World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Available online at: http://www.wjpsonline.org/
Original Article

Comparative antimicrobial activity of *pogostemon cablin* (patchouli) essential oil (PEO) and conventional antimicrobials against clinically important microbes

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Received: 14-06-2019 / Revised Accepted: 24-07-2019 / Published: 01-08-2019

ABSTRACT

Pogostemon cablin (patchouli), an important herb with multiple uses, has antimicrobial activity in its infusions, extracts and oil. Patchouli essential oil (PEO), an important perfumery ingredient has till date been tested for its potential antimicrobial activity on a limited number of strains. Inquest to determine the antimicrobial spectrum and real potential of PEO as an antimicrobial using data on 4598 bacteria and 67 fungi of 74 genera isolated from clinical infections and associated environment, this analytical study was conducted. Almost 3/4th of isolates tested were resistant PEO. The PEO had poor antimicrobial activity against most the common groups of pathogenic bacteria. However, PEO was active against most of the *Aggregatibacter, Acinetobacter, Actinomyces, Moraxella, Dermatophilus* and *Staphylococcus* species strains often associated with topical infections. Antimicrobial activity of PEO was significantly (p, <0.01) better against oxidase and Gram-positive (O+G+) bacteria than against O-G- bacteria. The MIC of PEO varied greatly from one part million (ppm) to more than 10000 ppm for members of the different species of bacteria. The study indicated that the potential of PEO can be explored further for developing an alternative antimicrobial therapy against topical infections.

Keywords: Herbal antimicrobials, ESKAPE pathogens, *Enterobacter, Escherichia coli, Bacillus, Staphylococcus, Streptococcus, Pseudomonas, Acinetobacter, Salmonella, Aeromonas*

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How to Cite this Article: Bhoj Raj Singh, Dharmendra Kumar Sinha, Vinodh Kumar O.R, Abhijit M. Pawde, Ujjwal Kumar D.E, Vinod Kumar Gupta. Comparative antimicrobial activity of *Pogostemon cablin* (patchouli) essential oil (PEO) and conventional antimicrobials against clinically important microbes. World J Pharm Sci 2019; 7(8): 47-65.

INTRODUCTION

Pogostemon cablin (patchouli), a plant of Lamiaceae, grows well in cultivated as well as wild forms in Southeast Asia [1]. It is a widely used herb in the form of decoction, infusion and spirits in many parts of Southeast Asia and South Asia to cure nausea/ emesis, common cold, diarrhoea, pyrexia, chronic fatigue and headache and as an appetizer [2-11]. Earlier studies have revealed a variety of pharmacological activities like anti-oxidant, anti-emetic, anti-inflammatory, analgesic, antimutagenic, antithrombotic, immunomodulatory and antimicrobial in its essential oil (PEO) extracted from leaves [11-15]. The PEO has been reported in recent past as neuroprotective, termite repellent, antiviral, antibacterial and antifungal agent [16-24]. Besides essential oil (PEO), antimicrobial activity of patchouli have also been reported in patchouli alcohol against *Staphylococcus* and *Streptococcus* strains [25], in aqueous and alcoholic extracts of patchouli leaves against *Enterobacter aerogenes, Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Streptococcus pyogenes* [26-27].

Except a few [28-32], most of the studies on antimicrobial activity of PEO and other patchouli preparations are conducted on a few strains that too on the reference strains isolated long ago and stored for long period in laboratory conditions [19-22, 26, 27]. Only a few studies have reported activity of patchouli essential oil or its extract on known multiple drug-resistant bacteria [25], that too only on a limited number of isolates of *Staphylococcus* [23, 24], *Pseudomonas* [24] and a few members of Enterobacteriaceae [25]. A few more studies on fresh bacterial isolates [28-32] were also targeted on bacteria associated with specific disease or source or food. Therefore, this study was designed to evaluate PEO for its antimicrobial potential and spectrum of activity against a sizeable number of clinically important bacteria isolated from clinical cases and associated environment having different types of drug resistance patterns.

MATERIALS AND METHODS

Patchouli essential oil: Pure patchouli essential oil (PEO) was purchased from Naga Fragrance Ltd. Dimapur, India and was stored at room temperature (25-27^{o}C) throughout the study period.

Microbial strains: A total of 4598 isolates of bacteria belonging to 68 genera of different groups of bacteria (Table. 1) isolated from different sources (Table. 2) during 2011 to 2018 and available at Epidemiology Laboratory of Indian Veterinary Research Institute, Izatnagar were tested for purity on blood agar and identity through morphological, cultural and biochemical characterization [33, 34]. All bacterial isolates were stored in buffered glycerol stock at –20^{o}C and on nutrient agar slants as described earlier [34] till tested for their sensitivity to PEO and selected antibiotics within 3-5 days of their isolation.

A total of 67 fungal isolates belonging to six genera (Table 3) and isolated from different sources (Table. 2), tested for purity and identified at Mycology laboratory of Indian Veterinary Research Institute using conventional morphological, growth and fermentation characteristics [33] were revived. All isolates were maintained on Mueller Hinton agar (MHA, BBL, Difco, USA) slants till tested for their sensitivity to PEO.

Antimicrobial sensitivity assay: Disc diffusion method was performed following guidelines of CLSI (35) to determine the sensitivity of the isolates of bacteria using standard antimicrobial discs (BBL, Difco, USA). Bacterial isolates were tested against amoxicillin + clavulanic acid, ampicillin, aztreonam, cefepime, cefotaxime, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, colistin, cotrimoxazole, erythromycin, gentamicin, linezolid, moxalactam, nitrofurantoin, oxacillin, penicillin, piperacillin, tetracycline, tigecycline, and vancomycin on MHA plates. For fastidious organisms including *Avibacterium, Bordetella, Brucella, Campylobacter, Gallibacterium, Listeria, Moraxella, Pasteurella* and *Streptococcus* species isolates bovine serum added (5%) MHA was used instead of MHA. For testing, bacterial isolates were grown in trypticase soy broth (TSB, BBL Difco) overnight under a suitable environment at 37^{o}C and then cultures were suitably diluted to the optical density of 0.1 at 590 nm. The suitably diluted culture was swab inoculated on to the desired MHA plates in duplicate and antibiotic discs were applied. After suitable incubation, the diameter of the zone of growth inhibition around antimicrobial discs was measured in mm. The sensitivity of the bacterial strains was determined using cut off limits as per CLSI guideline [35] where so ever applicable. For bacteria not having reference limits in CLSI guidelines, limits used for *E. coli* and *S. aureus* were used for Gram-negative (GNBs) and Gram-positive bacteria (GPBs), respectively. A reference sensitive *E. coli* strain (E-382) available in the laboratory was used as control.

Antimicrobial sensitivity assay for PEO: All bacterial and fungal isolates were tested for their sensitivity for PEO using disc diffusion assay on MHA plates or bovine serum added MHA (for fastidious organisms including *Avibacterium, Bordetella, Brucella, Campylobacter, Gallibacterium, Listeria, Moraxella, Pasteurella* and *Streptococcus* species isolates) as described above for bacterial strains. Filter paper discs (6 mm diameter cut from Whatman Filter No. 3) were loaded with suitably diluted PEO so that each disc contained 1 µL of PEO [36]. The PEO discs were stored at 4^{o}C throughout the study. The control

reference sensitive *E. coli* strain (E-382) available in the laboratory was used throughout the study. Any measurable zone around the PEO disc was taken as an indication of the sensitivity of the microbe for PEO.

Determination of minimum inhibitory concentration (MIC) of PEO for microbes: A total of 80 strains of 21 species of bacteria were tested for determining MIC of PEO using agar well diffusion assay [36]. To determine MIC, nine wells of 6 mm diameter each were cut in MHA plates under sterile environment and bottoms of wells were sealed with the same medium. Culture prepared for antimicrobial sensitivity assay for test microbe (described earlier) was swab inoculated and wells were filled with 50 µL of serially diluted herbal antimicrobial in sterile dimethyl sulphoxide (DMSO, SDFCL, India) so that well number one to nine in plate one contained 1, 2, 4, 8, 16, 32, 64, 128 µL and 256 µL of the PEO, and in second plate contained 128, 256, 512, 1024, 2048, 4096, 8192, 10000 µL and 0 µL of the PEO, respectively. In the second plate 9th well was filled with DMSO without MEO as a negative control. Plates were incubated under appropriate growth conditions for 2 h without inversion to get the oil absorbed in the medium and then overnight after inversion in an appropriate environment required for the optimum growth of the microbe. Measurable zone of growth inhibition around the well containing the highest dilution of herbal antimicrobial was marked as MIC value for the microbe. Tests were conducted in triplicate for confirmation.

Statistical analysis: For finding the difference in sensitivity patterns of microbes of different genera and sources, odds ratio and Chi-square ($\chi 2$) tests were used. To determine similarity in the activity of PEO with different antimicrobials on different microbes correlation coefficient (r) was calculated using MS Excel worksheet.

RESULTS

PEO as antimicrobial in relation to conventional drugs: In the study on 4665 cultures of different microbes (Table. 1) from diverse sources (Table 2) belonging to 74 genera (Table. 3) tested for sensitivity to PEO and conventional antimicrobials, 76.8% were not inhibited by discs containing 1µL of PEO, 12.4% were carbapenem-resistant (CR), 49.8% produced extended spectrum β-lactamases (ESBL) and 50.6% were resistant to three or more group of antibiotics commonly used for the treatment.

In the current study, irrespective of type of bacteria, the most effective antimicrobial was tigecycline followed by chloramphenicol, gentamicin, moxalactam, cefepime, nitrofurantoin, cefotaxime, ciprofloxacin, ceftriaxone, colistin, ceftazidime, linezolid, amoxiclav, tetracycline, cotrimoxazole, piperacillin, aztreonam, clindamycin, ampicillin, penicillin, erythromycin, oxacillin, and vancomycin (Table 4). In general, GPBs were often more commonly sensitive to most of the antimicrobials than GNBs. Tigecycline was the most effective (97.2%) on GPBs followed by linezolid (93.8%), nitrofurantoin (87.9%) and chloramphenicol (86.9%) while other antimicrobials could inhibit less than 78% GPBs, and aztreonam was the least effective (17.7%) antibiotic on GPBs. Oxacillin resistance, an indicator of methicillin resistance, was one of the most common types of resistance in GPBs. Tigecycline, chloramphenicol and gentamicin could inhibit ≥80% of GNBs in the study and ampicillin being the least effective inhibited only 30.9% isolates of GNBs. On oxidase positive bacteria (OPBs), four drugs including gentamicin (89.8%), ciprofloxacin (83.6%), tigecycline (82.1%) and colistin (80.3%) were effective to limit the growth of >80% of the bacteria (Table. 4). However, only tigecycline (95.4%) and chloramphenicol (87.6%) could inhibit more than 80% of the ONBs and PEO was one of the least effective (19.3%) antimicrobials. On 2328 MDR isolates, tigecycline was the most effective (89.5% isolates inhibited) followed by chloramphenicol (75%) while none of the other antimicrobials inhibited more than 70% of the isolates tested and PEO as well as ampicillin failed to inhibit even 20% of the MDR isolates. The ESBL producer isolates (1788) were the most often sensitive to tigecycline (92.8%) followed by ceftriaxone (86.4%) and chloramphenicol (77%) while other antimicrobials were effective on less than 75% of the isolates. The PEO was one of the least effective antimicrobials on ESBL producers inhibiting only 20.4% of the isolates, even colistin also failed on >53% ESBL positive isolates. Except for tigecycline none of the antimicrobials was effective to inhibit >73% isolates resistant to carbapenem group of antibiotics and PEO inhibited only 18% CR isolates in the study.

Sensitivity pattern and source of isolation of bacteria: Isolates from contaminated biologicals and food samples (≥50%) were significantly (p, <0.01) more often PEO sensitive than isolates from semi-domestic mithuns (8.7%), laboratory animals (5%) and fish (0.0%). An almost similar pattern was recorded for carbapenem resistance (CR) and MDR, the most CR resistant isolates were from fish (45.5%) and most sensitive were of non-domestic birds (100%) and mithuns (98.8%). However, ESBL production ability was most commonly detected in bacteria isolated from contaminated biologicals (92.3%) followed by fish (72.7%) and laboratory animals (66.5%) and the least count was in non-domestic birds (0%) and mithuns (7.1%).
Analysis of 3291 isolates from animals with different food habits revealed no significant (p, >0.05) difference in their sensitivity to PEO or ESBL production potential. However, resistance to multiple drugs (MDR) and CR was more often (p,

<0.05) detected in isolates from carnivores followed by omnivores and herbivores (Table. 2).
Of 3430 isolates from clinical samples, isolates associated with pyrexia (23) of unknown origin (PUO) were more often sensitive (56.5%) while >85% isolates associated with gastrointestinal disorders were resistant to PEO. However, PEO sensitivity of bacterial isolates associated with respiratory, urinary, genital and gastrointestinal tract infections not differed significantly (p, >0.05) than isolates from apparently healthy animals (84.5% resistant) but PEO resistance in those isolates was significantly (p, <0.02) more common than in isolates associated with PUO, skin, eye and ear infections (Table. 2). Similar to sensitivity to PEO, only 4.3% isolates from PUO cases had CR but >20% isolates from apparently healthy, gastrointestinal and urinary tract infections were resistant to one or more carbapenems. Almost similar to CR was the pattern for ESBL production and MDR potential among bacteria of different origin.

Comparative sensitivity of different types of bacteria: Gram-positive bacteria were significantly (p, <0.01) more often sensitive to PEO (48.5%) and less often produced ESBL (40.2%) than GNBs (13.2% and 53.7%) but difference was insignificant with respect to their carbapenem and multidrug-resistance (p, >0.05). Similarly, oxidase positive bacteria were more commonly (p, <0.01) sensitive to PEO (36.1%) than oxidase negative bacteria but no such difference was evident for ESBL production and carbapenem resistance (p, >0.05). However, MDR was more often (p, <0.01) observed in oxidase negative bacteria (52%) than in oxidase positive bacteria (45.1%). Yeast had 5.1 times more odds (p, 0.01, 2.5-10.4) of being sensitive to PEO than bacteria but no significant (p, >0.05) difference was evident between bacteria and moulds. Among all groups of bacteria, oxidase-positive and Gram-positive (O+G+) bacteria group was among the most sensitive groups for PEO (p, <0.01) followed by O-G+, O+G-, O-G- bacteria.
Among 4665 strains of 74 different genera of microbes, a wide variation in sensitivity to PEO was observed (Table. 3). All the 61 strains of microbes belonging to 22 genera *Achromobacter, Campylobacter, Geotrichum, Gordonia, Haemophilus, Leclercia, Listeria, Morganella, Obesumbacterium, Ochrobacterium, Pediococcus, Plesiomonas, Providencia, Rhodotorula, Roseomonas, Shewanella, Sphingomonas, Streptobacillus, Trichophyton, Xanthomonas, Xanthorhabdus,* and *Yersinia* were resistant to PEO (Table. 2). Whereas, isolates of 11 genera (*Aggregatibacter, Arsenophonus, Branhamella, Brevibacillus, Corynebacterium, Cytophaga, Dermatophilus, Ewingella, Lactobacillus, Paenibacillus,* and *Stomatococcus*), were sensitive to PEO). Among the genera where more number of isolates tested members of Enterobacteriales (earlier Enterobacteriaceae) (*Citrobacter, Salmonella, Proteus, Escherichia, Erwinia, Enterobacter, Klebsiella* and *Edwardsiella*), aeromonads, pseudomonads and enterococci were significantly (p, <0.05) more often resistant to PEO than members of *Pasteurella, Alcaligenes, Brucella, Streptococcus, Acinetobacter, Staphylococcus,* and *Bacillus* genus. Most of the *Citrobacter* isolates were resistant and most of the *Bacillus* isolates were sensitive to PEO (p, <0.001; OR, 114; CI, 23.84-546.81).
None of the 131 isolates from 32 genera (*Campylobacter, Gordonia, Leclercia, Listeria, Obesumbacterium, Ochrobacterium, Pediococcus, Plesiomonas, Sphingomonas, Streptobacillus, Xanthomonas, Yersinia, Kluyvera, Hafnia, Agrobacterium, Avibacterium, Leminorella, Gallibacterium, Vibrio, Flavobacterium, Geobacillus, Aggregatibacter, Arsenophonus, Branhamella, Brevibacillus, Corynebacterium, Cytophaga, Dermatophilus, Ewingella, Lactobacillus, Paenibacillus, Stomatococcus*) was resistant to carbapenems while CR resistance was detected in varying proportion of strains of 36 genera (*Haemophilus, Roseomonas, Shewanella, Actinomyces, Acinetobacter, Achromobacter, Pseudomonas, Morganella, Actinobacillus, Raoultella, Proteus, Bordetella, Streptococcus, Aeromonas, Alcaligenes, Enterococcus, Moraxella, Staphylococcus, Budvicia, Escherichia, Burkholderia, Micrococcus, Erwinia, Providencia, Enterobacter, Xanthorhabdus, Klebsiella, Edwardsiella, Serratia, Aerococcus, Brucella, Bacillus, Pragia, Salmonella, Pasteurella, Citrobacter*), being common in *Acinetobacter* and rare in *Citrobacter* (p, <0.01; OR, 184.7; CI, 12.7-2712.9) strains (Table. 3).

None of 62 isolates of *Roseomonas, Shewanella, Budvicia, Pragia, Campylobacter, Gordonia, Plesiomonas, Streptobacillus, Yersinia, Kluyvera, Avibacterium, Branhamella,* and *Stomatococcus* species produced ESBL while all the 20 isolates in the study belonging *Haemophilus, Morganella, Listeria, Obesumbacterium, Ochrobacterium, Pediococcus, Sphingomonas, Xanthomonas, Leminorella, Brevibacillus, Corynebacterium, Cytophaga,* and *Paenibacillus* species expressed ESBL activity. However, among strains of other genera tested for ESBL activity wide variation (15.6% to 78.1%) was observed (Table. 3), the most common in *Citrobacter* and least common in *Brucella* species isolates (p, <0.001; OR, 16.3; CI; 3.8-69.7).

None of the 26 isolates belonging to 16 genera (*Leclercia, Ewingella, Lactobacillus, Haemophilus, Listeria, Pediococcus, Sphingomonas, Cytophaga, Paenibacillus, Aggregatibacter, Campylobacter, Plesiomonas, Streptobacillus, Yersinia, Stomatococcus*) was resistant to more than two antimicrobials tested while MDR was detected in isolates of other 53 genera in the study to a variable (8.6% to 100%) extent (Table. 3). On comparing

the commonly isolated bacteria in the study, MDR was significantly (p, <0.05) more common among isolates of *Proteus, Pseudomonas, Escherichia, Alcaligenes, Acinetobacter, Staphylococcus, Streptococcus, Enterococcus,* and *Brucella* species than among isolates belonging to *Enterobacter, Erwinia, Aeromonas, Klebsiella, Bacillus, Edwardsiella, Pasteurella, Salmonella* and *Citrobacter* species. The MDR was the most common among *Proteus* and the least common among *Citrobacter* isolates, although both belong to the same order (p, <0.001; OR, 23.9; CI; 9.4-61.2).

On further analysis on bacterial genera with >50 isolates tested in the study (*Acinetobacter, Aeromonas, Alcaligenes, Bacillus, Citrobacter, Edwardsiella, Enterobacter, Enterococcus, Erwinia, Escherichia, Klebsiella, Pasteurella, Proteus, Pseudomonas, Salmonella, Staphylococcus, Streptococcus*) the difference in the antimicrobial activity of different antibiotics was evident (Table. 5). Tigecycline was the most effective antibiotic against isolates of most the genera but 64.8% of the pseudomonads followed by *Proteus* (18.2%), *Klebsiella* (14%) and *Enterobacter* (10.3%) species strains were resistant while all the *Bacillus, Citrobacter* and *Pasteurella* species isolates were sensitive to tigecycline. Among *Acinetobacter* (54) isolates, 46.3% were resistant to carbapenems but 91.8%, 84.9% and 76.5% were sensitive to tigecycline, gentamicin and chloramphenicol, respectively, while PEO inhibited only 40.7% isolates of *Acinetobacter*, much more than aztreonam (38%), nitrofurantoin (36.5%) and ampicillin (36%).

Aeromonads (191) were sensitive to many of the antibiotics (Table. 5), the most effective antibiotic was ceftriaxone inhibiting 93.2% isolates followed by gentamicin (93%), tigecycline (92.5%), cefotaxime (91.6%), chloramphenicol (91.4%), but PEO failed to control growth of 82.2% isolates.

The most effective antibiotic on *Alcaligenes* isolates (63) was tigecycline inhibiting 92.2% isolates followed by gentamicin (92.1%), colistin (87.7%), and chloramphenicol (85.7%), PEO failed on 73% isolates, more than ampicillin (58.2%).

Bacillus isolates (187) were one of the most sensitive groups of bacteria, all susceptible to tigecycline but 4.3% were resistant to carbapenems and 26.7% to PEO. However, *Bacillus* species isolates more often resistant to cefalosporins as 42.9% were resistant to cefepime (4th generation) and 76.8% to ceftazidime.

All isolates (128) of *Citrobacter* species were sensitive to tigecycline and moxalactam but 97.7% and 100% were resistant to PEO and penicillin, respectively. On *Edwardsiella* isolates (50) ceftazidime was the most effective antibiotic (inhibited 94.1% isolates) followed by carbapenems, tigecycline, cefotaxime, chloramphenicol and gentamicin inhibiting >90% isolates, tetracycline a drug of choice also inhibited 87% of edwardsiellae but PEO failed to inhibit 86% of edwardsiellaee.

On *Enterobacter* species isolates (300) instead of tigecycline (89.7%), gentamicin was the most effective (91.3%) antibiotic followed by carbapenems (91%) while PEO could inhibit only 11.7% of the isolates.

On enterococci (227) tigecycline was effective on >98% of the isolates and the next were linezolid (89.4%), carbapenems (84.1%), chloramphenicol (81.2%) and vancomycin (74.6%) while PEO failed to inhibit the growth of 76.2% of the isolates.
More than 90% of *Erwinia* species isolates (86) were inhibited by ceftriaxone (98.5%), cefepime (96.8%), moxalactam (95.8%), tigecycline (93.8%), cefotaxime (93.2%), chloramphenicol (93%), gentamicin (93%) and ciprofloxacin (90.7%). But PEO inhibited only 10.5% isolates even less than penicillin (15%). However, only tigecycline could inhibit >90% of the *Escherichia* species isolates (1356), and PEO (9%) was more effective than penicillin (5.8%).

More than 90% klebsiellae strains (246) were inhibited by chloramphenicol (93.7%), moxalactam (92.9%) and carbapenems (92.3%) while tigecycline inhibited only 86% of the isolates and PEO failed to inhibit 86.2% isolates.
Four antibiotics including moxalactam, gentamicin, tigecycline and colistin inhibited all 50 isolates *Pasteurella* species and eight antibiotics (carbapenems, 98%; ceftriaxone 97.1%; amoxicillin+ clavulenic acid, 96.6%; nitrofurantoin, 96%; chloramphenicol, 94%; ciprofloxacin, 94%; piperacillin, 93.3%; and cefepime, 92.3%) inhibited >90% of isolates. However, PEO failed to inhibit 76% of the isolates.

Proteus isolates (143) were among the most resistant types of bacteria tested, even the most effective tigecycline could inhibit only 81.8% of the isolates, next in affectivity were moxalactam (80.8%) and carbapenems (79.1%) while colistin (8%) and PEO (6.3%) were among the least effective antimicrobials.

On pseudomonads (189) most of the high-end antimicrobials failed to inhibit > 80% of the isolates but gentamicin (85.9%), colistin (85.7%) and ciprofloxacin (80.6%) hold the promise. Even carbapenems and 4th generation cephalosporins (cefepime) failed to contain the growth of 26.5% and 34.1% of the isolates, respectively. However, PEO was more effective (22.8%) than ampicillin (22%).

Salmonella, a common pathogen in India, was quite susceptible to most of the antimicrobials and 10 antibiotics (carbapenems 97.5%, ceftriaxone 97.4%, chloramphenicol 97.2%, tigecycline 97.1%, cefepime 97.1%, moxalactam 97.1%, cotrimoxazole 95.3%, aztreonam 94.6%, cefotaxime 94.5%, and gentamicin 94.1%) inhibited >90% of the isolates but >55% were of MDR type and PEO inhibited only 4.2% of the isolates tested (119).

Only tigecycline (96.7%), linezolid (96.5% and nitrofurantoin (93%) were the antimicrobial inhibiting >90% of the staphylococci isolates tested (458), vancomycin resistance was as common as PEO (>44%) resistance and oxacillin was one of the least effective antibiotic (15%) and almost 51% isolates were resistant to ciprofloxacin.

On streptococci (286), tigecycline (97.3%) and linezolid (96.5%) inhibited >90% isolates, next in affectivity were the chloramphenicol (86.8%), nitrofurantoin (85.5%) and vancomycin (84.5%). Gentamicin, often considered a drug of choice, was ineffective in controlling the growth of >40%, and PEO failed on >63% isolates.

Sensitivity to PEO not only varied among microbes of different genera but within species of the same genus viz., *Acinetobacter calcoaceticus* isolates were significantly (p, ≤0.05) more often resistant to PEO than isolates of *A. lwoffii* and *A. schindleri. Aeromonas bestiarum, A. sobria* and *A. hydrophila* isolates were more (p, ≤ 0.04) commonly sensitive to PEO than *A. veronii* isolates. Similarly, *Enterococcus faecium* isolates were more often (p, 0.02) resistant than *E. raffinosus* isolates and, *Pseudomonas aeruginosa* and *P. fluorescens* isolates were more often (p, ≤0.02) resistant than *P. paucimobilis* isolates to PEO. Among staphylococci, *S. sciuri* isolates were more often (p, ≤0.05) resistant to PEO than isolates of *S. aureus, S. hyicus, S. haemolyticus*, and *S. intermedius* isolates.

Among streptococci, *S. porcinus* strains were the most resistant ones followed by *S. mobilis, S. pyogenese* (p, 0.03) and the *S. equi* ssp. *equisimilis* strains were the most often sensitive to PEO among all (p, <0.03).

For carbapenems, the last resort antibiotics, *Aeromonas hydrophila* isolates were more often (p, 0.04) resistant than *A. salmonicida, A. veronii* isolates. Among enterococci, *E. avium, E. gallinarum* and *E. caecorum* isolates were more often sensitive to carbapenems than *E. faecalis, E. faecium, E. raffinosus*, and *E. malodoratus* (p,≤0.02) isolates. *Proteus mirabilis* isolates were more often carbapenem resistant than *P. mirabilis* and *P. penneri* isolates. *Staphylococcus aureus* isolates were more often resistant to carbapenems than *S. epidermidis* (p, 0.04), and *S. xylosus* and *S. haemoyticus* isolates were more commonly carbapenem resistant (CR) than *S. capitis* ssp. *capitis* and *S. chromogenese* (p, ≤0.03) isolates. Isolates of *Streptococcus mobilis* were less often CR than *S. equi* ssp. *equisimilis, S. equi* ssp. *zooepidemicus, S. pneumoniae* and *S. pyogenes* (p, ≤0.02) isolates.

Aeromonas salmonicida isolates were more often tigecycline resistant (TR) than *A. bestiarum* and *A. media* isolates (p, ≤0.01) while TR was more often detected in isolates of *Pseudomonas areuginosa* than in isolates of *P. fluorescens* and *P. paucimobilis* (p, ≤0.002) but *Staphylococcus epidermidis* isolates were more often sensitive to tigecycline than *S. xylosus* ((p, 0.04) isolates in the study. *Streptococcus aglactiae* were more often TR than isolates of *S. mobilis* (p, 0.03), and isolates of *S. equi* ssp. *zooepidemicus* were more often tigecycline sensitive than *S. pneumoniae* (p, 0.02).

Linezolid, an effective antibiotic against Gram-positive cocci, was more often inactive against *Enterococcus gallinarum* than isolates of *E. caecorum, E. faecalis, E. malodoratus* and *E. raffinosus* (P, <0.04). *Streptococcus equi* ssp. *zooepidemicus* were more often linezolid sensitive than *S. pneumoniae* isolates (p, 0.01).

Cefepime, the 4th generation cephalosporin, was more often active on *Aeromonas media* than on *A. sobria* isolates (p, 0.02) and also more active on *Enterococcus caecorum, E. faecalis* isolates than on isolates of *E. malodoratus, E. gallinarum, E. faecium* and *E. raffinosus* (p, <0.02). *Proteus penneri* isolates were more often cefepime sensitive than *P. mirabilis* and *P. vulgaris* isolates (p, ≤0.03). *Pseudomonas paucimobilis* and *P. fluorescens* were more often resistant to cefepime than *P. aeruginosa* (p, <0.03) but *Staphylococcus aureus, S. capitis* ssp. *capitis, S. capitis* ssp. *urealyticus, S. chromogenes, S. epidermidis, S. haemolyticus, S. hyicus, S. intermedius, S. lentus* and *S. xylosus* isolates were more (p, <0.05) often sensitive to cefepime than isolates of *S. sciuri*. Though among streptococci cefepime sensitivity not varied much among strains of different species, more of *S. porcinus* isolates were cefepime resistant than *S. agalactiae* isolates (p, 0.04).

Colistin, a high-end antibiotic for Gram-negative bacterial infections, was more active on *Acinetobacter calcoaceticus* and *A. lwoffii* than on *A. schindleri* isolates (p, <0.05), similarly *Aeromonas media* isolates were more often sensitive than *A. bestiarum, A. hydrophila, A. sobria*, and *A. veronii* isolates (p, <0.05). *Edwardsiella tarda, Enterobacter gregoviae* and *Pseudomonas fluorescens* isolates were more commonly resistant to colistin than *E. hoshniae* (p, 0.02), *E. agglomerans* (p, 0.03), and *P. aeruginosa* (p, <0.001) isolates, respectively.

Vancomycin-resistant enterococci (VRE) were common in the study (25.4%), however, *E. caecorum* were more often vancomycin sensitive (all 34 tested) than *E. avium, E. faecalis, E. faecium, E. gallinarum, E. maloodoratus* and *E. raffinosus* isolates (p, <0.01). On the other hand >44% staphylococci were VR, *Staphylococcus capitis* ssp. *capitis* were more often vancomycin sensitive than *S. aureus* (p, 0.05), and *S. hyicus* (p, 0.03). Vancomycin resistance in streptococci (15.5%) was significantly less common than in *Staphylococcus* and *Enterococcus* species isolates (p, <0.01), 21.4% *S. porcinus* isolates had vancomycin resistance significantly more often than in *S. equi* ssp. *equisimilis* (12%) isolates (p, 0.04) but not varied much among other species.

Correlation between antibiotic and PEO sensitivity of bacteria: Growth inhibition zones (sensitivity) of bacteria to most of the antibiotics had significant (p, <0.01) positive correlation with their sensitivity to PEO. However, sensitivity to aztreonam (p, 0.01; r, -0.19) had a significant negative correlation with PEO activity. The strongest positive correlation of antimicrobial activity of PEO was with antibiotics more active on Gram-positive bacteria followed by broad-spectrum antibiotics, viz., erythromycin (r, 0.42), oxacillin (r, 0.37), clindamycin (r, 0.37), penicillin (r, 0.36), linezolid (r, 0.35), ampicillin (r, 0.31), amoxicillin + clavulanic acid (r, 0.29), nitrofurantoin (r, 0.26), tigecycline (r, 0.26), tetracycline (r, 0.24), vancomycin (r, 0.23), piperacillin (r, 0.19), cefotaxime (r, 0.15), gentamicin (r, 0.13), ceftriaxone (r, 0.12), chloramphenicol (r, 0.12), and streptomycin (r, 0.11). Significant (p, 0.05) but less positive (r, 0.08) correlation with PEO was also evident with carbapenems, moxalactam and cotrimoxazole antibacterial activity on bacteria in the study. However, PEO sensitivity of microbes had no significant (p, >0.05) correlation with microbial sensitivity to ciprofloxacin, colistin and 3rd (ceftazidime) and 4th (cefepime) generation cephalosporins. One of strongest antibiotic, tigecycline sensitivity had a positive correlation (p, 0.01) with all other antimicrobials tested except aztreonam (p, >0.05; r, -0.01) but best correlated (p, <0.001; r, >0.4) to sensitivity to nitrofurantoin, chloramphenicol, amoxicillin+ clavulanic acid, linezolid, erythromycin and tetracycline. The sensitivity to carbapenems, another very effective group of antibiotics, could be very strongly correlated (p, <0.001; r, >0.4) to sensitivity to moxalactam, cefepime, cefotaxime, ceftriaxone, piperacillin and amoxicillin+ clavulanic acid.

Minimum inhibitory concentration of PEO for selected bacteria: A total of 80 bacterial isolates belonging to 21 species (Table 6) were tested to determine the MIC of PEO. The MIC ranged between one nL/ mL (1 ppm) to >10 µL/ mL (>10000 ppm). The MIC was usually low for GPBs than for GNBs. The minimum MIC was observed for *Streptococcus pyogenes* (from a case of mastitis in a buffalo) and *Bacillus polymyxa* (from a vaginal swab of a bitch with pyometra) isolates (1 nL / mL). The MIC of PEO was >10 µL / mL for most of the PEO resistant GNBs. All isolates of *Bacillus polymyxa* (2), *Bordetella bronchiseptica* (2), *Dermatophilus congolensis* (1), *Staphylococcus aureus* (12), *S. haemmolyticus* (4), *Streptococcus porcinus* (2) and *S. pyogenese* (4) tested for MIC of MEO had a sensitivity to it with ≤128 nL/ mL MIC. On the other hand all isolates of *Acinetobacter haemolyticus* (2), *Citrobacter freundii* (4), *E. coli* (14), *Salmonella enterica* (6) and *Serratia fonticola* (2) tested were highly resistant to PEO with >10 µL/ mL MIC.

DISCUSSION

Patchouli, a fragrant herb growing wild and cultivated in most parts of Southeast Asia including India [1, 2], is well known among herbalists and Ayurvedic practitioners due to its numerous pharmacologically important biological activities [2-18]. In recent past, emergence of antimicrobial drug resistance (AMR), multiple drug resistance (MDR) and superbugs (resistant to almost all available antibiotics) herbal antimicrobials are looked like an alternative to antibiotics [37]. Patchouli essential oil, its alcoholic and aqueous extracts have been shown to possess not only pleasant fragrance but also antimicrobial potential [12-25]. Observations revealed that PEO inhibited different types of bacteria (Table 1, 3) to a varying extent similar to most of the antibiotics. Gram positive bacteria were significantly more often sensitive to PEO than GNBs. In the study, isolates of *Citrobacter, Salmonella, Proteus, Escherichia, Erwinia, Enterobacter, Klebsiella* and *Edwardsiella* species, aeromonads, pseudomonads and enterococci were significantly (p, <0.05) more often resistant to PEO than *Pasteurella, Alcaligenes, Brucella, Streptococcus, Acinetobacter, Staphylococcus,* and *Bacillus* species isolates. Similar results on a limited number of isolates are reported in earlier studies [16-27]. In earlier studies, PEO has specifically being reported more active against *Bacillus, Staphylococcus* and *Streptococcus* species stains than of *E. coli* and *Enterobacter* strains [26-27].

Though GPBs were more often sensitive to PEO than GNBs, all were not equally sensitive as isolates belonging to *Enterococcus, Listeria* and *Pediococcus* species were as resistant as the GNBs. However, in earlier studies, no such comparison is reported for variation of sensitivity to PEO among GPBs [19-21, 24]. Similarly, all GNBs were not equally resistant to PEO, most of the isolates of *Aggregatibacter, Arsenophonus, Branhamella, Cytophaga, Dermatophilus, Ewingella,* and majority isolates of *Acinetobacter, Actinobacillus, Buvicia, Gallibacterium, Leminorella, Moraxella,* and *Pasteurella* species were as sensitive as many

of GPBs. Similar variation among strains of same species of bacteria has been reported earlier too (21, 24). Though no comparison has been made in earlier studies among oxidase positive and oxidase negative bacteria for their PEO susceptibility, this study indicated more susceptibility of oxidase positive bacteria (p, <0.01) to PEO than oxidase negative bacteria. However, neither Gram reaction nor oxidase production can be considered as PEO sensitivity determinants as there were several oxidase producing genera including *Campylobacter, Haemophilus, Plesiomonas, Roseomonas, Shewanella, Sphingomonas, and Xanthomonas* with no or very few PEO sensitive strains. Observations indicated that the type of bacterial cell wall may not be the only determinant for antimicrobial activity of PEO. Therefore, more explorations to determine factors responsible for sensitivity or resistance of bacteria to PEO may answer the riddle.

In the study, more than 60% of the yeast isolates were sensitive to PEO, much more than GPBs or GNBs. In earlier studies too yeast isolates have often been reported sensitive to PEO and other patchouli preparations [17, 19]. However, moulds appears to be resistant similar to most of the GNBs.

In the study, 12.4% isolates were carbapenem-resistant, 49.8% produced ESBL and 50.6% were resistant to three or more group of antibiotics commonly used for the treatment that is MDR. The observations are in the line of earlier reports on CR, ESBL and MDR among veterinary clinical isolates reported from India [28, 29, 32, 38, 39]. The study also revealed that CR, ESBL and MDR isolates were significantly (p, <0.001) more often resistant to PEO than carbapenem sensitive, ESBL negative and non-MDR isolates, respectively. Though, herbs are often claimed to be an alternative to antibiotics to kill AMR strains [37], observations for PEO are in contrast to the prevailing belief [37]. A similar type of association between antibiotic resistance and resistance to agarwood oil has been reported in bacteria associated with veterinary clinical isolates recently [38]. The observation indicated that drug resistance once developed and expressed by bacteria is often broad spectrum type as MDR strains had better chances of being resistant to PEO and several other antimicrobials.

In the analysis, MDR and CR were more often (p, <0.05) detected in isolates from carnivores followed by omnivores and herbivores. The observations are in concurrence of earlier observations indicating that bacteria in those lives at the higher end in the food chain are more often AMR type than bacteria in those at the lower end of the food chain [40, 41]. However, resistance in bacteria to PEO had no correlation with food habits of the source animal indicating that patchouli, though growing wild in Northern India, may not be in food chain affecting the microbiota of the animals. However, it needs a more elaborate study to estimate PEO contents in environmental sources and different types of food to conclude.

In the study, isolates causing pyrexia of unknown origin (PUO) were more often sensitive to PEO (56.5%) than bacterial isolates from GIT disorders (>85%). The pathogens associated with different systemic or local infections have been reported to vary in their sensitivity to antimicrobial drugs due to variation in pathogen type, predilection site and their pathogenesis is already known fact for different antibiotic drugs [38], however, have rarely been explored in relation to herbs. In an earlier study (23) community-acquired *S. aureus* infections were more often sensitive (55%) to PEO than the nosocomial strains of *S. aureus* (14.8%).

The MIC of PEO was the least (1 ppm) for *Streptococcus pyogenes* and *Bacillus polymyxa* but it exceeded 10000 ppm for PEO resistant GNBs. For all isolates tested sensitive to PEO with disc diffusion assay including *Bacillus polymyxa*, *Bordetella bronchiseptica*, *Dermatophilus congolensis*, *Staphylococcus aureus*, *S. haemmolyticus*, *Streptococcus porcinus* and *S. pyogenese* MIC was ≤1024 ppm. In contrast, for all isolates tested resistant by disc diffusion assay belonging to *Acinetobacter haemolyticus*, *Citrobacter freundii*, *E. coli*, *Salmonella enterica* ssp. *enterica* serovars, and *Serratia fonticola* species MIC of PEO was >10 µL/ mL (>10,000 ppm). The observations are in concurrence of earlier reports on the MIC of PEO for different bacteria [13-21, 23-27]. However, Das and co-workers [19] in their study on 9 bacterial strains reported MIC in range of 250 to 1000 µg/ mL; Yang and co-workers [20] reported MIC of PEO ranging from 1mg/ mL to 6.5 mg/ mL (minimum for *E. coli* and maximum for *Salmonella* Typhi), and Orchard and co-workers [21] studying 13 reference strains reported MIC in range of 0.25 mg/ mL (for *Staphylococcus epidermidis*) to 1 mg/ mL (for *P. aeruginosa* and methicillin- resistant *S. aureus*), which is less wide range of MIC than observed in the present study. It might be due to a large number of isolates studied in the present investigation leading to a wider gap in the MIC. Similar to observations in the present study, in a study on a few dozen clinical isolates [24] of *S. aureus*, *E. coli* and *P. aeruginosa* [24] isolates, *S. aureus* was reported to have much lower MIC (0.25 mg/ mL) of PEO than *E. coli* and *P. aeruginosa* (>30 mg/mL) isolates. Karimi [23] reported that 29.6% clinical isolates of *S. aureus* in community human hospital PEO MIC was ≤0.6 µL/ mL, in the present study PEO MIC for 37.8% isolates of veterinary cases *S. aureus* was ≤1.28 µL/ mL, the little difference in the two studies might be associated with origin of the isolates tested.

The positive correlation in antimicrobial activity of PEO and most of the antibiotics indicated that if

bacteria were resistant to the antibiotic it had a higher probability of being resistant to PEO too (p, <0.01). The observations appear to be in contrast of earlier study indicating PEO being more active against methicillin-resistant *S. aureus* (MRSA) than on methicillin-sensitive *S. aureus*. It might be due to the fact that >90% of the *S. aureus* isolates in the present study were MRSA. The negative correlation among sensitivity of microbes to PEO and aztreonam (p, 0.01; r, -0.19) might be due to the fact that more of the GPBs (inherently resistant to aztreonam) were sensitive to PEO. The similar reason may also be assigned for stronger positive correlation of PEO sensitivity with sensitivity to GPB to erythromycin (r, 0.42), oxacillin (r, 0.37), clindamycin (r, 0.37), penicillin (r, 0.36), linezolid (r, 0.35), and ampicillin (r, 0.31), the antibiotics selectively more effective against GPBs. Sensitivity to PEO cannot be correlated with sensitivity to most of the broad spectrum antibiotics viz., ciprofloxacin, and 3rd (ceftazidime) and 4th (cefepime) generation cephalosporins. Observations on PEO are in concurrence to observations reported on ajowan (*Trachyspermum ammi*) oil [30], agarwood (*Aquilaria malaccensis*) oil [38] and many other herbal essential oils [28, 29, 31, 32, 39] indicating that some similar mechanisms of antimicrobial resistance might be responsible for resistance to herbal and conventional antimicrobials [37, 42, 43].

CONCLUSION

The study concludes that PEO may be an important promising antibiotic alternative antimicrobial for the cure of topical infections like wounds and abscess as most of the strains of *Aggregatibacter, Acinetobacter, Actinomyces, Moraxella, Dermatophilus* and *Staphylococcus* species, often associated with topical infections, were susceptible to PEO (MIC, 1280 ppm). The positive correlation between sensitivity of microbes to conventional antibiotics and PEO indicated a somewhat similar pattern of sensitivity of microbes to PEO and antibiotics.

ACKNOWLEDGEMENT

Authors express their gratitude to the technical supporting staff of the laboratories specifically Mr. HC Joshi and Mr. Laiqur Rahman for their consistent help. We also thank the Director and Joint Director (Research) of Indian Veterinary Research Institute for granting permission and funds to conduct this study out of routine Institutional budget.

Table 1: Different groups of microbes tested for sensitivity to Patchouli (*Pogostemon cablin*) essential **oil (PEO) and other antimicrobials**

Types of microbes tested	Number of isolates tested	Resistant to PEO (%)	Carbapenem resistant (%)	Extended spectrum β-lactamase (ESBL) producers (%)	Multiple drug resistant (MDR) isolates (%)
Gram-positive (G+) bacteria (16 genera)	1238	51.5	13.2	40.2	49.4
Gram-negative (G-) bacteria (52 genera)	3360	86.8	12.1	53.7	51.1
Oxidase-positive (O+) bacteria (33 genera)	914	63.9	13.5	48.9	45.1
Oxidase-negative (O-) bacteria (35 genera)	3684	80.7	12.2	50.4	52.0
O+G+ bacteria (6 genera)	224	29.5	4.5	50.4	32.6
O+G- bacteria (27 genera)	690	75.1	16.4	48.5	49.1
O-G+ bacteria (10 genera)	1014	56.4	15.2	38.6	53.1
O-G- bacteria (25 genera)	2670	89.9	11.0	55.1	51.6
Yeasts and moulds (6 genera)	67	41.8	Not tested	Not tested	Not tested
Total isolates tested	4665	76.8	12.4	49.8	50.6

Table 2: Patchouli (*Pogostemon cablin***) essential oil sensitivity and antimicrobial resistance in microbes of different sources**

Source of the microbes	Number of isolates	% sensitive to PEO	% Carbapenem resistant	% ESBL producers	% MDR isolates
Non-Poultry Birds	82	28.0	0.0	0.0	33.3
Domestic animals	1675	20.7	13.5	52.5	51.6
Environment	410	18.5	4.1	44.6	18.0
Fish	11	0.0	45.5	72.7	81.8
Foods	286	49.3	1.8	55.6	20.4
Human	319	22.9	15.0	46.0	74.8
Laboratory animals	20	5.0	5.0	65.0	35.0
Pets (horse, dog, donkey etc.)	1002	25.7	17.6	49.8	69.9
Poultry birds	192	30.7	9.9	54.2	67.7
Reference strains	63	19.0	3.5	17.5	15.8
Semi-domestic Mithuns (*Bos frontalis*)	173	8.7	1.2	7.1	10.4
Contaminants from Veterinary biologicals	13	53.8	30.8	92.3	53.8
Wild animals	258	13.9	16.2	40.5	47.7
Animals in zoos and sanctuaries	161	21.1	17.4	66.5	52.8
Carnivores	811	24.3	22.2	51.4	78.6
Herbivores	1680	22.0	11.8	50.2	51.2
Omnivores	800	21.1	20.3	50.8	67.6
Apparently healthy	818	15.5	20.2	50.9	71.5
Ear infections	101	36.6	16.8	51.5	57.4
Eye infections	34	47.1	8.8	52.9	47.1
Gastrointestinal tract infections	436	14.7	21.8	53.8	62.1
Genital tract infections	578	16.1	11.6	46.5	50.0
Mastitis	125	44.8	5.6	45.2	50.0
Pyrexia and sickness of unknown origin	23	56.5	4.3	60.9	56.5
Respiratory tract infections	121	29.8	13.2	44.6	55.4
Septicaemia deaths	565	18.4	9.7	55.0	59.2
Skin affections, abscesses and wounds	414	37.0	16.1	49.8	61.3
Urinary tract infections	215	24.7	23.8	47.2	77.6

PEO, *Pogostemon cablin* essential oil; ESBL, extended spectrum β-lactamase; MDR, multiple drug resistance

Table 3: Patchouli (*Pogostemon cablin***) essential oil resistance and other types of antimicrobial resistance in isolates of different genera of microbes**

Genus of Microbes	Number of isolates tested	Species and number of isolates	Resistant to PEO	CR	ESBL producer	MDR
Achromobacter	9	*A. xylosoxidans* 5, *Acromobacter* sp. 4	100.0	33.3	66.7	66.7
Acinetobacter	54	*A. calcoaceticus* 10, *A. ewoffli* 4, *A. haemolyticus* 3, *A. lowffii* 22, *A. schindleri* 15	59.3	46.3	59.3	59.3
Actinobacillus	8	*A. equeli* 1, *A. seminis* 3 Actinobacillus sp. 4,	87.5	25.0	62.5	62.5
Actinomyces	5	*A. propionicus* 3, *A. pyogenes* 2	40.0	60.0	40.0	100.0
Aerococcus	22	*A. suis* 1, *A. sanguinicola* 1, *A. viridans* 1, *Aerococcus* sp. 19	45.5	4.5	27.3	59.1
Aeromonas	191	*A. bestiarum* 45, *A. caviae* 15, *A. eucranophila* 18, *A. hydophila* 24, *A. jandaei* 2, *A. media* 26, *A. popoffii* 5, *A. salmonici*da 14, *A. schubertii* 7, *A.*	82.2	16.8	63.1	35.6

		sobria 15, *A. trota* 4, *A. veronii* 18				
Aggregatibacter	2	*A. aphrophilus* 1, *A. actinomycetemcomitans* 1	0.0	0.0	50.0	0.0
Agrobacterium	6	*A. tumefaciens* 2, *Agrobacterium* Yellow group 4	67.7	0.0	16.7	66.7
Alcaligenes	63	*A. faecalis* 23, *A. denitrificans* 40	73.0	15.9	52.4	61.9
Arsenophonus	3	*A. nasoniae* 3	0.0	0.0	33.3	33.3
Aspergillus	10	*A. flavus* 5, *A. niger* 5	50.0	NT	NT	NT
Avibacterium	3	*A. gallinarum* 2	66.7	0.0	0.0	33.3
Bacillus	187	*B. alvei* 1, *B. anthracis* 2, *B. anthracoides* 3, *B. badius* 7, *B. brevis* 3, *B. cereus* 11, *B. circulans* 4, *B. coagulans* 12, B. firmus 2, *B. laterosporous* 1, *B. lentus* 9, *B. licheniformis* 2, *B. marcerans* 6, *B. mycoides* 7, *B. pantothenticus* 26, *B. polymyxa* 2, *B. sphaericus* 8, *B. steareotheromphilus* 7, *B. subtilis* 3, *Bacillus* sp. 71	26.7	4.3	55.4	32.6
Bordetella	10	*B. bronchiseptica* 10	90.0	20.0	70.0	90.0
Branhamella	1	*B. cuniculi* 1	0.0	0.0	0.0	100.0
Brevibacillus	1	*Brevibacillus* sp. 1	0.0	0.0	100.0	100.0
Brucella	45	*B. abortus* 35, *B. melitensis* 10	71.1	4.4	15.6	48.9
Budvicia	8	*B. aquatica* 8	50.0	12.5	0.0	12.5
Burkholderia	18	*B. cepecia* 3, *B. gladioli* 1, *B. mallei* 9, *B. pseudomallei* 2, *Burkholderia* sp. 3	61.1	11.1	38.9	38.9
Campylobacter	4	*C. jejunii* 4	100.0	0.0	0.0	0.0
Candia	47	*C. albicans* 3, *C. cruseii* 1, *C. famata* 1, *C. kefyr* 1, *C. pseudotropicalis* 4, *C. tropicalis* 10, *Candida* sp. 27	38.3	NT	NT	NT
Citrobacter	128	*C. amalonaticus* 16, *C. diversus* 8, *C. freundii* 104	97.7	0.8	75.0	8.6
Corynebacterium	2	*C. stationis* 2	0.0	0.0	100.0	100.0
Cytophaga	1	*Cytophaga* sp. 1	0.0	0.0	100.0	0.0
Dermatophilus	3	*D. congolensis* 3	0.0	0	66.7	33
Edwardsiella	50	*E. hoshniae* 11, *E. ictaluri* 2, *E. tarda* 37	86.0	6	41.2	20
Enterobacter	300	*E. aerogenes* 4, *E. agglomerans* 240, *E. amnigenus* 21, *E. canerogenus* 2, *E. gregoviae* 20, *E. hormaechaei* 1, *E. intermedius* 1, *E. nimipressuralis* 1, *E. sakazaki* 1, *Enteroacter* sp. 9	88.3	9	49.5	43
Enterococcus	227	*E. asaccharolyticus* 4, *E. avium* 17, *E. caecorum* 34, *E. casseliflavus* 5, *E. cloacae* 4, *E. dispar* 6, *E. durans* 5, *E. faecalis* 33, *E. faecium* 24, *E. gallinarum* 16, *E. hirae* 9, *E. malodoratus* 10, *E. mundtii* 3, *E. pseudoavium* 1, *E. raffinosus* 12, E. *solitarus* 8, *Enterococcus* sp. 36	76.2	16	42.5	50
Erwinia	86	*E. amylovora* 7, *E. ananas* 6, *E. cacticida* 20, *E. carotovora* 2, *E. chrysanthami* 21, *E. cyperipedi* 2, *E. mallotivora* 10, *E. nigrifulens* 4, *E. rhapontici* 3, *E. uredovora* 8, *Erwinia* sp. 3	89.5	10	68.8	42
Escherichia	1356	*E. blattae* 5, *E. coli* 1302, *E. fergusonii* 38, *E. hermanii* 1, *E. vulneris* 10	91.0	12	55.1	65
Ewingella	1	*E. americana* 1	0.0	0	NT	0
Flavobacterium	5	*F. aquatile* 1, *F. branchiophila* 3, *F. odoratum* 1	40.0	0	20.0	60
Gallibacterium	27	*G. anatis* biovar Anatis 13, *G. anatis* biovar Haemolytica 14	59.3	0	25.9	59
Geobacillus	3	*G. stearіothermophilus* 3	33.3	0	33.3	33

Geotrichum	1	*Geotrichum* sp. 1	100.0	NT	NT	NT
Gordonia	1	*Gordonia* sp. 1	100.0	0	0.0	100
Haemophilus	1	*Haemophilus* sp. 1	100.0	100	100.0	0
Hafnia	24	*H. alvei* 24	79.2	0	77.8	58
Klebsiella	246	*K. oxytoca* 24, *K. pneumoniae* 222	86.2	7	60.0	35
Kluyvera	11	*K. cryocrescens* 11	90.9	0	0.0	9
Lactobacillus	4	*L. acidophilus* 1, *L. fermentum* 3	0.0	0	NT	0
Leclercia	1	*L. adecarboxylata* 1	100.0	0	NT	0
Leminorella	3	*L. ghrimontii* 3	66.7	0	100.0	33
Listeria	1	*L. monocytogenes* 1	100.0	0	100.0	0
Micrococcus	38	*M. agilis* 1, *M. luteus* 2, *Micrococcus* sp. 35	39.5	11	44.1	42
Moraxella	32	*M. atlanatae* 4, *M. bovis* 1, *M. canis* 3, *M. catarrhalis* 1, *M. nonliquifaciens* 2, *M. osloensis* 15, *M. phenylpyruvica* 6	50.0	16	46.9	38
Morganella	4	*M. morganii* 4	100.0	25	100.0	50
Obesumbacterium	1	*O. proteus* 1	100.0	0	100.0	100
Ochrobacterium	1	*O. anthropi* 1	100.0	0	100.0	100
Paenibacillus	1	*P. macerans* 1	0.0	0	100.0	0
Pasteurella	49	*P. aerogenes* 2, *P. caballi* 4, *P. canis* 11, *P. dagmatis* 2, *P. langaaensis* 1, *P. multocida* 27, *P. pneumotropica* 2	75.5	2	69.4	16
Pediococcus	1	*Pediococcus* sp. 1	100.0	0	100.0	0
Plesiomonas	4	*P. shigelloides* 4	100.0	0	0.0	0
Pragia	24	*P. fontium* 24	95.8	4	0.0	25
Proteus	143	*P. mirabilis* 71, *P. myxofaciens* 1, *P. penneri* 36, *P. vulgaris* 35	93.7	20	56.4	69
Providencia	10	*P. alcalifaciens* 3, *P. heimbachae* 1, *P. rettgeri* 6	100.0	10	50.0	40
Pseudomonas	189	*P. aeruginosa* 145, *P. alcaligenes* 1, *P. diminuta* 1, *P. fluorescens* 12, *P. paucimobilis* 11, *P. pseudoalcaligenes* 9, *P. stutzeri* 6, *P. testosteronii* 2, *P. vesicularis* 2	77.2	26	45.6	67
Raoultella	39	*R. planticola* 1, *K. terrigena* 38	97.4	21	78.1	56
Rhodotorula	1	*Rhodotorula* sp. 1	100.0	NT	NT	NT
Roseomonas	1	*Roseomonas* sp. 1	100.0	100	0.0	100
Salmonella	119	*S. enterica* ssp. *enterica* 71, *S. enterica* ssp. *houtenae* 3, *S. enterica* ssp. *indica* 45	95.8	3	25.0	10
Serratia	43	*S. ficaria* 5, *S. fonticola* 2, *S. mallotivora* 4, *S. marcescens* 4, *S. odorifera* 18, *S. plymuthica* 4, *S. rubidaea* 5, *S. proteomaculans* 1	90.7	5	71.4	42
Shewanella	2	*Shewanella* sp. 2	100.0	100	0.0	100
Sphingomonas	2	*S. echinoides 2*	100.0	0	100.0	0
Staphylococcus	458	*S. arlettae* 8, *S. aureus* 82, *S. auricularis* 9, *S. capitis* 38, *S. caprae* 5, *S. carnosus* 5, *S. caseolyticus* 7, *S. chromogenes* 13, *S. cohnii* 7, *S. delphini* 4, *S. epidermidis* 48, *S. equorum* 2, *S. felis* 4, *S. gallinarum* 4, *S. haemolyticus* 59, *S. hominis* 5, *S. hyicus* 18, *S. intermedius* 45. *S. kloosii* 3, *S. lentus* 14, *S. lugdunerisii* 3, *S. sciuri* 22, *S. simulans* 1, *S. warneri* 5, *S. xylosus* 11, *Staphylococcus* sp. 36	44.5	13	44.0	55
Stomatococcus	1	*Stomatococcus* sp. 1	0.0	0	0.0	0

Streptobacillus	1	*S. moniliformis* 1	100.0	0	0.0	0
Streptococcus	286	*S. adjascens* 1, *S. agalactiae* 10, *S. alactolyticus* 3, *S. bovis* 8, *S. canis* 3, *S. defactivus* 4, *S. dysgalactiae* 5, *S. equi* ssp. *equi* 4, *E. equi* ssp. *equisimilis* 27, *E. equi* ssp. *zooepidemicus* 48, *S. faecalis* 1, *S. gallinarum* 1, *S. iniae* 8, *S. intestinalis* 5, *S. macacae* 1, *S. mitis* 2, *S. mobilis* 22, *S. morbilorum* 1, S. pneumoniae 14, S. porcinus 14, *S. pyogenes* 36, *S. rattus* 1, *S. sanguis* 6, *S. suis* 3, *S. uberis* 7, *Streptococcus* sp. 51	63.3	18	27.4	51
Trichophyton	1	*Trichophyton* sp. 1	100.0	NT	NT	NT
Trichosporon	7	*Trichosporon* sp. 1	28.6	NT	NT	NT
Vibrio	14	*V. alginolyticus* 2, *V. cholerae* Non O1 1, *V. damsela* 4, *V. fluvalis* 1, *V. metschnikovii* 1, *V. mimicus* 1, *V. natrigenes* 2, *Vibrio* sp. 2	42.9	0	50.0	29
Xanthomonas	1	*X. multophila* 1	100.0	0	100.0	100
Xanthorhabdus	12	*X. bovienii* 9, *X. luminescens* 1, *P. pionarii* 2	100.0	8	33.3	58
Yersinia	1	*Y. enterocolitica* 1	100.0	0	0.0	0

PEO, *Pogostemon cablin* essential oil; CR, carbapenem resistant; ESBL, extended spectrum β-lactamase; MDR, multiple drug resistance .

Table 4. Antimicrobial activity of Patchouli (*Pogostemon cablin*) essential oil and other conventional antimicrobials against different types of bacteria isolated from clinical samples, environmental and food samples shown as percent resistant isolates.

Isolates tested For sensitivity to	Total 4598	Gram +ve 1238	Gram -ve 3360	Oxidase +ve 914	Oxidase -ve 3684	Oxidase +ve Gram +ve 224	Oxidase +ve Gram -ve 690	Oxidase -ve Gram +ve 1014	Oxidase -ve Gram-ve 2670	MDR 2329	Non-MDR 2269	ESBL* +ve 1788	ESBL -ve 1783	CR 571	Non -CR 4027
Patchouli	77.3	51.5	86.8	63.9	80.7	29.5	75.1	56.4	89.9	80.4	74.2	79.6	76.0	82.0	76.7
Amoxicillin + clavulanic acid (Amoxiclav)	37.6	23.3	43	36.2	38	21.5	41.7	23.6	43.3	54	7.2	39.5	35.2	64.3	5.2
Ampicillin	60.3	34.2	69.1	59.9	60.4	32.2	67.8	34.6	69.4	82.3	32.2	63.2	51.7	79	12.3
Aztreonam	52	82.3	41.4	47.1	53.1	70.3	42.1	84.1	41.2	64.4	27.6	57.8	28.9	73.8	38.6
Cefepime	28.4	29.5	28	22.7	29.5	33.3	19.8	28.9	29.7	38.8	7.4	34.5	25.5	65	25.8
Cefotaxime	28.8	23.3	30.8	24.2	29.9	18.3	26	24.3	32	49	2.5	26.8	28.3	54	29.7
Ceftazidime	32.6	48.8	27.5	34.8	32.1	61.9	29.9	47	26.8	45.6	14.8	39.4	19.2	29.3	18
Ceftriaxone	30.6	24.3	32.8	22.8	32.5	25	22.2	24.2	35.6	45.6	2.2	16.4	13.6	27.4	26.3
Chloramphenicol	14.2	13.1	14.7	21.5	12.4	11.9	24.4	13.3	12.1	25	2.4	23	23.6	66.3	20.8
Ciprofloxacin	30.6	33.9	29.4	16.4	34.1	10.9	18.1	38.7	32.3	54.2	4.4	38.9	28	69.9	37
Clindamycin	53.6	26.3	81.7	59.8	51.8	21.9	78.3	27	83.1	68.1	37	51.2	53	72.5	26.9
Colistin	32.4	67.6	20.1	19.7	35.2	53.6	12.5	69.6	22.1	38.7	20.1	53.8	41.7	64.1	36.9
Cotrimoxazole	40.5	46.2	38.6	39	40.9	35.2	40	48.1	38.2	65.1	11.7	43.3	33.4	72.1	23.6
Erythromycin	69.5	27.3	90.7	65.6	70.6	16.6	84.4	29.6	92.7	81	55.5	81.9	66.6	85.9	13.6
Gentamicin	22.7	32.8	18.6	10.2	25.7	10.3	10.2	37.2	20.7	38.8	4	30.9	25.5	43.9	22.3
Linezolid	37.2	6.2	77.2	27.2	39.7	9.3	55.7	5.4	82.1	32.7	41.5	78.8	49.4	85.1	31.1
Moxalactam	23.3	31.7	20.4	34.8	21.1	31.6	35.5	31.7	17.3	32.1	4.4	29.6	27.3	64.1	47.6

Nitrofurantoin	28.5	12.1	33.5	32.8	27.4	12.9	36.6	12	32.7	41.2	11.5	30	35.7	45.3	38.3
Oxacillin	70.5	69.3	72.4	63.8	72.3	52.3	76.8	73.1	71	93.6	48.4	78.2	54.3	82	69.9
Penicillin	64.8	36.1	84.1	59.8	66.6	23.6	74.9	39.2	88.4	78.5	49.6	74.3	61.3	75.9	61.6
Piperacillin	44.7	26.2	50.9	34.8	46.8	21.3	38.1	27	53.9	58.7	15.7	50.2	38.4	64.4	76.7
Tetracycline	40.5	26.5	45.6	25.3	44.3	11.5	29.7	29.7	49.8	68.1	10.1	41.5	32.8	60	56.7
Tigecycline	7.1	2.8	8.5	17.9	4.6	1	21.6	3	5.2	10.5	0.4	7.2	6.6	16.4	66.9
Vancomycin	70.8	31.1	93.9	72	70.4	21.8	85.7	32.5	97.1	74.2	67.4	94.9	82.9	100	66

*1027 isolates were not tested for ESBL activity.

Table 5. Resistance (shown as percent resistant isolates) of commonly isolated bacteria from clinical samples, environmental and food samples.

Antimicrobial drugs tested	Types of bacteria tested (number of isolates tested)															
	Acinobacter (54)	*Aeromonas* (191)	*Alcaligenes* (63)	*Bacillus* (187)	*Citrobacter* (128)	*Edwadsiella* (50)	*Enterobacter* (300)	*Enterococcus* (227)	*Erwinia* (86)	*Escherichia* (1356)	*Klebsiella* (246)	*Pasteurella* (50)	*Proteus* (143)	*Pseudomonas* (189)	*Salmonella* (119)	*Staphylococcus* (458)	*Streptococcus* (286)
Patchouli essential oil	59.3	82.2	73.0	26.7	97.7	86.0	88.3	76.2	89.5	91.0	86.2	76.0	93.7	77.2	95.8	44.5	63.3
Cefotaxime	39.2	8.4	34.5	21.6	3.6	7.1	19.0	33.3	6.8	47.2	12.2	11.4	22.0	54.9	5.5	21.6	21.0
Ciprofloxacin	26.9	15.7	15.9	6.0	2.5	11.4	19.6	25.5	9.3	45.5	18.1	6.0	35.6	19.4	10.8	48.8	33.9
Ampicillin	66.0	80.4	58.2	35.7	53.0	34.2	70.3	41.1	73.1	74.7	91.8	24.0	57.8	78.0	29.6	41.0	27.2
Gentamicin	15.1	7.0	7.9	8.1	3.3	8.9	9.0	41.4	7.0	29.2	12.7	0.0	29.0	14.1	5.9	36.1	40.5
Chloramphenicol	23.5	8.6	14.3	13.8	3.3	8.7	10.7	18.8	7.0	13.1	6.3	6.0	34.1	58.8	2.8	10.1	13.2
Tetracycline	27.8	21.0	15.9	8.0	8.7	13.0	34.4	27.1	29.1	65.8	29.5	16.0	76.1	55.2	17.1	30.2	31.9
Cotrimoxazole	29.2	24.3	36.7	35.9	5.0	27.3	28.4	64.6	11.8	52.4	22.1	12.2	44.3	68.4	4.7	40.6	47.9

Nitrofurantoin	63.5	13.9	57.1	8.0	18.5	43.9	33.2	28.6	31.6	25.1	61.4	4.0	77.2	74.2	14.9	7.0	14.5
Oxacillin	80.0	85.0	75.0	51.8	76.9	33.3	63.0	47.8	78.6	80.2	90.9	62.5	67.6	58.8	44.4	85.0	80.1
Erythromycin	83.7	91.1	81.8	13.3	95.1	85.7	84.3	30.6	96.1	96.4	88.5	86.5	99.0	91.4	71.4	31.0	28.7
Clindamycin	75.0	88.9	96.0	18.3	88.2	25.0	71.8	28.7	78.9	91.1	86.2	65.6	96.0	82.2	62.8	23.5	31.4
Vancomycin	82.4	98.7	86.8	20.5	100.0	95.7	96.4	25.4	96.4	97.1	98.5	80.6	98.8	86.9	100.0	44.2	15.5
Ceftazidime	39.5	16.3	31.3	76.8	8.6	5.9	19.3	76.9	14.3	35.2	11.6	22.2	25.6	48.0	30.3	42.8	42.5
Amoxiclav	44.9	56.6	26.8	25.0	40.6	13.3	44.2	41.4	50.0	46.6	32.7	3.4	26.8	68.9	13.9	24.7	17.9
Penicillin	73.9	93.5	79.4	26.4	100.0	72.2	79.8	31.8	85.0	94.2	91.7	29.2	84.9	94.3	81.0	45.9	27.6
Linezolid	100.0	58.3	57.1	9.8	88.9	33.3	72.3	10.6	93.3	92.2	93.3	16.7	100.0	88.2	61.8	3.5	4.9
Colistin	25.5	17.0	12.3	54.9	6.1	56.7	28.5	91.5	20.9	13.7	38.0	0.0	92.0	14.3	11.7	65.2	72.0
Tigecycline	8.2	7.5	7.8	0.0	0.0	6.9	10.3	1.6	6.2	2.4	14.0	0.0	18.2	64.8	2.9	3.3	2.7
Ceftriaxone	35.3	6.8	25.4	34.7	3.2	11.8	18.9	54.5	1.5	46.5	18.4	2.9	29.6	48.0	2.6	17.0	26.9
Moxalactam	50.0	14.5	52.5	41.4	0.0	13.8	13.9	70.2	4.2	19.5	7.1	0.0	19.2	61.8	2.9	22.5	34.6
Cefepime	39.1	10.1	26.2	42.9	9.7	10.3	14.1	62.1	3.2	38.9	14.3	7.7	20.9	34.1	2.9	22.9	26.5
Aztreonam	62.0	23.7	54.2	74.7	6.5	11.8	29.4	86.1	13.4	49.9	17.9	33.3	37.5	51.3	5.4	88.8	73.5
Piperacillin	51.0	50.5	29.8	22.1	13.8	32.0	43.8	41.8	41.5	60.6	65.9	6.7	32.9	54.6	15.2	27.6	20.4
CR	46.3	16.8	15.9	4.3	0.8	6.0	8.7	15.9	10.5	12.2	7.3	2.0	20.3	26.5	2.5	13.1	18.2
ESBL	59.3	63.1	52.4	55.4	75.0	41.2	49.5	42.5	68.8	55.1	60.0	68.0	56.4	45.6	25.0	44.0	27.4
MDR	59.3	35.6	61.9	32.6	8.6	20.0	43.3	49.6	41.9	65.2	35.4	16.0	69.2	67.2	10.1	55.2	51.0

PEO, *Pogostemon cablin* essential oil; ESBL, extended spectrum β-lactamase; MDR, multiple drug resistance

Table 6: Minimum inhibitory concentration (MIC) of Patcouli (*Pogostemon cablin*) essential oil (PEO) for different bacteria using agar well diffusion assay

Bacteria	Number of isolates tested	MIC of PEO in parts per million (ppm) v/v
Acinetobacter haemolyticus	2	>10000
Aeromonas hydrophila	3	512, 2048, >10000
Bacillus polymyxa	2	1, 16
Bordetella bronchiseptica	2	2
Brucella abortus	3	2, 8, 64
Citrobacter freundii	4	>10000
Dermatophilus congolensis	1	16
Edwardsiella tarda	3	512 , 1024, >10000
Enterobacter agglomerans	2	512, >10000
Enterococcus faecium	2	512, >10000
Enterococcus feacalis	2	512, >10000
Escherichia coli	14	>10000
Pasteurella multocida	3	512, 1024, >10000
Proteus mirabilis	4	512, >10000
Pseudomonas aeruginosa	3	512, 4096, >10000
Salmonella enterica subspecies *enterica* serovars	6	>10000
Serratia fonticola	2	>10000
Staphylococcus aureus	12	4 to 128
Staphylococcus haemolyticus	4	16- 32
Streptococcus porcinus	2	2, 16
Streptococcus pyogenes	4	1- 16

Note: All the isolates showing zone of inhibition (≥ 7 mm, were considered sensitive) around PEO discs (6 mm) in disc diffusion assay had MIC ≤1.024 µL/ mL (1024 ppm).

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