each strain was separately inoculated into 5 mL nutrient broth and was incubated at 37 °C for 24 hours. Filter paper discs (Whatman filter paper No.1) were prepared using paper punch and

sterilized. Lawn cultures of the test organisms were made on nutrient agar plates using a sterile cotton swab under aseptic conditions. The filter paper discs were loaded with plant extracts (aqueous and ethanolic) and nanoparticle solution using a micropipette under aseptic conditions. Discs impregnated with Amoxycillin served as positive control (standard) and the filter paper disc soaked in solvents were used as negative control. The discs were placed on the surface of nutrient agar with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar plate. The prepared plates were incubated at 37 °C for 24 hours. Inhibition zones were measured after incubation period.

RESULTS

Phytochemical screening

The result of phytochemical screening of *Hamelia patens* is shown in Table 1. It reaveals the presence of alkaloids, tannins and phenols, glycosides, saponins, flavonoids, protein, carbohydrates and terpenoids in both aqueous and ethanolic extracts.

Silver Nanoparticle Assay

Silver nanoparticles were biosynthesized with *Hamelia patens.* Jacq extract. The colour change after adding silver nitrate solution was from pale brownish colour to dark reddish-browncolour. After 5 hours the nanoparticles started to settle down at the bottom and was centrifuged and washed. Under 40X resolution of the microscope (Biolinkz M2000 series), the nanoparticles synthesized was found to be spherical in shape and grayish in color.

Antibacterial Assay

The zones of inhibition of the plant extracts and nanoparticle solution obtained are given in the Table 2.

Both the extracts showed similar activities against the four strains of bacteria. Nanoparticle solution showed considerable activity against *Escherichia coli* with an inhibition zone of 11mm. Maximum zones of inhibition were exhibited by the standard antibiotic drug Amoxycillin. The zone of inhibition in aqueous extract was higher for *Escherichia coli* with an inhibition zone of 7 mm and for *Bacillus cereus, Staphylococcus aureus* and *Vibrio harveyi,* the inhibition zones were 6mm. In the case of ethanolic extract, the zone of inhibition was higher for *Vibrio harveyi* with 7mm followed by *Bacillus cereus, Staphylococcus aureus,* and *Escherichia coli* with an inhibition zone of 6mm each. The zone of inhibition of nanoparticle solution against *Escherichia coli* was maximum, *i.e.* 11 mm followed by *Vibrio harveyi, Staphylococcus aureus* and *Bacillus cereus* with 8mm, 7mm and 6mm respectively. For Amoxycillin the zone of inhibition was higher in *Escherichia coli* with 19mm followed by *Vibrio harveyi, Staphylococcus aureus* and *Bacillus cereus* with inhibition zone of 18, 14 and 10 respectively.

DISCUSSION

Hamelia patens Jacq. is traditionally used as a medicinal plant in many parts of the world. A

Table 1. Preliminary phytochemical screening of *Hamelia patens* Jacq.

Sl No.	Phytoconstituents	Test	Aqueous	Ethanol
1.	Alkaloids	a. Mayer's test	+ve	- ve
		b. Wagner's test	+ve	+ve
		c. Hager's test	+ve	+ve
2.	Tannins and phenols	a. $FeCl_3$ test	-ve	+ve
		b. Lead acetate test	+ve	+ve
3.	Glycosides	a. Borntrager's test	+ve	-ve
		b. Keller-Killiani's test	-ve	+ve
4.	Saponins	a. Foam test	+ve	+ve
5.	Flavonoids	a. Shinoda test	-ve	+ve
		b. Con. H_2SO_4	+ve	-ve
6.	Protein	a. Biuret test	-ve	+ve
		b. Millon's test	+ve	+ve
7.	Carbohydrate	a. Molish test	+ve	+ve
		b. Benedict's test	+ve	+ve
8.	Terpenoids	a. Salkowski test	+ve	+ve

Table 2. Antibacterial activity of *Hamelia patens* Jacq.

Sample	Zones of inhibition of different bacteria (in mm)			
	Bacillus cereus	*Staphylococcus aureus*	*Escherichia coli*	*Vibrio harveyi*
Aqueous	6	6	7	6
Ethanol	6	6	6	7
Nanoparticle solution	6	7	11	8
Amoxycillin	10	14	19	18

variety of compounds that are of plant origin are found to act against a wide range of microbes. Hence such compounds can be exploited as antimicrobial agents to treat a variety of diseases (Tom *et al.,* 2016; Abubacker *et al.,* 2013). In the present study, preliminary phytochemical screening of *Hamelia patens* Jacq. was carried out and the result showed that both aqueous and ethanolic extract contain chemical compounds like alkaloids, glycosides, saponins, flavonoids, protein, carbohydrate, terpenoids, tannins and phenols. Poornima *et al.* (2018); Samriti *et al.* (2019); Woldeyes *et al.* (2012); Nisha *et al*. (2017); Annam *et al.* (2016) and Ogundipe *et al*. (2008) reported that *Zanthoxylum rhetsa* (Roxb). DC., *Sida acuta* Burm. F., *Sida rhombifolia*, *Gomphrena serrata* L, and *Trianthema portulacastrum* Linn. possessed alkaloids which were found to be antimicrobial against bacteria such as *Escherichia coli, Staphylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa.*

In the present investigation, *Hamelia patens* Jacq. showed the presence of alkaloids. As per the earlier reports, alkaloids are a promising source of antimicrobial agent. *H.patens* Jacq. also contain tannins and phenols which can also act against a variety of strains of bacteria (Ajaykumar *et al*., 2016; Arshad *et al.,* 2012). Both aqueous and ethanolic extract showed significant antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. The standard antibiotic Amoxycillin was used to compare the antibacterial activity of *H. patens* Jacq. Earlier studies (Tom *et al.,* 2016; Abubacker *et al.,* 2013) revealed that hydro alcoholic extract of *H. patens* Jacq. showed antibacterial activity. In the present study, the antibacterial properties were studied against bacterial strains like *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Vibrio harveyi*. *Bacillus cereus* is a facultatively anaerobic bacterium responsible for food borne illness like severe nausea and vomiting. *Staphylococcus aureus* are round shaped bacterium responsible for common cause of skin infections including abscesses, respiratory infections such as sinusitis and food poisoning. *Escherichia coli* are coli form bacterium commonly found in the lower intestine of warm blooded organism which causes food poisoning in their hosts. *Vibrio harveyi* are bioluminescent, rod shaped bacterium responsible for Luminous Vibriosis. Hence, the present study revealed that *H. patens* Jacq. is a potential source of antibacterial components. However further fractionations and characterisations are required to identify the exact principle.

Silver nanoparticle synthesized with various plants showed antibacterial activity. In the present investigation, the antibacterial activity of the biosynthesized nanoparticles was tested. Silver nitrate solution was used as the control (Nisha *et al*., 2017; Swarup and Tapan, 2015). The percentage of inhibition was found to be more than that of aqueous and ethanolic extracts. This indicates that biosynthesized nanoparticles possessed a better antibacterial activity than the aqueous and ethanolic extracts.

The present study revealed that the antimicrobial property associated with *H. patens* Jacq. was promising for further investigation. Hence, further characterisation and screening is needed to ascertain the exact molecule behind the antibacterial property of *H. patens* Jacq.

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