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RESEARCH ARTICLE

ANTIMICROBIAL PROPERTIES AND PHYTOCHEMICAL SCREENING OF MEDICINAL PLANTS AGAINST CLINICAL PATHOGENS BY *IN-VITRO* METHODS

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ABSTRACT

Antimicrobial properties of aqueus extracts of 6 different plant extracts were tested against bacterial pathogens such as *Staphylococcus aureus* and *Pseudomonas* species. The determination of the antibacterial activity by agar well diffusion method and Disc diffusion method showed that 6 plant extracts tested exhibited antibacterial activity against *Staphylococcus aureus* and *Pseudomonas* species. The present experimental results indicated that out of 6 crude extracts from different plant species *Chromolaena odorata*, *Tectona grandis*, *Musa paridisiaca*, *Hemigraphis colorata*, *Curcuma longa* and *Psidium guajava* possessed antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas* species. Phytochemical screening of selected plants showed the presence of Saponin, Proteins, Tannins, Flavanoids, Steroids and reducing sugars. The above observations confirmed that some of these plant extracts possess bactericidal compounds, which inhibit the growth of *Staphylococcus aureus* and *Pseudomonas* species.

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INTRODUCTION

Skin flora is usually non-pathogenic, and either commensals (are not harmful to their host) or mutualistic (offer a benefit). The benefits bacteria can offer include preventing transient pathogenic organisms from colonizing the skin surface, either by competing for nutrients, secreting chemicals against them, or stimulating the skin's immune system. However, resident microbes can cause skin diseases and enter the blood system creating life - threatening diseases particularly in immunosuppressed people. Hygiene to control such flora is important in preventing the transmission of antibiotic resistant hospital-acquired infections (Cogen.AL. *et al*., 2008).

In 1882, Gessard first discovered *Pseudomonas*, a strictly aerobic, gram-negative bacterium of relatively low virulence. The organism is ubiquitous, with a predilection to moist environments, primarily as waterborne and soil borne organisms. *Pseudomonas aeruginosa* colonization reportedly occurs in more than 50% of human's skin, and *it* is the most common pseudomonal species. *Pseudomonas* is a clinically significant and opportunistic pathogen, often causing nosocomial infections. In addition to causing serious and often life-threatening diseases, these organisms exhibit innate resistance to many antibiotics and can develop new resistance after exposure to antimicrobial agents.

Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance (Lowy, 1998). The skin and mucous membrane are excellent barriers against local tissue invasion by *S. aureus*. However, if either of these is breached due to trauma or surgery, *S. aureus* can enter the underlying tissue, creating its characteristic local abscess lesion (Elek, 1956), and if it reaches the lymphatic channels or blood can cause septicaemia (Waldvogel, 1990). The basic skin lesion caused by an *S. aureus* infection is a pyogenic abscess. However, *S. aureus* can also produce a range of extracellular toxins, such as enterotoxin A-E, toxic shock syndrome toxin- 1 (TSST-1) and exfoliative toxins A and B (Projan and Novick, 1997). Ingestion of enterotoxin produced by *S. aureus* in contaminated food can cause food poisoning (Howard and Kloos, 1990). TSST-1 is the toxin responsible for toxic shock syndrome (TSS) and is only caused by strains carrying the TSST-1 gene (Waldvogel, 1990). TSS infections are commonly

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associated with menstruating women, particularly those using tampons. The exfoliative toxins are associated with staphylococcal scalded skin syndrome (SSSS). SSS consists of three entities, toxic epidermal necrolysis, scarlatiniform erythema, and bullous impetigo (Howard and Kloos, 1987), all of which damage the epidermal layer of the skin.

Antibiotics are among the most frequently prescribed medications in modern medicine. Antibiotics cure disease by killing or injuring bacteria. Antibiotics such as penicillin and erythromycin have been reported to prevent bacterial infections in humans but they are not at all used clinically because of many unfavorable effects such as hypersensitivity reaction, supra infections, fever and nausea to major allergic reactions, including photo dermatitis and anaphylaxis. Furthermore, these organisms become resistant to antibiotics.

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Nowadays, the use of phytochemicals for pharmaceutical purpose has gradually increased in many countries. Medicinal plants have a promising future because there are about half million plants around the world, and most of their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. Medicinal plants have many characteristics when used as a treatment. The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects. This type of medicines referred as Synergic medicine. Some plants used as support of official medicines in the treatment of complex cases like cancer diseases the components of the plants proved to be very effective. In case of Preventive medicine it has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Bassam Abdul Rasool Hassan, 2012).

Hence in our present study we investigate the antibacterial activity of the aqueous extracts of selected indigenous medicinal plants against *Staphylococcus aureus* and *Pseudomonas sps.*

MATERIALS AND METHODS

Bacterial strains: *Staphylococcus aureus* and *Pseudomonas sps* isolated from the clinical sample obtained from DDRC Laboratory, Palakkad, Kerala used for our study. The isolated microorganisms were identified on the basis of morphological, biochemical and cultural characteristics on Manitol salt agar and Pseudomonas isolation agar. Strains were brought to pure culture on Nutrient agar plates and maintained at 4ºC.

Preparation of Plant Extracts: Medicinal plants were obtained from Kottakal arya vaidyasala, Malappuram and the extracts were ready by the method of Uhegbu *et al.*, (2005) using distilled water as the solvent. 20 g of powdered sample of the aromatic plant was extracted by soaking wet in 180 mL of distilled water in a beaker, stirred for about 6 min and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. 1) and the extracts were lyophilized.

Antimicrobial activity of various plant extracts against clinical pathogens

Well diffusion method: The agar well diffusion method was adopted according to Kavanagh, (1972) to assess the antibacterial activity of the prepared extracts. Loop full of bacterial stock suspensions was thoroughly mixed with 60 ml of sterile nutrient agar and kept for overnight incubation. 1m.l of overnight culture mixed with sterile Muller Hinton agar plates. 20 ml of the Muller Hinton agar were distributed into sterile Petri dishes. The agar was left to set and in each of these plates 4 cups, 10 mm in diameter, were cut using a sterile cork borer No. 4 and the agar discs were removed. The wells were filled with different extracts of 20µl, 40 µl , 60µl and 80 µl respectively and allow diffusing of plant extract into the medium for about 45minutes and then kept in an incubator at 37ºC. After 24 hours, the agar plates were examined for inhibition zones, and the zones were measured in millimeters. Microbial growth was determined by measuring the diameter of zone of inhibition. After incubation the diameters of the results and growth inhibition zones were measured, averaged and the mean values were tabulated.

Disc diffusion method: The Disc diffusion method was used to assess the antibacterial activity of the prepared extracts. 20 ml of the Muller Hinton agar were distributed into sterile Petri dishes. Swab culture was made using sterile cotton swabs using bacterial stock suspension. Sterile Band aid clothes were immersed in crude plant extracts and kept on Muller Hinton agar Plates. The plates were incubated at 37^0c for 24 hrs.

Phytochemical Studies of Plant Extracts

The crude plant extract was subjected to phytochemical analysis for detecting the chemical compounds in it.

RESULTS

Clinical pathogens *Staphylococcus aureus* and *Pseudomonas* sps were obtained from Kottakkal Arya Vidya sala Malappuram. On gram staining isolates showed Gram positive cocci and gram negative rods respectively. Motility was checked by using hanging drop method and the isolate was found to be Non motile (Gram positive cocci) and Motile (Gram negative rods). The isolates showed gram negative rods on Gram staining, subjected for biochemical and cultural characterisation for further confirmation. (Table - 1)

Table 1 Biochemical characterisation for Gram negative rods

S.I No	Tests	Results
1	Indole Test	-
2	Methyl red	-
3	Voges Proskauer Test	-
4	Citrate utilization Test	+
5	Urease Test	-

Inhibitory properties of various plant extracts were tested against *Staphylococcus aureus and Pseudomonas sps.* Different aqueous extracts from 7 different plant species were investigated by antibacterial activity by agar well diffusion method and disc diffusion method.

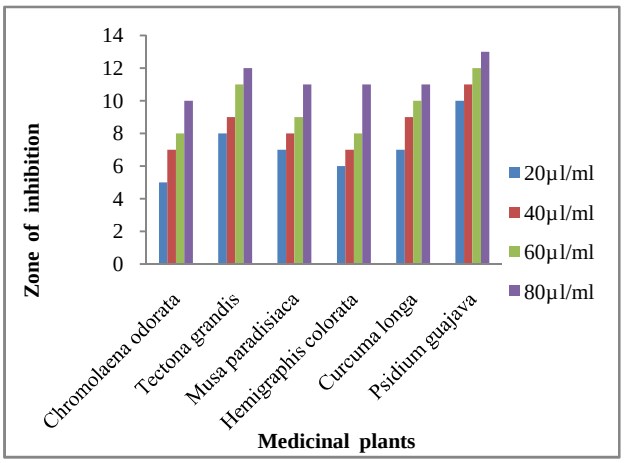
Table 2 Antibacterial activity of crude extracts of various medicinal plants against *Staphylococcus aureus* and *Pseudomonas* sps - Well cut Method

Plant extracts	Conc. µl/ml	Zone of inhibition (mm)	
		Staphylococcus aureus	*Pseudomonas* sps
Chromolaena odorata	80	9.0	10
	60	8.0	8.0
	40	6.0	7.0
	20	4.0	5.0
Tectona grandis	80	11	12
	60	10	11
	40	9.0	9.0
	20	7.0	8.0
Musa paridisiaca	80	10	11
	60	8.0	9.0
	40	7.0	8.0
	20	6.0	7.0
Hemigraphis colorata	80	9.0	11
	60	8.0	8.0
	40	6.0	7.0
	20	5.0	6.0
Curcuma longa	80	10	11
	60	9.0	10
	40	8.0	9.0
	20	6.0	7.0
Psidium guajava	80	12	13
	60	11	12
	40	9.0	11
	20	8.0	10

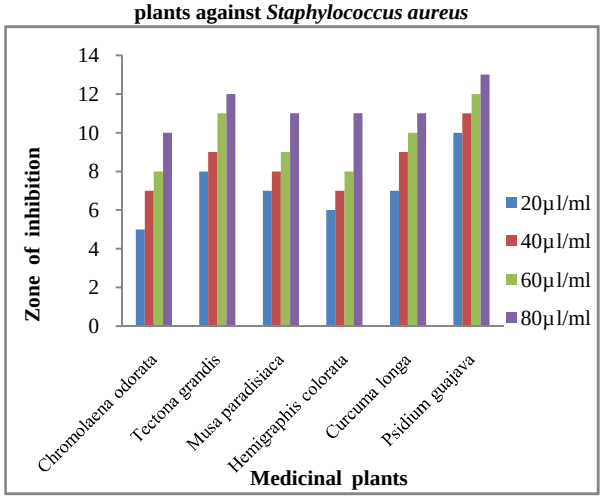
Table 3 Antibacterial activity of crude extracts of various medicinal plants against *Staphylococcus aureus* and *Pseudomonas* sps – Disc diffusion method

Plant extracts	Zone of inhibition (mm)	
	Staphylococcus aureus	*Pseudomonas* sps
Chromolaena odorata	18.0	20.0
Tectona grandis	20.0	24.0
Musa paridisiaca	16.0	20.0
Hemigraphis colorata	20.0	22.0
Curcuma longa	19.0	23.0
Psidium guajava	21.0	25.0

showed the presence of Saponin, Proteins, Tannins, Flavanoids, Steroids and reducing sugars. The above observations confirmed that some of these herb extracts possess bactericidal compounds, which inhibit the growth of *Staphylococcus aureus* and *Pseudomonas* species.



Comparison of antimicrobial activity of crude extracts of various plants against *Staphylococcus aureus*



Comparison of antimicrobial activity of crude extracts of various plants against *Pseudomonas* sps.

Table 4 Phytochemical result

Tests	*Chromolaena odorata*	*Tectona grandis*	*Musa paridisiaca*	*Hemigraphis colorata*	*Curcuma longa*	*Psidium guajava*
Test for Saponin	+	-	+	-	+	+
Test for Amino acids	-	-	-	-	-	-
Test for proteins	+	+	+	+	+	+
Test for tannins	+	+	-	+	-	+
Test for Anthraquinone	-	-	-	-	-	-
Test for flavanoids	-	-	-	+	+	+
Test for phenols	-	-	-	-	-	-
Salkowsky test	+	+	+	+	+	+
Benedict test	-	+	-	-	+	+
Anthrone test	+	+	+	+	+	+

Abbreviation:- + (Positive**) - (**Negative)

The determination of the antibacterial activity by agar well diffusion method and Disc diffusion method showed that 5 plant extracts tested exhibited antibacterial activity against *Staphylococcus aureus and Pseudomonas* (Table: 2 and 3) About 80µl of *Psidium guajava* extracts produced zone of inhibition in the range of 12mm for *Staphylococcus aureus* and 13mm for *Pseudomonas* sps. When the concentrations of the extracts were decreased showed slight diminish in inhibition zones were observed. Phytochemical screening of selected herb

DISCUSSION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated

phytochemically and the fraction submitted to biological or pharmacological screening is even smaller. (Srivastava. *et al.*, 1996). The screening of plant products for antimicrobial activity has shown that the higher plants represent a potential source of novel antibiotic prototypes. There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants (Wu. *et al.*, 1999).

Bacterial pathogens were obtained from DDRC, Microbiological laboratory, Palakkad. A total of 6 plant extracts from different plant species were investigated. Each plant extracts were tested at four different concentrations (20µl, 40µl, 60µl, and 80µl/ml) to see their inhibitory effects against bacterial pathogens such as *Staphylococcus aureus* and *Pseudomonas* species.

The present experimental results indicated that out of 6 crude extracts from different plant species *Chromolaena odorata* [Communist pacha], *Tectona grandis* [Teak], *Musa paridisiaca* [Banana leaf], *Hemigraphis colorata* [Murikooti], *Curcuma longa* [Turmeric] and *Psidium guajava* [Guava] possessed antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas* species.

Some studies demonstrated that *Chromolaena odorata* leaves extracts display antibacterial activities against *Pseudomonas aeruginosa*, *Streptococcus faecalis* (Irobi. 1992), *Neisseria gonorrhoeae* (Cáceres. *et al.*, 1995) and antifungal activities against *Cryptococcus neoformans*, *Microsporum gypseum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum.* In addition, methanol/dichloromethane (1:1) extraction of *C. Odorata's* roots, showed a significant antibacterial activity against *Escherichia coli and Salmonella typhi* (Wafo. *et al.*, 2011). *C. odorata* also displays antiinflammatory activities (Owoyele *et al.*, 2005) and contributes to wound healing (Thang. *et al.*, 1995), hemostasis and blood coagulation, antioxidant activity (Srinivasa Rao. *et al.*, 2009) as well as cytotoxic effects against cancerous cells (Taylor. *et al.*, 2012). The antimicrobial activities of methanolic extract of *Tectona grandis* on various strains were confirmed and synergism was possible with the antimicrobial drug tested. Tetracycline presented good synergism with methonolic extract of *Tectona grandis* (Purushotham. *et al.*, 2010).

Some studies showed the ethanolic extracts of *Musa paradisiaca* (Bananana), investigated individually for antimicrobial activity by disc diffusion method and investigated against selected species of *Escherichia coli*, *Bacillus subtilis*, *Vibrio cholerae*, *Klebsiella pneumoniae* to find the inhibitory activities of the microbes (Valarmathy. *et al.*, 2010).

Hemigraphis colorata have great potentials as antimicrobial agents against bacterial pathogens like *E.coli*, *S.aureus*, and *P.aeruginosa*. The antibacterial effect may be attributed to the phytoconstituents present in the cream (Jayaprakasan. *et al.*, 2014).

Antibacterial activity was studied with oil and *curcuminoid* extracts. Agar well diffusion method was used to determine the zone of inhibition of bacterial growth at particular concentration of both oil and curcuminoid. Both curcumin and the oil dilutions suppress growth of several bacteria (Bhavani & Sreenivasa. 1979). Turmeric is well known indigenous herbal medicine having many biological activities (Ammon & Wahl. 1991).

Antimicrobial potential of *Psidium guajava* leaves extract by using various solvents. The results indicated that ethanol and methanol are better than n-hexane and water for the extraction of the antibacterial properties of guava.

The results also indicated that the plant extracts have no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the organisms (Bipul Biswas *et al.*, 2013). In our present study the crude extracts of *Psidium guajava* showed good antimicrobial activity against both gram positive and Gram negative bacteria.

The result from this preliminary study indicated that these plant extracts could be used for therapeutic purpose. Phytochemical screening of selected plants showed the presence of Saponin, Proteins, Tannins, Flavanoids, Steroids and reducing sugars. Further investigations are needed for identification and purification of the specific antimicrobial components from these plants against bacterial pathogens.

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