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Original Article

Antibacterial and Antifungal Activity Analysis of Essential Oil of *Pogostemon cablin* (Blanco) Benth

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The essential oil of *Pogostemon cablin* (Blanco) Benth, also known as Patchouli oil was subjected for its antimicrobial investigation against a panel of ten human pathogenic bacteria and six human pathogenic fungi by Agar well diffusion method and Macrobroth dilution technique using Ampicillin (20ìg/well) and Nystatin (20µg/well) as control. Antibacterial activity revealed that, the essential oil was more active against Gram positive bacteria than Gram negative bacteria. The largest zone of inhibition was 35 mm (against *Bacillus cereus*) with 20 µl of oil. Ditermination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) showed that, *Bacillus cereus* exhibited the lowest MIC (250 µg/ml) and MBC (750 µg/ml). The oil showed moderate antifungal activity against all tested organisms. *Candida albicans* showed greater zone of inhibition (16 mm) than *Saccharomyces cerevisiae* (14 mm) with 20 µl and *Candida albicans* showed lowest MIC and MFC (both were 750 µg/ml). The zone of inhibition was 25 mm for each filamentous fungal strain with 20 µl, except for *Rhizopus oligosporus* (15 mm) and the lowest MIC (250 µg/ml) and MFC (500 µg/ml) were reported for *Aspergillus fumigatus*.

Keywords: *Pogostemon cablin* (Blanco) Benth, Patchouli oil, Antimicrobial activity

Introduction

Over the past years, the problem of antimicrobial resistance has received increasing attention and has become a global concern[1]. In addition, the increased magnitude of emergence of bacterial drug resistance, high dosage and prolonged antimicrobial therapy could eliminate commensal and beneficial bacteria and be predisposing to pathogen invasion[2,3]. Moreover, food-borne disease is still a major problem in the world, even in well-developed countries[4]. Nowadays, there is a growing interest in the screening of extracts and essential oils from plants in order to discover new antimicrobial agents. *Pogostemon cablin* (Blanco) Benth, also known as "Patchouli" or "Putchaput" is a species belonging to the family of Lamiaceae from the genus *Pogostemon.* The plant is native to tropical regions of Asia and is now extensively cultivated in China, Indonesia, India, Malaysia, Mauritius, Philippines, Thailand, Vietnam, and West Africa. It was introduced in India in 1942 and various cultivars were being evaluated for its suitability to tropical humid South Indian conditions[5]. The main constituents in essential oil are patchouli alcohol (49.06ÿ), á-bulnesene (14.34 %), α-patch-oulene (5.24%), β-Carophyllene (3.90%), β-patchoulene (3.11%), Globulol (1.41%), Caryophyllene oxide (1.08%), spathulenol (0.79%) etc[6]. History says that, Patchouli oil is very effective in sorting out rough, cracked and overly dehydrated skin and is used to treat acne, eczema, sores, ulcers, fungal infections, as well as scalp disorders. Chinese medicine uses the herb to treat headache, cold, nausea, diarrhea and abdominal pain[7]. Previous data showed that, crude hexane extraction patchouli leaves shows strong antibacterial activity against Gram positive bacteria *e.g. Staphylococcus aureus* and *Bacillus subtilis*[8-9]. There is no substantial data on patchouli oil. The aim of the present work was to investigate antimicrobial activities of patchouli oil against a diverse range of human pathogenic organisms including Gram positive and Gram negative bacteria and fungi in order to look for natural antimicrobial agents.

Materials and Methods

Collection of the essential oil

The patchouli oil was collected from BCSIR Laboratories, Chittagong which formerly cultivated, harvested and extracted essential oil by hydrodistillation. Chemical composition was analyzed and identified by GC-MS electron impact ionization (EI) method on GC-17A gas chromatography (Shimadzu, Japan) under BCSIR Laboratories, Chittagong[6].

Test organisms

The patchouli oil was tested for its antimicrobial activities against ten human pathogenic bacteria, two human pathogenic yeasts and four human pathogenic molds. Among ten human pathogenic bacteria, three were Gram positive, viz, *Bacillus subtilis* BTCC17, *Bacillus cereus* BTCC19, *Staphylococcus aureus* ATCC6538 and seven were Gram negative bacteria, viz., *Salmonella Typhi*

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AE14296, *Salmonella Paratyphi* AE14298, *Pseudomonas aeruginosa* ICDDR,B, *Shigella sonnei* ICDDR'B, *Shigella dysenteriae* AE14612, *Vibrio cholerae* AE14748, and *Escherichia coli* ATCC25922.The two human pathogenic yeasts were *Candida albicans* ATCC10231 and *Saccharomyces cerevisiae* (Proff. Scarsman, Australia) and the four human pathogenic molds were *Rhizopus oligosporus* ATCC22959, *Aspergillus flavus* ATCC9807, *Aspergillus fumigatus* ATCC16903, *Fusarium equisetti* ATCC15622. Bacterial test organisms were collected from the Department of Microbiology, University of Chittagong. Fungal cultures were collected from BCSIR Laboratories, Dhaka.

Determination of antibacterial and antifungal activity

In vitro sensitivity of the bacterial and fungal strains to the test materials was carried out by using Agar well diffusion method using growth media, e.g. Muller Hington Agar (MHA) for bacteria, Sabouroud Dextrose Agar (SDA) for yeast and Potato Dextrose Agar (PDA) for mold[10]. In order to perform the antimicrobial screening, colonies collected from each twenty-four hours bacterial culture were diluted in sterile saline and the optical density was adjusted according to the tube 0.5 of McFarland scale to prepare a standardized inoculum (1.5×10^8 cfu/ml)[11]. Forty-eight hours old culture of yeast from SDA media were used for preparing a standardized inoculum (1.5×10^6 cfu/ml) and spore suspension containing 1.5×10^8 cfu/ml of five days old culture of mold from PDA media were used. Fixed volumes (20 µl and 10 µl) of the essential oil of the plant was used. The control was 0.1ml of soybean oil. After plating and inoculation the plates were kept at low temperature (4ºC) for 2-4 hrs to allow maximum diffusion of the material. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel[12]. The agar cup-plates were incubated in an upright position and readings were then taken. The results were obtained by measuring the diameters of the zones of complete inhibition after 24 hrs at 37ºC for bacteria, 48 hrs at 37± 1ºC for yeast and 5 days 27 ± 2ºC for molds. Bacterial results were compared with standard bacterial antibiotic ampicillin (20 µg/well, Beximco Pharma Bangladesh Ltd, Dhaka) and fungal results were compared with standard fungal antibiotic Nystatin (20 µg/well, Beximco Pharma Bangladesh Ltd, Dhaka).

Determination of MIC, MBC and MFC

MIC, MBC and MFC of the essential oil of *Pogostemon cablin* against all the above test organisms were performed against all the teste organisms by using Macrobroth dilution technique[13]. Peptone broth (2%) for bacteria, Sabouroud Broth for yeast and Potato Dextrose Broth (PDB) for mold were used in MIC test. DMSO (Dimethyl sufoxide) was used to dissolve the essential oil and then diluted to the highest concentration ranging from 125 to 3000 ìg/ml in the case of bacteria and from 250 to 4000 µg/ml in the case of yeast. One ml of suspension (for bacteria containing approx. 1.5×10^6 CFU/ml and for yeast containing approx. 1×10^4 CFU/ml) of test organism was used as inoculum and control. After incubation at 37ºC for 24-48 hours for bacteria and at 25 ºC for 48 hours for yeast MIC results were recorded. For MBC testing Nutrient Agar (NA) medium for bacteria and SDA media for yeast were used. After incubation at 37ºC for 48 hours for bacteria and at 25ºC for 48 hours for yeast the lowest concentration that shows no colony or growth was determined as MBC. For MFC test, PDA used as basal medium and incubated at 27ºC for 5 days.

Results and Discussion

Patchouli oil obtained from *P. cablin* was screened for its *in vitro* antibacterial activity against ten human pathogenic bacteria comparing to standard antibacterial antibiotic Ampicillin. The results of the sensitivity test are summarized in Table 1. It was observed that the essential oil was more effective against all the test organisms when compared with Ampicillin. The zones of inhibition varied from a highest of 35 mm to a lowest of 20 mm using 20 µl and from a highest of 23 mm to lowest of 12 mm using 10 µl. The biger zone of inhibition was recorded against *Bacillus cereus* (35 mm) followed by *Shigella sonnei* (33 mm) whereas in case of Ampicillin (20 µg/well) the zone size were 25mm for *Bacillus cereus*. The lowest zones of inhibition were found against *Salmonella Typhi* and *E. coli* (20 mm) using 20 µl. Using 10µl concentration the highest zone was found against *Shigella sonnei* (23 mm) followed by *S. aureus* (20 mm) and *S. Paratyphi* (20 mm).

Table 1. *Antibacterial activity of essential oil of Pogostemon cablin*

Test Bacteria	Zones of inhibition (mm in diameter)		
	Dose (µl/well) of essential oil		
	10	20	Ampicillin (20 µg/well)
Bacillus subtilis	15	30	22
Bacillus cereus	20	35	25
Staphylococcus aureus	20	25	20
Salmonella typhi	15	20	25
Salmonella paratyphi	20	30	30
Pseudomonas aeruginosa	17	25	19
Shigella sonnei	23	33	20
Shigella dysenteriae	12	24	25
Vibrio cholerae	22	30	24
Escherichia coli	16	20	15

The MIC values of the essential oil varied against different test bacteria ranging from 250 µg/ml to 1000 µg/ml (Table 2). The results showed that *Bacillus cereus* and *Salmonella Paratyphi* exhibited the lowest MIC (250 µg/ml). Their MBC (750 µg/ml) was higher than MIC which means that this oil is bacteriostatic for these bacteria. MIC against *B. subtilis*, *S. dysenteriae* and *Pseudomonas aureginosa* were found to be (500 µg/ml) and MBC the highest resistance at 750 µg/ml, 1000 µg/ml and 500 µg/ml respectively. *Salmonella typhi* showed resistance against this oil, because the MIC was 4500 µg/ml for this bacterium, which was higher than others. No MBC was found for this bacterium within the range of 4500 µg/ml.

Table 2 *MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacteriocidal Concentration) of essential oil (EO's) of Pogostemon cablin against test bacteria*

Test bacteria	Dose (concentration of EO's in µg/ml)	
	MIC (µg/ml)	MBC (µg/ml)
Bacillus subtilis	500	750
Bacillus cereus	250	750
Staphylococcus aureus	1000	1250
Salmonella Typhi	-*	-*
Salmonella Paratyphi	250	750
Pseudomonas aeruginosa	500	500
Shigella sonnei	750	750
Shigella dysenteriae	500	1000
Vibrio cholerae	750	750
Escherichia coli	500	750

* - *Not detected*

In the present study it was found that both gram-positive and gram-negative bacteria (except *S. typhi*) were susceptible to the oil. It can be noted that, an important characteristic of essential oil and their components is their hydrophobicity, which enables them to partition in the lipids of the bacterial cell membrane, disturbing the structures and rendering them more permeable[14]. Leakage of ions and other cell contents can then occur. *Bacillus cereus* was the most susceptible bacterium. This may attributed to the presence of single membrane of the organism that makes it more accessible to permeation by active constituents of this oil. In contrast, *Salmonella typhi* and *E. coli* showed least susceptibility (lowest zone of inhibition) to this oil. This may be due to the presence of outer membrane that serves as an effective barrier in gram-negative species[15-16]. Previous studies revealed that many terpenes, terpenoid, sesquiterpenoids show strong antibacterial activity. Patchouli alcohol is a terpene, α- patchoulene is a terpenoid and α-bulnesene is a sesquiterpenoid that could be considered as answerable for the antimicrobial activity. In a brief, we can say that, the oil has showed strong bacteriocidal effect except *S. typhi.*

The results of *in vitro* antifungal activity of patchouli oil against six human pathogenic fungi by agar cup method with 10 and 20µl are presented in Table 3. The oil exhibited strong antifungal activity against all the tested fungi, but inhibition of the mycellial growth were more remarkable against four human pathogenic molds than the pathogenic yeasts. *Candida albicans* (16 mm) showed greater sensitivity against this oil than *S. cerevisiae* (14 mm) with 20 µl. At 10 µl *Candida albicans* showed 8 mm of zone of inhibition, where *Saccharomyces cerevisiae* showed 7 mm zone of inhibition. On the case of mold, almost all strains showed sensitivity against this oil. In most cases, the oil exhibited better antifungal activity than the standard antibiotic nystatin. In case of nystatin the highest zone was 25 mm for *A. flavus.* For oil it was 25 mm for each fungal strain with 20 µl except for *Rhizopus oligosporus* (15 mm). At 10 µl *F. equisetti* showed highest zone of inhibition (12 mm) followed by *A. flavus* (11 mm).

Table 3 *Antifungal activity of essential oil of Pogostemon cablin*

Test organisms	Zones of inhibition (mm in diameter)		
	Dose (µl/well) of essential oil		
	10	20	Nystatin (20µg/well)
Candida albicans	8	15	15
Saccharomyces cerevisiae	7	15	12
Rhizopus oligosporus	7	16	20
Aspergillus flavus	11	25	25
Aspergillus fumigatus	10	25	23
Fusarium equisetti	12	25	21

The MIC values against different test fungi are presented in Table 4. In case of yeasts, *Candida albicans* showed lower MIC and MFC (both were 750 µg/ml) than *Saccharomyces cerevisiae*, which were 1500 µg/ml and 2500 µg/ml, respectively. In case of molds, the lowest MIC (250 µg/ml) and MFC (500 µg/ml) were reported for *A. fumigatus.* Though *Rhizopus oligosporus* showed lowest zone of inhibition (16mm), its MIC was 500 µg/ml, which was lower than *A. fumigatus* and *Fusarium* sp., but it showed MFC 3000 µg/ml which was same as *A. flavus.* This means that this oil inhibits the growth of *Rhizopus oligosporus* at low concentration, but does not kill this fungus. For killing it require higher dose as same as *A. flavus*. The low MIC of the essential oil against fungal strains indicates that the main compounds present in the oil, patchouli alcohol, a terpene hydrocarbons had a stronger antifungal activity. In addition, α-caryophyllene and caryophyllene oxide, detected in our experiments, could be responsible for this property[17]. In brief we can say that the oil has moderate fungicidal effect that may provide a renewable source for useful as bacteriocidal and fungicidal drugs.

Table 4 *MIC (Minimum Inhibitory Concentration) and MFC (Minimum Fungicidal Concentration) of essential oils (EO's) of Pogostemon cablin against test fungi*

Test organisms	Dose (concentration of EO's at µg/ml)	
	MIC (µg/ml)	MFC (µg/ml)
Candida albicans	750	750
Saccharomyces cerevisiae	1500	2500
Rhizopus oligosporus	500	3000
Aspergillus flavus	2000	3000
Aspergillus fumigatus	250	500
Fusarium equisetti	1500	1500

Conclusion

The essential oil of *P. cablin* is a natural product that can be used against antibiotic resistance organisms and also could be used in food preservation. It may provide tomorrow's antibiotic source for useful bactericidal and fungicidal drugs that can be utilized against many food borne pathogens, in many opportunistic infection *e.g.* against *Candida* spp. in oral candidiasis as well as against *A. fumigatus* and *A. flavus* infection in patients suffering from pulmonary tuberculosis. It would be a valuable source to find out leading compounds having antimicrobial activity.

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