Research Article

Antimicrobial activity of selected essential oils against antibiotic resistant organisms

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Abstract

Objective: Antimicrobial resistance is one of the major public health problems especially in developing countries where relatively higher incidence of inappropriate use of antibiotics and greater levels of resistance compared to developed countries. The current study is focused to analyze the effectiveness of natural essential oils to overcome the resistance. **Materials and methods:** The causative agent was isolated from various Clinical Samples by standard microbiological method. The isolates were subjected to antibiotic senstivity testing for common three antibiotics such as Ampicillin, Amoxycillin, and Cefriaxone, combinational drugs such as sulbactam and clavulanic acid, natural essential oils such as Lemongrass, Palmarosa, Cinnamon, Rosemary. **Results:** The isolates were found to be resistant to to Ampicillin, Amoxycillin, and Cefriaxone,but sensitive to combinational drugs such as sulbactam and clavulanic acid and in the same way to essential oils. **Conclusion:** The results have clearly indicated that natural products such as essential oils could be used to overcome the resistance of bacteria. Aromatherapy is a well established field and the selected oils have therapeutic value for diseases caused by those isolates.

Keywords: Isolation of bacteria, antimicrobial, MIC, MBC, essential oils

Introduction

HIV/AIDS pandemic is still extremely dynamic and growing worldwide. In the world, about 33.2 million people are estimated to be infected with HIV/AIDS. From this more than 22.5 million are expected to live in Sub-Saharan Africa. HIV is now the leading cause of death worldwide among the age group of 15-24. Among the most heavily HIV/AIDS affected countries, Ethiopia is the third next to India and South Africa in 2005 (Andualem, 2012). HIV prevalence among adults (aged 15-49) was an estimated 0.2%. This figure is small compared to most other middle-income countries but because of India's huge population (1.3 billion people) this equates to 2.1 million people living with HIV. Overall, India's HIV epidemic is slowing down. Between 2010 and 2017, new infections declined by 27% and AIDS-related deaths more than halved, falling by 56%. However, in 2017, new infections increased to 88,000 from 80,000 and AIDS-related deaths increased to 69,000 from 62,000 (UNAIDS, 2018).

In India the infectious disease burden is among the highest in the world and recent report showed the inappropriate and irrational use of antimicrobial agents against these diseases, which led to increase in development of antimicrobial resistance (Arora et al., 2007). Besides, it has shown that health sector in India suffers from gross inadequacy of public finance which will result in the conditions favorable for development of drug resistance (Bang, 1990). A recent study highlighted the importance of rationalizing antibiotic use to limit antibiotic resistance in India (Behera and Mathur, 2011). Antimicrobial resistance will result in difficulty in controlling the diseases in the community and ineffective delivery of the health care services. Little is known regarding the epidemiological aspects of antimicrobial resistance in most of South East Asian countries (Bhatia and Narain, 2010). Although many International agencies like World Health Organization, European Centre for Disease Control and World Health Assembly resolutions highlighted the

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antimicrobial resistance as a major public health issue, it will be a big challenge to tackle the problem for the policy makers and health care providers. World Health Organization (WHO) has proposed regional strategy on antimicrobial resistance with the goal to minimize the morbidity and mortality due to antimicrobial resistant infection to preserve the effectiveness of antimicrobial agents in the treatment and prevention of microbial infections (Arora Ray et al., 2007).

Though there are many interventional studies in developing countries (World health organization, 2012. Before recommending a series of interventions, it will be necessary to investigate the relative effectiveness of different strategies in the Indian context (Chande et al., 2009). Implementation and follow up of intervention research should be strengthened by health care planners, managers and practitioners to identify the most appropriate strategies to improve drug use and prevent the emergence of drug resistance. Challenges Strengthening of Surveillance Data Standard Operating Guidelines Improvement in antibiotic prescription practices over the counter sale of antibiotics, Poor sanitation, endemic infections and malnutrition. Limited public awareness and government commitment Lack of coordination and fragmentation of effort perverse incentives. A critical issue at the regional level is the need for and difficulty in taking effective measures as the responsibility for health remains essentially a national problem (World Health Organization, 2016).

Opportunistic infections (OIs) are infections that take advantage of a weak immune system. When the immune system is weakened by HIV disease, bacteria and fungi can get out of control and cause a wide range of illnesses resulting in mild to life threatening illnesses such as diarrhoea, bacterial meningitis, respiratory infections, fungal infections of the skin, headache, weight loss, night sweats, fever, cryptococcal meningitis, herpes zoster, candidiasis (fungal infection of the oral cavity, throat and vagina), tuberculosis (a bacterial infection that attacks the lungs), *Pneumocystis carinii* pneumonia and *Mycobacterium avium* complex which causes recurring fevers (Murray and Pizzorno, 1999; Vermani and Garg, 2002). Patients with HIV infection are particularly prone to infections with organisms such as *Candida albicans, Cryptococcus neoformans, Mycobacterium tuberculosis, Pseudomonas aeruginosa, Streptococcus pneumoniae, Klebsiella pneumoniae and Staphylococcus aureus* (Methicillin susceptible and -resistant strains) are some of the most common pathogens of immunocompromised individuals (Zgoda and Porter, 2001).

The ancient Egyptians recognized that oils could be used in treating illness, including infection and inflammation. So valuable were these oils, that King Tutankhamen was entombed with roughly 350 liters of aromatic oil including cedar wood, frankincense, and myrrh (Baser, 2008). These oils, alone or in combination, were used extensively for the treatment of wounds, inflammation, cystitis, rheumatic joints, skin sores, bleeding, fungal infections, burns, pharyngitis, syphilis, and leprosy (Cockburn, 1964; Cox et al., 2000). Other cultures across the globe have long-standing, medical practices which incorporate the use of aromatic oils and other plant-based therapies, including those found in the Americas, Australia, and the Far East such as the Ayurvedic, Unani, and Chinese traditions. Among the more well-recognized remedies still in use today from North and South America and Australia are purple coneflower (*Echinacea purpurea*), cat's claw (*Uncaria tomentosa*), and eucalyptus (*Eucalyptus globulus* (Baser and Demirci, 2007) Ayurvedic traditions include the use of camphor (*Cinnamomum camphora*) and cardamomum (*Elettaria cardamomum*) (Baser and Demirci, 2007). China's use of herbal medicine dates as far back as 3000 B.C., when the mythological and legendary ruler Shen Nong Shi (or Shennong) taught humans the use of medicinal plants. His cumulative work, 'Shennong Bencao Jing', is considered one of the earliest medical collections in China (Davidson, 1997).

Documentation of plant species with active compounds against HIV/AIDS opportunistic infections helps to preserve or keep important indigenous knowledge safe. The key to medicinal plants research revolves around the detection, isolation and characterization of active compounds as therapeutic agents. Scientific validation of herbal medicines provides basic understanding of a plant's efficacy and this may lend further support to the widespread use of TM in health care systems, provided toxicological investigations are carried out. Several herbs have been attributed to improve immune response and could reduce symptoms of HIV/AIDS; however, this needs scientific validation in terms of safety and efficacy. Information on safety and toxic levels of the plants can be a starting point for the formulation of dosage levels of the herbal remedies.

This was done stemming from the first principles of use which is indigenous knowledge information. This is why this study was set out to document and validate the herbal and nutritive plants; and their practices in the management of HIV/AIDS- related infections. Efforts geared towards improving the quality of herbal medicines will in turn improve the quality of health, nutritional status and economic empowerment of the people involved in the conservation, collection, preparation and administration of these plant products. Hence the value of this research lies not only in promoting the value of nutri-medicinal plants in the context of HIV/AIDS epidemic, but also in the need for improved primary health care. This research will also create

new knowledge in the area of pharmacology and nutrition.

Materials and methods

Cleaning solution and cleaning of glassware

All the glassware were immersed in the cleaning solution for 24 hours and then the glassware were washed thoroughly with tap water followed by the detergent solution and finally rinsed with distilled water. The cleaned glass ware dried in the hot air oven and stored.

Potassium dichromate 60 g dissolved in warm waters, cooled and Conc H_2SO_4 acid added slowly. It was mixed thoroughly make 1000ml cleaning solution and used for cleaning the glass wares. The media and glassware were sterilized in autoclave at 121 °C with pressure for 20 minutes.

Chemicals

All chemicals, reagents and solvents used in this study were of analytical grade (Sigma-Aldrich, Mumbai). The media used in the microbiological analysis were of high grade (Hi Media, Mumbai). Double distilled deionized water was used for all the experiments. Following are the essential oils subjected in the present investigation: 1.Lemongrass (*Cymbopogon citratus)* 2.Palmarosa *(Cymbopogon martinii)* 3.Cinnamon *(Cinnamomum zeylanicum)* 4.Rosemary *(Rosmarinus officinalis)* 5.Geranium *(Pelarogonium graveolens)* 6.Peppermint (*Mentha piperita)* 7. Nochi oil *(Vitex negundo)* Samples such as pus, oral swab, throat swab, sputum, urine and stool were collected from the HIV/AIDS patients and subjected to the standard microbiological analysis to find out the causative agents. Attention was paid only on the bacterial and fungal species. Following are the bacterial and fungal species isolated from those samples and identified.

Identification of bacteria

Morphological study

Samples such as pus, oral swab, throat swab, sputum, urine and stool were collected from the patients and subjected to the standard microbiological analysis to find out the causative agents.

Microscopic observation

A small portion of the sample was streaked on Nutrient agar plate and incubated at 37°C for 24 h (Benson and Harold, 1990).

Gram's staining

A smear of the suspected colony was made on a clean glass slide and heat fixed. It was flooded with crystal violet solution and allowed to remain for one minute. Then it was washed with water, flooded with iodine solution and left for two minutes. It was then dried and decolorized with 95% ethanol, washed gently in running water and counter stained for 30 seconds to one minute with safranine and washed with water. Then it was air dried and stained smear was observed under microscope (Bergey et al., 1994).

Motility test

A drop of culture suspension was placed on a clean cavity glass slide cover slip was placed over it carefully and examined under microscope for the motility of the test organism (Bartholomew James, 1977).

Capsule staining

Colonies in the culture were identified to see the capsule by India ink preparation (Negative staining). One drop of India ink was placed on the centre of the slide. Pure culture of isolate was mixed with the stain and then the17mixture was made into thin smear as making blood smear and it was microscopically observed for the presence capsules (Ellen et al., 1990).

MacConkey agar

A small portion of suspected colony from nutrient agar plate was streaked on MacConkey agar plate and incubated at 37°C for 24 hours (Benson and Harold, 1990).

Mannitol salt agar

The suspected colony was aseptically streaked on selective mannitol salt agar media for *S.aureus* and incubated at 37°C for 24 h. Strains of *S. aureus* ferment mannitol and form colonies surrounded by yellow zones due to acid production (Boyd Robert and Joseph Marr, 1988).

Eosin Methylene Blue agar

One loopful of suspected colony from nutrient agar plate was streaked on Eosin Methylene Blue agar and incubated at 37°C for 24 h (Benson and Harold, 1990).

Bismuth sulphite agar

One loopful of suspected colony from nutrient agar plate was streaked on *Salmonella* agar and incubated at 37°C for 24 h (Benson and Harold, 1990).

Baird Parker agar

A small portion of suspected colony from nutrient agar plate was streaked on Baird Parker agar**,** a selective medium for *S. aureus* and incubated at 37°C for 24 h (Ellen et al., 1990).

Deoxycholate citrate agar

A small portion of suspected colony from nutrient agar plate was streaked on Deoxycholate citrate agar, a selective medium for *Shigella sp.* and incubated at 37°C for 24 h (Ellen et al., 1990).

Cetrimide agar

A small portion of suspected colony from nutrient agar plate

was streaked on cetrimide agar, a selective medium for *Pseudomonas* and incubated at 37°C for 24 h (Ellen et al., 1990).

Christenson urea agar

A small portion of suspected colony from nutrient agar plate was streaked on Christenson urea agar base and incubated at 37°C for 24 h (Ellen et al., 1990). Following biochemical tests were done to characterize the bacterial isolates.

Biochemical tests

Catalase test

A drop of 3% hydrogen peroxide was placed on a glass slide and a loopful of culture was added and observed for the occurrence of brisk effervescence, which indicated a positive result (MacFaddin Jean, 1980).

Oxidase test

A small amount of culture was streaked smoothly on an oxidase disc (*tetra* methyl *para* phenylene diamino dihydrochloride) with an applicator19stick. The reaction was assessed by an intense deep purple colour appearing with in five to ten seconds (MacFaddin Jean, 1980).

IMViC tests Indole test

6 mL of sterile Indole broth was inoculated with the test culture and incubated at 37°C for 24 hours. Cultures that produce a red ring following the addition of Kovac's reagent was noted (MacFaddin Jean, 1980).

Methyl-Red Test

6 mL of sterile MR-VP broth was inoculated with the test culture and incubated at 37°C for 24h. Methyl red reagent was added. After incubation, change in colour of the broth was noted (Clarke and Kirner, 1941).

Voges Proskauer Test

6 mL of Sterile MR-VP broth was inoculated with the test culture and incubated at 37°C for 24 h. After incubation 40% NaOH, Barrit's napthol reagent was added. After inoculation, change in colour of the broth was recorded (MacFaddin Jean, 1980).

Citrate utilization test

The test organisms were inoculated in Simmon's citrate agar slants. The tubes were incubated at 37^0C for 24 h. Following incubation, citrate positive were identified by change in the colour was noted. Citrate negative cultures showed no growth and the medium remained green (MacFaddin Jean, 1980).

Coagulase test

0.5 mL of diluted (1:4) citrate human plasma in a small sterile tube was inoculated with heavy saline suspension of the organism and was incubated at37°C for 1-4 h. It was examined every 15 min for the formation of plasma clot and compared with the controls (Blazevic Donna and Grace Mary Ederer, 1975).

Nitrate reductase test

6 mL of Sterile Nitrate broth was inoculated with the test culture and incubated at 37°C for 24 h. After incubation alpha-naphthylamine and sulphanilic acid reagents were added. The change in colour of the broth was recorded (MacFaddin Jean, 1980).

Bile solubility test

Suspected bacterial isolate was inoculated in a test tube containing 1 mL serum broth for overnight at 37°C. After incubation, a few drops of 10%sodium deoxycholate was added into the culture and observed for clearing from turbid condition (MacFaddin Jean, 1980).

Antibacterial assay

Antibacterial analysis was followed using standard agar well diffusion method to study the antibacterial activity of essential oils (Perez et al., 1990; Erdemoglu et al., 2003; Bagamboula et al., 2004). Each bacterial isolate was suspended in Brain Heart Infusion (BHI) broth and diluted to approximately10^5 colony forming unit (CFU) per mL. They were flood-inoculated onto the surface of BHI agar and then dried. Five millimeter diameter wells were cut from the agar using a sterile cork-borer and 100µL (50µL oil in ethanol) of the sample solution were poured into the wells. The plates were incubated for18h at 37C. Antibacterial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Ethanol was used as solvent control. Chloramphenicol was used as reference antibacterial agent. The tests were carried out in triplicates. The estimation of the Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) were carried out by the broth dilution method**.** Dilutions of essential oil from 2.0 to 0.075 mg/mL were used. Test bacteria culture was used at the concentration of 105 CFU/mL. MIC values were taken as the lowest essential oil concentration that prevents visible bacterial growth after 24h of incubation at 37C, and MBC as the lowest concentration that completely inhibited 25 bacterial growths. Chloramphenicol was used as a reference antibacterial agent and appropriate controls with no essential oil were also used. Experiments were triplicate.

Subculture

The same type of colony was simultaneously taken from the plate aseptically and streaked on the prepared nutrient agar plate. Then the plates were incubated at 37°C for 24 hours. After one day, the results were noted for their colony morphology and pigment production and also the colonies grown on the plate were used for performing biochemical tests and antibiotic sensitivity tests.

The clinical isolates were isolated from clinical samples and conducted antimicrobial susceptibility test by disc diffusion method (Kirby Bauer method).

Preparation of inoculum

24 hours old cultured of selected bacteria and fungi were mixed with physiological saline and turbidity was corrected by adding standard physiological saline until a McFarland turbidity standard of 0.5 (10^{-6} cfu/ml).

Antibiotic sensitivity test

Test for the isolates

After confirming the quality of medium and discs using standard strains, antibiotic sensitivity test was performed in Mueller Hinton agar medium. A lawn culture was prepared on the media with the swab from the culture in nutrient broth. Antibiotic discs were placed on the media using sterile forceps. After 24 hours of incubation the clear zone of inhibition around the disc was measured and the results were noted (Modified Kirby – Bauer technique).

Test for the isolates resistant to penicillin and cephalosporin derivatives

Sulbactam with ampicillin/sulbactam with amoxycillin medium

Mueller Hinton agar was prepared and sterilized at 121°C for 15 mins, after sterilization, pinch of Ampicillin was added to the medium and poured in to the sterile Petri plates. After solidification different types of environmental samples were streaked into the medium. Incubated at 37°C for 24 hours, on the next day bacteria resistant organisms were isolated and the bacteria resistant organisms were streaked into sulbactam with ampicillin incorporate medium. Similar test was carried out for amoxicillin also separately.

Clavulanic acid with ampicillin/clavulanic acid with amoxycillin medium

Mueller Hinton agar was prepared and sterilized at 121°C for 15 mins, after sterilization, pinch of ampicillin was added to the medium and poured in to the sterile Petri plates. After solidification different types of environmental samples were streaked into the medium. Incubated at 37°C for 24 hours, on the next day bacteria resistant organisms were isolated and the bacteria resistant organisms were streaked into clavulanic acid with Ampicillin incorporate medium. Similar test was carried out for amoxicillin also separately.

Sulbacactum with ceftrioxone/clavulanic acid with ceftrioxone medium

Mueller Hinton agar was prepared and sterilized at 121°C for 15 mins, after sterilization, pinch of ceftriaxone was added to the medium and poured in to the sterile Petri plates. After solidification different types of environmental samples were streaked into the medium. Incubated at 37°C for 24 hours, on the next day bacteria resistant organisms were isolated and the bacteria resistant organisms were streaked into sulbactam with ceftriaxone incorporate medium. Similar test was carried out for Clavulanic acid also separately.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1

Results and discussion

Following are the bacterial agents isolated from those samples collected and identified by following standard microbiological analysis such as morphological, cultural and biochemical tests, as shown in tables 1, 2, 3, 4 and 5.

Table 1. Morphology and cultural characters of the bacterial isolates

S. No	Gram's staining	Motility	Capsule	Nutrient agar	Blood agar	MacConkey agar	Baird Parker agar	EMB agar	Bismuth sulphite agarr	DC agar	Cetrimide agar
1	G+VE COCCI IN BUNCHES	-VE	-VE	Golden yellow	Beta Haemolytic	LF	Black colonies	ND	ND	ND	ND
2	G+VE COCCI IN Chains(diplococcic)	-VE	+VE	No growth	Alpha-haemolytiic	LF	ND	ND	ND	ND	ND
3	G-VE RODS	+VE	-VE	Greyish white, smooth	Non-haemolytiic	LF	ND	Metallic sheen	ND	No growth	Growth inhibited
4	G-VE RODS	+VE	-VE	Large, circulasr	Non-haemolytiic	NLF	ND	ND	Jet black- metallic sheen	Colourless growth	Growth inhibited
5	G-VE RODS	+VE	-VE	Green, irregular, musty	Beta Haemolytic	NLF	ND	ND	ND	ND	Growth occurs
6	G-VE RODS	-VE	-VE	Small, pale circular	Non-haemolytiic	NLF	ND	ND	No growth	Good growth	Growth inhibited
7	G-VE RODS	+VE	-VE	Thin filmy-Swarming	Non-haemolytiic	NLF	ND	ND	ND	ND	Growth Inhibited

EMB agar –Eosin methylene blue agar, DC agar- Deoxy cholate agar, NLF- Non Lactose fermenting- LF- Lactose fermenting, ND- Not Done

Table 2. Biochemical characters of the bacterial isolates

Indole	Methyl Red	Voges Proskauer	Citrate utilization	Coagulase	Catalase	Oxidase	Bile solubility test	Urease	Nitrate reductase	Sugar Fermentation						Isolates	
										Glucose	Lactose	Sucrose	Mannitol	Maltose	Inulin	Name of the organism	positive sample no.
										+ve	+ve	+ve	+ve	ND	ND	*Staphylococcus aureus*	87
ND	ND	ND	ND	ND	-ve	-ve	+ve	ND	ND	+ve	+ve	+ve	+ve	+ve	+ve	*Streptococcus pneumoniae*	12
+ve	-ve	+ve	-ve	ND	+ve	-ve	ND	-ve	ND	+ve	+ve	+ve	+ve	+ve	ND	*Escherichia coli*	43
-ve	+ve	-ve	+ve	ND	+ve	-ve	ND	-ve	ND	+ve	-ve	-ve	+ve	+ve	ND	*Salmonella typhi*	27
-ve	-ve	-ve	-ve	ND	+ve	+ve	ND	+ve	+ve	-ve	-ve	-ve	-ve	-ve	ND	*Pseudomonas aeruginosa*	46
-ve	+ve	+ve	-ve	ND	-ve	-ve	ND	-ve	+ve	+ve	-ve	-ve	-ve	-ve	ND	*Shigella dysenteriae*	19
-ve	+ve	-ve	d	ND	+ve	-ve	ND	+ve	ND	+ve	-ve	-ve	-ve	-ve	ND	*Proteus mirabilis*	38

ND - Not Done

Bacterial isolates

Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Shigella dysenteriae, Proteus mirabilis. Among the bacterial species *S. aureus* has been found to be predominant over other organisms (36.6%). *S. aureus* is causing localized pyogenic infection and generalized infection in most of the immunocompromised individuals. Penicillin and their derivatives have been successfully used, but these organisms have developed resistance and are involved in fatal infections. Next to *S. aureus*, *Pseudomonas aeruginosa (*20.4%), the problematic opportunistic pathogen, causing skin, respiratory and urinary tract infections (UTIs), resistant to various chemicals and antibiotics was isolated. The prevalence of other organisms such as *Streptococcus pneumoniae, Escherichia coli, Salmonella typhi, Shigella dysenteriae,* and *Proteus mirabilis* have been identified to be 5.3%, 19.1%, 12.0%, 8.4% and 16% respectively. *Streptococcus pneumoniae* is the causative agent of pneumonia and one among the major threat to immunocompromised individuals with AIDS. *E.coli,* the common causative agent of gasterointestinal and UTI, along with other serious infections by different antigenic forms causing travellers diarrohea etc. They are trasmitted by food and water,and infact they are a mjor opportunistic problem to AIDS patients. *Salmonella typhi,* the causative agent for typhoid (enteric fever), is a food and waterborn disease,seen in all, but a serious problem in AIDS patients. *Shigella dysenteriae*, the aetiological agent of bacillary dysentry, transmitted as typhoid bacilli, is common in children as well as in adults, must be considered as a risk factor in immunocompromised individuals. *Proteus mirabilis* is a well known opportunistic infection, causing UTI, and some other rare systemic infection. The isolated bacterial and fungal pathogens which cause opportunistic infections in AIDS patients were tested against the essential oils subjected in the present investigation. The following are the essential oils used for analysis such as Lemongrass (*Cymbopogon citratus),* Palmarosa *(Cymbopogon martinii),* Cinnamon *(Cinnamomum zeylanicum),* Rosemary *(Rosmarinus officinalis).*

Antibacterial activity

Antibacterial studies have been made with different concentration and finally 1:2 concentrations in ethanol have been selected for all the essential oil in agar well diffusion method. Essential oil of *C. citratus* has been highly active against *S. typhi* and the zone of inhibition was recorded as 12.85mm and least against *S. aureus* (9.98 mm). *C. martinii* has been found to be more active against *S. typhi* (14.16mm) and least against *S. aureus* (11.28mm). *C. zeylanicum* has been found to be highly effective against *S. pneumoniae* (22.39mm) and it was lesser against P. *mirabilis* (12.08mm). The activity of *R.officinalis* has been found to have a higher activity against *P. mirabilis* (14.06mm) and it was least against *P. aeruginosa* (8.21mm). *C. zeylanicum* has been found to be superior in its antibacterial activity compared to others. Other oils have been found to be moderately active against all bacterial isolates. Though the results are less effective than the control, they are found to have the expected range of activity. No organism has been found to be resistant to the tested essential oils. The results are shown in table 5.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of essential oils

The MIC of *C. citratus* against *S. aureus* has been 1.00 mg/mL, for *S. pneumoniae* 1.00 mg/mL, for *S. typhi* 2.00 mg/mL, for *E. coli* 2.00 mg/mL *P. aeruginosa* was 2.00 mg/mL, *S. dysenteriae* was 2.00 mg/mL, and for *P.*

Table 3. Antibacterial activity of essential oils against clinical isolates from HIV positive individuals

Essential oils	Bacterial isolates /Zone of Inhibition in mm						
	Staphylococcus aureus	***Streptococcus pneumoniae***	***Salmonella typhi***	***Escherichia coli***	***Pseudomonas aeruginosa***	***Shigella dysenteriae***	***Proteus mirabilis***
Cymbopogon citratus	9.98±0.53[a]	11.30±0.90 [a]	12.85±0.56[a]	11.58±0.61[ah]	7.98±0.45 [a]	12.24±0.81[a]	13.16±1.00[a]
Cymbopogon martini	11.28±0.87 [a]	12.96±0.51 [b]	14.16±0.84[b]	11.95±0.39 [a]	11.54±0.54 [b]	12.92±0.36[a]	11.98±0.45[a]
Cinnamomum zeylanicum	20.15±1.31 [b]	22.39±0.91 [c]	19.03±0.61[c]	20.98±1.46[b]	17.23±0.65 [c]	14.10±0.64[b]	12.08±0.60[a]
Rosmarinus officinalis	10.12±0.36 [a]	10.11±0.56 [ae]	13.03±0.64[ab]	11.12±0.37 [a]	8.21±0.44 [a]	11.08±0.59[c]	14.06±0.47[ae]
Chloramphenicol©	20.27±1.15[fb]	20.30±0.91[f]	18.65±0.72[gcd]	16.43±0.52[e]	12.51±0.51[gb]	18.08±0.59[g]	17.05±0.65[dc]

© - control antibiotic disc in 100 µg concentration. Different superscripts in the same column are significantly different at $P<0.05$ level (Least Significance Difference) mean followed by ± S.D

Table 4. MIC of essential oils against clinical bacterial isolates from HIV positive individuals

Organisms	Essential oil / MIC in mg/mL				
	Cymbopogon citratus	***Cymbopogon martini***	***Cinnamomum zeylanicum***	***Rosmarinus officinalis***	**Chloramphenicol©**
Staphylococcus aureus	1.00	0.50	0.25	0.75	0.25
Streptococcus pneumoniae	1.00	0.50	0.25	0.75	0.25
Salmonella typhi	2.00	1.50	0.25	1.00	0.25
Escherichia coli	2.00	1.50	0.25	1.00	0.25
Pseudomonas aeruginosa	2.00	2.00	0.25	1.50	0.25
Shigella dysenteriae	2.00	2.00	0.25	2.00	0.25
Proteus mirabilis	2.00	1.50	0.25	1.00	0.25

© - control antibiotic disc in 100µg concentration, MIC- Minimum Inhibitory Concentration

mirabilis it was 2.00 mg/mL. The results were moderate when compared with the reference drug Chloramphenicol (0.25 mg/mL).

The MIC of *C. martinii* against *S. aureus* has been 0.50 mg/mL, for *S. pneumoniae* 0.50 mg/mL, for *S. typhi* 1.50 mg/mL, for *E. coli* 1.50 mg/mL *P. aeruginosa* was 2.00 mg/ml, *S. dysenteriae* was 2.00 mg/ml, and for *P. mirabilis* it was 1.50 mg/mL. The results have been identified to be moderate when compared with the reference drug. The MIC of *C. zeylanicum* against all test organisms has been found to be similar and it has been found to be 0.25 mg/mL and it has been quite comparable with the reference drug. The MIC of *R. officinalis* against *S.aureus* has been 0.75 mg/mL, for *S. pneumoniae* 0.75, for *S. typhi* 1.00 mg/mL, for *E. coli* 1.00 mg/mL. *P. aeruginosa* was 1.50 mg/mL, *S. dysenteriae* was 2.00 mg/mL, and for *P. mirabilis* it has been 1.00 mg/mL. The results have been found to be moderate when compared with the reference drug. The results for Minimum Bactericidal Concentration (MBC) have been found to be similar to MIC results, but in MBC confirmation was made by the presence and absence of growth in culture media.

The spread of drug-resistant microorganisms and the search for natural antimicrobial substances for use in food preservation has increased the interest in EOs, which have been demonstrated to have strong antimicrobial properties (Davidson, 1997; Manso et al., 2010). The present study confirms that both oregano and cinnamon essential oils have a high antimicrobial activity against reference strains and clinical isolates of Gram-negative bacilli. The results indicated that this activity does not depend on the antibiotic susceptibility pattern, even in bacteria with high antimicrobial resistance rates including extended-spectrum beta-lactamase producers. This same conclusion was obtained by other authors studying different EOs, including oregano and cinnamon (Opalchenova et al., 2003; Mayaud et al., 2008; Doran et al., 2009). MICs and MBCs similar to those found by other authors were obtained in some cases in this study, but different values were found in other cases. For example, Mayaud et al. (2008) obtained similar MICs

Table 5. MBC of essential oils against clinical bacterial isolates from HIV positive individuals

Organisms	Essential oil / MBC in mg/mL				
	Cymbopogon citratus	***Cymbopogon martinii***	***Cinnamomum zeylanicum***	***Rosmarinus officinalis***	**Chloramphenicol©**
Staphylococcus aureus	1.00	0.50	0.25	0.75	0.25
Streptococcus pneumoniae	1.00	0.50	0.25	0.75	0.25
Salmonella typhi	2.00	1.50	0.25	1.00	0.25
Escherichia coli	2.00	1.50	0.25	1.00	0.25
Pseudomonas aeruginosa	2.00	2.00	0.25	1.50	0.25
Shigella dysenteriae	2.00	2.00	0.25	2.00	0.25
Proteus mirabilis	2.00	1.50	0.25	1.00	0.25

© - control antibiotic disc in 100µg concentration; MBC- Minimum Bactericidal Concentration

against Gram-negative bacilli for cinnamon, while they obtained different values for oregano.

The disparity in the results could be attributed to variations in the chemical composition of EOs obtained from the same plant species (Kalemba et al., 2003). Because EOs are composed of a large number of chemical constituents, it is not surprising that different combinations of them show synergistic or antagonistic effects (Burt, 2004; Goni et al., 2009; Tajkarimi et al., 2010). However, according to the results obtained in the present study, there is no interaction observed between cinnamon and oregano. As far as we know, no interactions have been described in the literature between cinnamaldehyde and carvacrol (Michiels et al., 2007; Pei et al., 2009).

According to the results obtained by Lo´pez et al. (2005), the major compounds of cinnamon (90%) and oregano (80%), respectively. It is well documented that bacteria can develop resistance to antimicrobials due to continuous and prolonged exposure to antimicrobial agents. The frequency of resistance acquisition depends on the type of antimicrobial and bacteria. We observed that resistance development was only detected for two out of four bacteria species tested to oregano essential oil, but not cinnamon. Other natural substances such as honey or tea tree oil have also been studied, and the results also varied depending on both the substance and the bacteria (McMahon et al., 2007; Cooper et al., 2010).

Due to the complex composition of EOs, it is likely that their antibacterial activity is due to different mechanisms of action that implies several targets in the cell (Burt, 2004). For this reason, it is expected that bacteria rarely develop a resistance mechanism for EOs. In this work, this hypothesis is supported for cinnamon, since after 50 passages with Cinnamon; an increase in resistance was not detected. However, the development of resistance to oregano was observed after exposure to this EO for 50 passages in the cases of *M. morganii* and *P. mirabilis.*

Two parenteral penicillins with unusual chemical structures, *mecillinam* and *temocillin),* were introduced to treat infections caused by enteric bacteria before the global emergence of *extended-spectrum b-lactamases* (ESBLs) in the late 1980s. *Mecillinam* (also known as *amdinocillin)*, with a 6-b-amidino side chain, is a *narrow-spectrum b-lactam* that binds exclusively to PBP2 in enteric bacteria. Because of this specificity, it shows synergy in vitro in combination with other *b-lactams* that bind to PBPs 1a/1b and or PBP3 in Gram-negative bacteria, thus decreasing the possibility that a point mutation in a single PBP would lead to resistance **(**Hickman et al., 2014). Temocillin, the 6-a-methoxypenicillin analog of *ticarcillin*, had greater stability than *ticarcillin* to hydrolysis by serine *b-lactamases,* but lost antibacterial activity against Gram-positive bacteria, anaerobic Gram-negative pathogens, and some enteric bacteria that included the important pathogens *Enterobacter spp.* and *Serratia marcescens* . *Mecillinam* and *temocillin* are currently enjoying resurgence in interest owing to their stability to many ESBLs, often resulting in greater than 90% susceptibility when tested against many contemporary ESBL-producing *Enterobacteriaceae* (Giske 2015; Zykov et al., 2016).

Sulbactam has been combined with *ampicillin* for general global use and with *cefoperazone* to provide additional synergistic activity against nonfermentative and anaerobic bacteria, primarily in Japan. *Tazobactam* has been combined with *piperacillin* and, more recently, with cefoperazone and ceftolozane for *nosocomial* infections, including those caused by *P. aeruginosa* (Lister 2000). The number of hydrolytic events before inactivation was at least 25-fold higher for *sulbactam* than for *clavulanic acid or tazobactam* for the TEM-2 *b-lactamase*. In contrast to *clavulanic acid,* the sulfone inhibitors do not function as inducers of chromosomally mediated *AmpC b-lactamase* (Weber and Sanders 1990).

Conclusion

Bacterial isolates such as *Staphylococcus aureus, Streptococcus pneumoniae, Salmonella typhi ,Escherichia coli, Pseudomonas aeruginos, Shigella dysenteriae and Proteus mirabilis* were isolated from various clinical samples such as pus, oral swab, throat swab, sputum, urine and stool collected from the patients and subjected to the standard microbiological analysis to find out the causative agents and subjected to antibiotic senstivity testing for common three antibiotics such as Ampicillin, Amoxycillin, and Cefriaxone. All the isoaltes were found to be resistant to the the selected antibiotics.To overcome this problem, commercial antibiotics such as sulbactam and clavulanic acid used as a combination to the same drugs. And now the organisms were found to sensitive. As they are chemicals, they induce various side effects in human body. Hence natural products like essential oils from plants such as, Lemon-grass, Palmarosa, Cinnamon, and Rosemary, when tried, all the isolates were found to be sensitive. Sulbactam and clavulanic acid are effective in overcoming resistance to Ampicillin, Amoxycillin, and Cefriaxone by inhiting the enazyme, Beta lactamase produced from the organisms. But the effectiveness of the essential oils in overcoming the resistance to Ampicillin, Amoxycillin, and Cefriaxone is not clearly known. This has to be analyzed further. The results have clearly indicated that natural products such as essential oils could be used tom overcome the resistance of bacteria. Though they are effective, their toxicity, side effects, dose, cost, therapeutic valued shold also be studied in detail.

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