

Evaluation of Antimicrobial Activity and Minimum Inhibitory Concentration of Ethanolic Extract of Three Medicinal Plants against Bacteria causing Skin Infection

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Medicinal plants are widely used for the treatment of different diseases. The purpose of this study is to observe the antibacterial and minimum inhibitory concentration of ethanolic leaves extract of three medicinal plants (*Psidium guajava*, *Ficus religiosa*, *Cardiospermum halicacabum*) against gram positive (*Streptococcus pyogenes*, or Group A *Streptococcus*) and gram negative bacteria (*Escherichia coli*, *Proteus species*) isolated from clinical skin samples. The antibacterial activity and minimum inhibitory concentration of plant extracts were determined by using agar well diffusion method and well plate method respectively. The plants extracts showed varied levels of antimicrobial activity against the gram positive and gram negative bacteria. The ethanolic extracts of three medicinal plants showed a broad spectrum antibacterial activity against the panel of pathogens. This study indicated clear evidence supporting the traditional use of the plants in treating skin diseases related to bacteria. The present study thus suggests the use of these medicinal plants in the treatment of various skin infections.

Keywords: Bacterial skin infection, Medicinal plants, Minimum Inhibitory Concentration, *Streptococcus pyogenes*, *Escherichia coli*, *Proteus sp.*, *Psidium guajava*, *Ficus religiosa*, *Cardiospermum halicacabum*.

For centuries, the therapeutic properties of various medicinal plants have been used to treat human diseases. In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases. Although their efficacy and mechanisms of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses due to their active chemical constituents (Barnes *et al.*, 2007)¹. Plant-derived products contain a great diversity of phytochemicals such as phenolic

acids, flavonoids, tannins, lignin, and other small compounds (Cowan, 1999)². These compounds possess numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic and vasodilatory activities (Bidlack *et al.*, 2000)³.

The expanding bacterial resistance to antibiotics has become a growing concern worldwide (Gardam, 2000)⁴. Increasing bacterial resistance is prompting resurgences in research on the antimicrobial role of herbs against resistant strains (Alviano and Alviano, 2009)⁵. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds (Mahady, 2005)^[6]. Medicinal plant extracts offer considerable potential for the development of new agents effective against

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infections which are difficult to treat (Iwu *et al.*, 1999)⁷. A wide range of phytochemicals present in plants is known to inhibit bacterial pathogens (Cowan, 1999; Medina *et al.*, 2005; Romero *et al.*, 2005).^{2, 8, 9}

Apart from the expensive costs of some antibiotics, most of the clinically important antibiotics have major setbacks. A good number of conventional antibiotics have been found to be neurotoxic, nephrotoxic and hypertensive, and few others cause severe damage to the liver and bone marrow depression (Chong and Pagano, 1997)¹⁰. The primary benefit of using herbal drugs is that they are relatively safer and cheaper than the synthetic alternatives (Aiyegoro and Okoh, 2009)¹¹. In addition, herbal medicine is a complex mixture of different phytochemicals acting by different mechanisms, which makes it difficult for pathogens to develop resistance (Daferera *et al.*, 2003)¹².

In this study, the three medicinal plants, (Table 1) dried leaves extract was made in ethanol solvent. These extracts were used to examine antibacterial activity by agar well diffusion method and minimum inhibitory concentration, by microdilution method against *Escherichia coli*, *Proteus species*, *Streptococcus pyogenes*, or *Group A Streptococcus*, which are common cause of skin infection.

MATERIALS AND METHODS

Collection of Plants Materials

Medicinal plants reported were collected from Trivandrum, India. The plants leaves were rinsed with water to remove soil and dust particles. Then they were shade dried and grinded. The grinded plants materials were passed through 0.125 mesh sieve to remove debris and store in a sterile air tight container.

Extraction of Plant Material

Ethanol leaf extracts of all the three selected plants were prepared. 100gm of air dried and powdered leaves of each plant were soaked in 100 ml of 70% ethanol for 72 hours. Each mixture was stirred for every 24 hours using a sterile glass rod. At the end of extraction each extract was passed through Whatman Filter Paper No.1. The ethanolic extract obtained was concentrated at 30°C and then stored in air tight containers at 4°C.

Antimicrobial assay

Isolation and identification of bacterial strains

Three pathogenic bacterial strains, *Escherichia coli*, *Proteus species*, *Streptococcus pyogenes*, and *Group A Streptococcus* were collected from Metro Scans and Laboratories, Trivandrum, India. The three bacterial strains were maintained in blood agar at 37°C for further studies.

Preparation of Inoculum

For the antibacterial activity test, the bacteria were aerobically cultures in nutrient broth at 37°C for 24 hours and then suspended in sterile saline at a density equivalent to that OD 0.5 McFarland standards. The culture was inoculated over Muller Hinton Agar (MHA) plates. Bacterial suspension with a concentration of 10⁵cfu/ml was used in vitro antibacterial activity test.

Antibacterial activity by Well Diffusion Method

The antimicrobial activity of the plant extracts were evaluated using Agar Well Diffusion Method (Eloff 1998, Perez *et al.*, 1990)^{13, 14}. 0.1 ml of diluted inoculum (10⁵cfu/ml) of the bacterial strains were swabbed on the Muller Hinton Agar plates. Wells of 5mm diameter were punched into the agar plates with the help of sterilized cork borer (5mm). Using micropipette, 100 µl of the plant extracts were added to the wells made in the plate. The plates were incubated aerobically at 37°C for 24 hours. Antibacterial activity was evaluated by measuring the zone of inhibition (mm). (Table 2)

Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of each plant extract was determined using broth microdilution method (Tripathi 2013; Fabry *et al.*, 1998)^{15, 16}. Two fold serial dilutions of each plant extract were added to the wells of sterile 96 well plates containing inoculated nutrient agar medium 100 µl with a bacterial concentration of 10⁵cfu/ml. *Escherichia coli*, *Proteus species* and *Streptococcus pyogenes* were used as the test organism. Samples were dissolved in the solvent to a final concentration of 10mg/ml and added in increasing concentration such as 31.25, 62.5, 125, 500, 1000µg. Following 24 hour incubation at 37°C, the MIC was determined as the lowest concentration that completely inhibited the growth of the bacteria by measuring the optical density

(OD) at 630 nm using a spectrophotometer. The OD was measured immediately after the visual reading. The growth inhibition for the test wells at each plant extracts dilution was determined by the formula,

Percentage of Inhibition = (OD of Control - OD of Test) / (OD of Control) X 100%

RESULTS

In this study, three medicinal plants were selected according to their traditional usage for the treatment of skin infections and associated symptoms. The plants by their scientific and common names, traditional use, plant part used are listed in the Table.1. All plants are extracted with

ethanol. In this study ethanol is used as extraction solvent because it is relatively safe when compared with other organic solvent. Further, ethanol extraction is widely used to obtain crude extracts of phytochemicals from plant materials in the herbal medicine industry for therapeutic applications.

All the plants extracts exhibited different levels of antibacterial activities and appeared to have the best activity at the extract concentration 10mg/ml in the agar well diffusion method. (Table.2). The result in the Table.3 shows the average minimum inhibitory concentration (MIC) of the selected medicinal plants extracts on gram positive and gram negative bacteria obtained using microdilution method.

Table 1. Profile of medicinal plants used

Botanical Name	Family Name	Common Name	Plant Parts Used	Therapeutic Uses
<i>Psidium guajava</i>	Myrtaceae	Guava	Leaves	Ulcers, boils, rheumatic places and the chewing of the leaves to relieve toothache, oral ulcers, treating diarrhea, type 2 diabetes, hyperlipidemia, and hypertension
<i>Ficus religiosa</i>	Moraceae	Banyan tree	Leaves	Treatment of ulcers, asthma, heart disease, viral infection, bacterial infection, and protozoan infections
<i>Cardiospermum halicacabum</i>	Sapindaceae	Balloon Wine	Leaves	Treatment of rheumatism, nervous diseases, stiffness of the limbs, skin infection, Asthma, Abdominal disease, anemia etc.

Table 2. The antibacterial activity of ethanolic extracts of medicinal plants against selected skin pathogens

Ethanolic Extract	Concentration (mg/ml)	Zone of Inhibition (mm)		
		<i>Streptococcus pyogenes</i>	<i>Proteus sp.</i>	<i>Escherichia coli</i>
<i>Psidium guajava</i>	10	17	16	12
	7.5	13	13	11
	5.0	12	12	8.0
	2.5	-	-	5.0
<i>Ficus religiosa</i>	10	13	13	14
	7.5	11	11	11
	5.0	10	6.0	10
	2.5	8.0	8.0	6.0
<i>Cardiospermum halicacabum</i>	10	12	8.0	12
	7.5	11	5.0	10
	5.0	10	6.0	9.0
	2.5	8.0	4.0	7.0

Table 3. Minimum Inhibitory Concentration of Medicinal plants against Tested organisms

Plants	Minimum Inhibitory Concentration		
	<i>Streptococcus pyogenes</i>	<i>Proteus sp.</i>	<i>Escherichia coli</i>
<i>Psidium guajava</i>	483.940µg	557.462µg	602.460µg
<i>Ficus religiosa</i>	501.082µg	611.216µg	621.096µg
<i>Cardiospermum halicacabum</i>	480.201 µg	516.459 µg	558.099 µg

DISCUSSION

Nature has bestowed a very rich botanical wealth, and a large number of diverse types of plants grow in different parts of the world. Antimicrobial agents of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases, and simultaneously they also mitigate many of the side effects that are often associated with synthetic antimicrobials (Rios and Recio)¹⁷.

Hence, the purpose of the present study is to evaluate the role of antimicrobial agents of plant origin in inhibition of the growth of *Escherichia coli*, *Proteus species* and *Streptococcus pyogenes*. The results demonstrate that the effectiveness of the mentioned plant extracts against the pathogens tested. All strains were susceptible to the extracts. The extracts show significant activity against the investigated microbial strains. Although the bacterial used in the study are different, the active components present in the extracts have the capability of destroying bacterial cell wall which inevitably inhibits the growth of bacteria. Isolation and purification of phytoconstituents from these plants may yield significant novel antimicrobials, as plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any adverse effects that are often associated with synthetic compounds.

CONCLUSION

These results provide a rationalization for the use of these medicinal plants for the treatment and control of various infections in traditional medicine. Further studies, toxicity study can be

conducted on the extracts of these plants to evaluate the safe limit of their dosage and consumption and formulation of the selected medicinal plants.

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