

Full Length Research Paper

Comparitive studies on antibacterial activity of Patchouli [*Pogostemon cablin* (Blanco) Benth] and Geranium (*Pelargonium graveolens*) aromatic medicinal plants

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Solvent (Hexane, Ethanol, Methanol) extracts of Patchouli (*Pogostemon cablin*) and Geranium (*Pelargonium graveolens*) were compared for their potential antibacterial activity against four bacterial species using disc diffusion assayed method. DMSO and Chloramphenicol were used as negative and positive controls respectively. The growth inhibitory effect of the various solvents on *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Enterobacter aerogenes* were obtained and the most effective extract was the hexane extract of Patchouli compared to that of Geranium, which showed a maximum zone of inhibition (18 to 21 mm) against *S. aureus*. Ethanol and methanol extracts of Geranium showed maximum zone of inhibition (10 to 11 mm) against *S. aureus*. There was no zone of inhibition for aqueous, ethanol and methanol extracts of patchouli with 20 to 80 µl concentration and no zone of inhibition for aqueous, hexane extracts of Geranium with 20 to 80 µl concentration. The minimum inhibitory concentration (MIC) ranged from 40 to 80 µl for Patchouli hexane extract and MIC ranged from 60 to 80 µl for Geranium ethanol and methanol extracts. The potency of these extracts based on the zones of inhibition and MIC values were higher indicating that leaves have a potential broad spectrum antibacterial activity. The combination of these antimicrobial plant extracts can be used to treat infectious diseases in the near future.

Key words: Patchouli, geranium, plant extracts, disc diffusion, bacterial species, MIC, zone of inhibition, infectious diseases.

INTRODUCTION

Medicinal plants are the greatest economic source of the world. Nature has bestowed on us a very rich botanical

resources and a large number of various types of plants grow in several parts of the world (Bishnu et al., 2011).

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Plants are the richest resource of drugs of modern medicines, traditional systems of medicine, food supplements, nutraceuticals, pharmaceutical intermediates, folk medicines, and chemical entities for synthetic drugs (Hammer et al., 1999; Bishnu et al., 2011). Herbal medicine is still the basis of about 75 to 80% of the whole population, and the plant extracts and their active constituents are used in the major part of traditional therapy (Akerele, 1993). Indigenous medicinal systems like Ayurveda, Unani and Siddha are used around 1500 plants systematically (Bishnu et al., 2011). Plant originated antimicrobials have more therapeutic potential (Salau and Odeleye, 2007; Girish and Satish, 2008). Lot of interest has been shown towards the exploitation of natural compounds for their antimicrobial activity over the past 2 decades (Werner et al., 1999; Samy and Ignacimuthu, 2000; Girish and Satish, 2008). Earlier findings have reported the efficacy of various herbal extracts against microorganisms confirming the fact that plants are the bedrocks for identifying novel antimicrobial molecules (Evans et al., 2002; Girish and Satish, 2008). The increasing interest on traditional ethno medicine may lead to discovery of novel therapeutic agents.

Human infections caused by microorganisms and fungi are rampant in the tropical and subtropical areas of the world (Girish and Satish, 2008). Traditional therapy involves the use of plant extracts or their active principles which may serve as source for modern drugs and intermediate compounds for synthesizing analog drugs with better desirable properties (Jones, 1996; Hammer et al., 1999). The aim of this work was to prepare aqueous organic (solvent) extracts of Patchouli (*Pogostemon cablin* (Blanco) Benth) and Geranium (*Pelargonium graveolens*) and compare their antibacterial activities with selected microorganism strains.

Patchouli (*P. cablin*) is a herb belonging to the Labiatae family originating from Southeast Asia. Patchouli leaves contain an essential oil which is made up of patchouli alcohol (patchoulol) as a major component and several other minor components such as caryophyllene, α -, β -, γ - and δ -patchoulene, pogostol, seychellene, cycloseychellene, α - and β -bulnessene, α - and δ -guaiene and norpatchoulol (Akhila and Nigam, 1984; Akhila et al., 1988). The other species (*Pogostemon horthensis*, *Pogostemon hyneanus* and *Pogostemon plectranthoide*) of genus *Pogostemon* also yield patchouli oil in a lesser amounts than *P. cablin* (Ngampong et al., 2009).

P. graveolens (L.) belongs to the family Geraniaceae also called as Geranium is an erect, much branched shrub, which can reach a height of up to 1.3 m and a spread of 1 m. The essential oil produced from this plant has been used in the treatment of hemorrhoids, dysentery, heavy menstrual flows, inflammation and cancer (Ben et al., 2013). The French medicinal community currently treats diabetes, diarrhoea, gall bladder problems, gastric ulcers, jaundice, liver problems, sterility and urinary stones with this oil ((Peterson et al.,

2006). The essential oil fraction of *P. graveolens* and its main components, geraniol and citronellol, exhibited additive effects with amphotericin B and with ketoconazole against both *Aspergillus* species, resulting in fractional inhibitory concentration (FIC) indices ranging from 0.52 to 1.00 (Shin, 2003).

MATERIALS AND METHODS

Collection of medicinal plants for the study

Patchouli (*Pogostemon cablin* (Blanco) Benth) and Geranium (*Pelargonium graveolens*) producing aromatic oil of medicinal importance were selected based on ethnomedicinal importance. Healthy and disease free leaves of *P. cablin* (Blanco) Benth collected from University of Agriculture, Bengaluru and Authenticated (Authentication No. 11) by Dr. Vasundhara, Professor, GKVK, Bengaluru. Geranium (*P. graveolens*) plants were collected in and around Hyderabad (CIMAP), Andhra Pradesh (India) and authenticated from Department of Botany, Osmania University (Voucher No. 094), Hyderabad, India. These leaves were used for the preparation of solvent extracts.

Test microorganisms

Authentic pure cultures of human pathogenic Gram positive bacteria like (*Bacillus subtilis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*, *Enterobacter aerogenes*) were obtained from Department of Microbiology, Osmania University, Hyderabad, India. They were pre-cultured in nutrient broth culture overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min and pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm) (Suseem and Mary, 2012).

Preparation of extracts

Grinding of the selected plant materials

After drying under shade at 37°C for 7 days the plant materials were ground. To prevent the loss of active components exposure to direct sunlight was avoided as suggested by Girish and Satish (2008).

Aqueous extraction

50 g of dry powdered *P. cablin* and *P. graveolens* leaves were separately infused in distilled water, and the mixture was heated for 15 min at slow heat (Jigna et al., 2005; Soniya et al., 2013). The extract was then filtered using gauze and Whatman filter paper No.1, followed by sterilization via filtration through sterile syringe filter (0.2 -0.45 μ m pore). The filtered extract was stored as aliquots for future use (Haitham et al., 2009).

Preparation of leaf solvent extract

Soxhlet extraction

The finely ground sample (100 g) of *P. cablin* and *P. graveolens* leaves was successively extracted with hexane, ethanol and methanol using the Soxhlet apparatus for 48 h each. The solvent extracts were concentrated under reduced pressure separately.

Table 1. Patchouli extracts concentration (40 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Patchouli extract concentration (40 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	16	--	--	--	24
<i>Bacillus subtilis</i>	--	12	--	--	--	19
<i>Staphylococcus aureus</i>	--	18	--	--	--	18
<i>Enterobacter aerogenes</i>	--	10	--	--	--	25

Table 2. Patchouli extracts concentration (60 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Patchouli extract concentration (60 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	20	--	--	--	24
<i>Bacillus subtilis</i>	--	19.3	--	--	--	19
<i>Staphylococcus aureus</i>	--	20	--	--	--	18
<i>Enterobacter aerogenes</i>	--	15.6	--	--	--	25

The mixture was filtered and dried using a rotary evaporator (Abah and Egwari, 2011). The dried materials were stored in sterile labeled bottles and kept as aliquots until further use.

Antibacterial assay

Leaf extracts (aqueous and organic) of Patchouli (*Pogostemon cablin* (Blanco) Benth) and Geranium (*Pelargonium graveolens*) were then tested for their antibacterial activity. The preparations of leaf extracts for antimicrobial activity were done by slight modifications to Alade and Irobi protocols (Kedarnath et al., 2012).

Disc diffusion method

Disc diffusion method was employed for the determination of antibacterial activities of the *P. cablin* and *P. graveolens* leaf extracts (NCCLS, 1997; Haitham et al., 2009). Nutrient agar and the petriplates were sterilized by autoclaving under aseptic conditions. 20ml of the agar medium was dispensed into petriplates in sterile conditions in a laminar flow to obtain plates of a uniform depth of 4mm. The overnight inoculums containing 10⁶ bacterial cells/ml was spread on the surface of the solidified nutrient agar plates.

Whatmann No.1 filter paper was cut into small discs of diameter 6 mm and autoclaved (Kensa and Yasmin, 2011). Filter paper discs were impregnated into each plant extracts of different concentrations namely 20, 40, 60, 80 µl and dried aseptically. Using a sterile forceps, the treated filter papers containing *P. cablin* and *P. graveolens* leaf extracts were laid down on the surface of inoculated agar plate. In addition a positive control disc (Chloramphenicol) and negative control disc (10% DMSO) were also added alongside the extract treated discs. All the plates were maintained as triplicates. The plates were incubated at 37°C for 24 h and the diameter of the zone of inhibition was measured in millimeter.

RESULTS

No positive results were found at a concentration of 20 µl in both the leaf extracts. The results of the antibacterial activity of Patchouli and Geranium aqueous and organic (Hexane, Ethanol, Methanol) extracts, assayed in vitro by the disc diffusion method are described in the tables that follow. The highest zone of inhibition and the effectiveness is the major consideration in the case of antibacterial activity. The growth inhibitory effect of *E. coli*, *B. subtilis*, *S. aureus* and *E. aerogenes* are presented in Tables 1 to 6 and Figure 1 to 2. In our study, the most effective activity was proven by Patchouli hexane extract in comparison to Geranium with a maximum zone of inhibition ranging from 16 to 20.5 mm against *E. coli*, 12 to 19.3 mm against *B. subtilis*, 18 to 21 mm against *S. aureus*, 10 to 15.8 mm against *E. aerogenes*. All other 3 extracts were ineffective. Ethanolic and methanolic extracts of Geranium showed effective activity against *S. aureus* than *E. coli*, *B. subtilis* and *E. aerogenes* with maximum zone of inhibition ranging from 08 to 8.5 mm against *E. coli*, 10 to 10.5 mm against *B. subtilis*, 10 to 11 mm against *S. aureus*, 08 to 09 mm against *E. aerogenes* ethanolic extract and 8.5 to 9 mm against *E. coli*, 10 to 11 mm against *B. subtilis*, 11 to 11.5 mm against *S. aureus*, 9 to 9.5 mm against *E. aerogenes* with methanolic extract, respectively. The aqueous and hexane extracts of Geranium were not effective in inhibiting *E. coli*, *B. subtilis*, *S. aureus* and *E. aerogenes*. Geranium showed very low activity than Patchouli. The minimum inhibition concentration (MIC) of patchouli hexane extract ranged from 40 to 80 µl. In case of

Table 3. Patchouli extracts concentration (80 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Patchouli extract concentration (80 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	20.5	--	--	--	24
<i>Bacillus subtilis</i>	--	19.3	--	--	--	19
<i>Staphylococcus aureus</i>	--	21	--	--	--	18
<i>Enterobacter aerogenes</i>	--	15.8	--	--	--	25

Table 4. Geranium extracts concentration (40 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Geranium extract concentration (40 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	--	--	--	--	24
<i>Bacillus subtilis</i>	--	--	--	--	--	19
<i>Staphylococcus aureus</i>	--	--	--	--	--	18
<i>Enterobacter aerogenes</i>	--	--	--	--	--	25

Table 5. Geranium extracts concentration (60 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Geranium extract concentration (60 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	--	08	8.5	--	24
<i>Bacillus subtilis</i>	--	--	10	10	--	19
<i>Staphylococcus aureus</i>	--	--	10	11	--	18
<i>Enterobacter aerogenes</i>	--	--	08	09	--	25

Table 6. Geranium extracts concentration (80 µl) and their zone of inhibitions.

Test organisms	Zone of inhibition in diameter (mm)					
	Geranium extract concentration (80 µl)					Standard reference
	Aqueous	Hexane	Ethanol	Methanol	DMSO	Chloramphenicol
<i>Escherichia coli</i>	--	--	8.5	09	--	24
<i>Bacillus subtilis</i>	--	--	10.5	11	--	19
<i>Staphylococcus aureus</i>	--	--	11	11.5	--	18
<i>Enterobacter aerogenes</i>	--	--	09	9.5	--	25

Geranium's ethanolic and methanolic extract; the MIC ranged from 60 to 80 µl. These results indicate that leaves have a potential broad spectrum antibacterial activity (Table 1a to 1c; Table 2a to 2c).

In future, these extracts can be combined as a formulation to treat infections caused by the test organisms.

DISCUSSION

The present study revealed the antibacterial potential of Patchouli [*P. cablin* (Blanco) Benth] and Geranium (*P. graveolen*). Extracts of these plants hold active constituents with antimicrobial properties and appear to be potential antimicrobial therapeutic agents against



Figure 1. Patchouli (*Pogostemon cablin*).



Figure 2. Geranium (*Pelargonium graveolens*).

infections caused by the tested pathogens in this study. Good results were achieved with nutrient agar showing visible zone formation indicating bacterial growth inhibition. Similar approach was used by other investigators but used different culturing media for growth of organisms (Senthilkumar et al., 2010). Use of different solvents helps us to isolate extracts containing higher active compounds from the plants. Many studies suggested that different solvent extracts of various plants

has tremendous biological activity (Senthilkumar et al., 2010). Such an effective extract can be subjected to isolation of the therapeutic compounds and antimicrobials agents for further Pharmacological studies (Parekh and Chanda, 2006). Ethnobotanical approach is one of the universal practices applied in choosing the plants for pharmacological study (Cox and Balick, 1994) although, these plants declared the antibacterial activity against 4 medically important human pathogens. Minimum inhibitory concentrations (MICs) and Minimum bactericidal concentrations (MBCs) are the matters under study. This study can be further extended to study other major pathogenic bacteria and develop a novel broad spectrum antibacterial formulation in future. Now, our research will be focused to develop a broad spectrum antibacterial combined herbal formulation with these plants.

Conclusion

The present study confirmed the antimicrobial properties of aqueous and organic (hexane, ethanol and methanol) extracts from Patchouli [*P. cablin* (Blanco) Benth] and Geranium (*P. graveolens*) that showed significant growth inhibition for *E. coli*, *B. subtilis*, *S. aureus* and *E. aerogenes*.

All these microorganisms pose serious threat to mankind because of their ability to produce resistant strains towards a spectrum of antibiotics thereby making them difficult to treat. The encouraging results obtained by us indicate the antimicrobial activity of *P. cablin* and *P. graveolens* which can be exploited as a natural antibiotic for the treatment of several infections caused by these organisms, and could be useful in understanding the relations between traditional cures and current medicines.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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