ANTIBACTERIAL ACTIVITY OF PEPPERMINT OIL AGAINST CLINICAL ISOLATES OF PSEUDOMONAS AERUGINOSA-A MICROBIAL STUDY

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Abstract

Background: Pseudomonasaeruginosais ubiquitous in nature and it is found as a commensal in the skin and gastrointestinal tract of humans. It causes opportunistic infections in immune-compromised patients, not commonly seen in immune-competent patients. Peppermint oil has antibacterial activity against both gram positive and gram-negativebacteria.Aim of the study is to determine the antibacterial activity of peppermint oil against isolates of Pseudomonas aeruginosa.
Materials and method: 20 quantity of isolates of pseudomonas aeruginosa which are non-repetitive were collected. Isolates were preserved in semi-solid trypticase soy broth stock and stored at 4^{o}C until further use. Antibiotic susceptibility testing was found for these twenty isolates to commonly used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracyclin, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method. Anti-bacterial activity of peppermint oil was tested against P. aeruginosa isolates by minimum inhibitory concentration method.
Results:40% of isolates were inhibited at 0.06%, 25% of isolates were inhibited at 0.125% of essential oil, 20% of isolates was inhibited at 0.25% of essential oil and 5% of isolates were inhibited at 0.5% of essential oil. Thus, the Minimal inhibitory concentration of peppermint oil against P. aeruginosa was found to be 0.06%.
Conclusion: The peppermint oil is found to have antibacterial activity against P. aeruginosa. However, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration.

Keywords: Pseudomonasaeruginosa,MIC, peppermintoil,plantessentialoil,Hospital infection

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1. Introduction

P. aeruginosa is a Gram-negative bacterium which thrives in soil, water, and animals, but it is also an opportunistic pathogen in humans. It can infect the pulmonary and urinary tracts, wounds, and burns and cause devastating medical complications by forming biofilms on medical devices, such as catheters. The biofilms formed by P. aeruginosa allow this pathogen to evade treatment with antibiotics and cause persistent, sometimes deadly, infections.[1]

Pseudomonasaeruginosahasbecome an important cause of gram-negativeinfection, especially in patients with compromised host defensemechanisms. Pseudomonasaeruginosais a pathogenwhich is commonlyisolated from patients who have been in hospitallongerthan 1 week, and it is a maincause of nosocomialinfections. Pseudomonalinfections are complicated and can be life-threatening.Pseudomonas aeruginosa is ubiquitous in nature and it is found as a commensal in the skin and gastrointestinal tract of humans. It causes opportunistic infections in immune-compromised patients, not commonly seen in immune-competent patients.[1] It also causes serious nosocomial infections such as ventilator associated pneumonia and various sepsis syndromes because it thrives on moist surfaces of the medical equipment like catheter, causing cross infections in clinic and hospitals.[2]It typically affects the airways, urinary tract, burns, wounds, gastrointestinal system andalso causes blood infections. The most prominentattributsis the resistance of this bacteria to multiple clinically important antibiotics like third generation cephalosporins (imipenem and aztreonam) and extended–spectrumcephalosporins (cefotaxime, ceftriaxone,ceftazidime).[3]

Essential oil obtained from plants have been used for 100 of years as natural medicines against a multitude of pathogens, including bacteria, fungi, and viruses.[4]Several essential oils confer antimicrobial activity by damaging the cell wall and membrane, leading to cell lysis, leakage of cell contents, and inhibition of proton motive force.[5]Pure coconut oil" (CO) and "pure groundnut oil" (GO) available throughout the tropical globe, are widely used vegetable oil. It is anti-toxic, secure in heat, slow to oxidize and also has the highest rancidity strength.[6,7,8]There is studysaying they effectively kill bacteria without promoting the acquisition of resistance.[9,10] Finally, many essential oils are relatively easy to obtain, have low mammalian toxicity, and degrade quickly in water and soil, making them relatively environmentally friendly.[11]

Menthapiperita L., a medicinally important plant belongs to the family Lamiaceae and commonly known as peppermint is a hybrid of M. spicata L. (spearmint) and Mentha aquatic. It was cultivated by the ancient Egyptians and documented in the Icelandic pharmacopoeia of the thirteenth century. It is widely grown in temperate areas of the world, particularly in Europe, North America and North Africa but nowadays cultivated throughout all regions of the world. The medicinal parts are the essential oil extracted from the aerial parts of the flowering plant, the dried leaves, the fresh flowering plant and the whole plant.Peppermint oil has antibacterial activity against both gram positive and gram negative bacteria. It also possesses antiviral and antifungal activities. The antiviral property shown to act against influenza, herpes viruses.[12] Thus, the objective of the studywas to determine the antibacterial activity of peppermint oil against isolates of Pseudomonas aeruginosa.

2. Materials And Methods

Bacterial Isolates

Twentyquantities of isolates of pseudomonas aeruginosa which are non-repetitive were collected.They were processed for a battery of standard bio chemical tests and confirmed. Isolates were preserved in semi-solid trypticase soy broth stock and stored at 4ºC until further use.

Antibiotic Susceptibility Testing

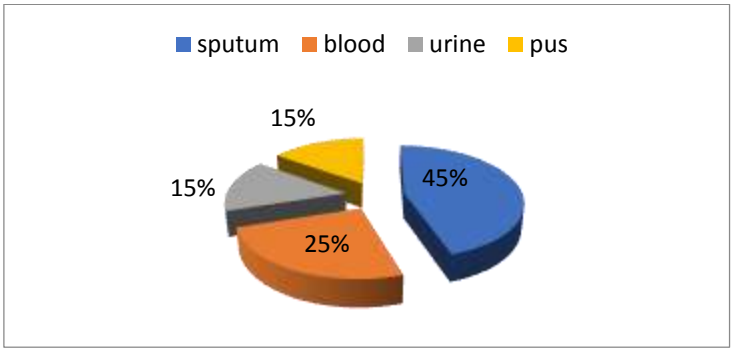
Antibiotic susceptibility testing was found for this twentyisolatesto commonly used antibiotics such as to piperacillin-tazobactam, cefotaxime, ceftazidime, tetracyclin, cotrimoxazole, aztreonam, gentamicin and imipenem by Kirby Bauer disc diffusion method as per CLSI guideline.[13]

Detection of Antibacterial Activity of Peppermint Oil Against Clinical Isolates fPseudomonas Aeruginosa

Anti-bacterial activity of peppermint oil was tested against P. aeruginosa isolates by minimum inhibitory concentration method. Mueller Hinton broth was supplemented with 0.002% (V/V) tween 80 (HiMedia, Mumbai) to enhance the dispersion of the essential oil. Agar dilution method was performed to attain the different concentrations of essential oils such as 0.03%, 0.06%, 0.125%, 0.25%, 0.5%, 1% and 2% in Mueller Hinton Agar (MHA). Media containing various concentrations of essential oil were poured over the sterile petridishes and allowed to dry. Media without essential oil was served as control plate. Spot inoculation of 0.5 McFarland standard turbidity adjusted isolates were made on the plates and incubated at 37ºC for 24 hours.The lowest concentration of the essential oil that completely inhibited the growth of isolates was considered as Minimal inhibitory concentration.

3. Results

A total of 20 clinical isolates of P.aeruginosa were taken from 9/20 (45%) isolates were from sputum, 5/20 (25%) from blood, 3/20 (15%) from urine, 3/20 (15%) from pus.(Graph.1)



Graph 1 : Sample wise distribution of clinical isolates of P. aeruginosa

Results of antibiotic susceptibility testing

In our isolates, we have observed that an increased percentage of isolates have shown to be resistant to most of the routinely used antibiotics. Only 2/20 (10%) isolates haveshowed sensitivity towardsimipenem. Other than that, for all other antibiotics such as piperacillin-tazobactam, cefotaxime, ceftazidime, tetracycline, cotrimoxazole, aztrionum, gentamicin isolates showed complete resistance 20/20 (100%).(Table.1)The clinical isolates of P. aeruginosawere inhibited from 0.06-1% of peppermint oil. The Minimal inhibitory concentration of peppermint oil was appeared to be 0.06% for P.aeruginosa. (Table.2)

Table1: Results of antibiotic susceptibility pattern of P. aeruginosa

Antibiotics	Sensitivity (20)(%)	Intermediate (20)(%)	Resistant (20)(%)
piperacillin-Tazobactam	0(0)	0(0)	20(100)
Cefotaxime	0(0)	0(0)	20(100)
Ceftazidime	0(0)	0(0)	20(100)
Tetracycline	0(0)	0(0)	20(100)
Cotrimoxazole	0(0)	0(0)	20(100)
Aztreonam	0(0)	0(0)	20(100)
Gentamicin	0(0)	0(0)	20(100)
Imipenem	2 (10)	1 (5)	17 (85)

Table.2 The antibacterial activity of peppermint oil against clinical isolates of Pseudomonas aeruginosa

Dilutions of Peppermint oil	0.03%	0.06%	0.125%	0.25%	0.5%	1%	2%
No. of organisms	0	8 (40%)	5 (25)	4 (20)	1 (5)	2	0

4. Discussion

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium. It has a pearlescent appearance and grape-like or tortilla-like odour. P. aeruginosa grows well at 25°C to 37°C, and its ability to grow at 42°C helps distinguish it from many other Pseudomonas species. P. aeruginosa is a ubiquitous microorganism which can survive under a variety of environmental conditions. It not only causes disease in plants and animals, but also in humans, causing serious infections in immunocompromised patients with cancer and patients suffering from severe burns and cystic fibrosis (CF).[2]

Most strains of P. aeruginosa produce one or more pigments, including pyocyanin (blue-green), pyoverdine (yellow-green and fluorescent), and pyorubin (red-brown). Previous investigations have suggested that pyocyanin not only contributes to the persistence of P. aeruginosa in the lungs of CF patients, but also interferes with many mammalian cell functions, including cell respiration, ciliary beating, epidermal cell growth,

calcium homeostasis and prostacyclin release from lung endothelial cells.[1]However, the precise molecular mechanism mediated by pyocyanin pathology is unknown.

P. aeruginosa strains produce two distinct types of O antigen (O-Ag): a common polysaccharide antigen (A-band) composed of a homopolymer of d-rhamnose, and an O-specific antigen (B-band) composed of a heteropolymer of three to five distinct sugars in its repeat units. So far, P. aeruginosa isolates have been classified into 20 serotypes by the International Antigenic Typing Scheme (IATS).[14]The lipopolysaccharide (LPS) of P. aeruginosa is less toxic than that of other Gram-negative rods, facilitating its establishment of chronic infections by eliciting a low inflammatory response.Plant essential oils and extracts have been used for many thousands of years,in food preservation, pharmaceuticals, alternative medicine and natural therapies.[15]It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compoundsespecially against bacterial pathogens.[16,17]Among the medicaments, chlorhexinde showed the highest antimicrobial activity on both the time intervals , followed by 10% bamboo salt, 5% bamboo salt and the least efficacy was observed in calcium hydroxide group. Chlorhexidine and 10% bamboo salt exhibited the highest depth of penetration, which was proximate to the penetration of E. faecalis.[18]Sunayana et al evaluated anti-bacterial and anti-fungal action on four medicinal plants extract the A.arabica, T.chebula, A.indica, and V.vinifera against Streptococcus mutans and Candida albicans. They concluded that the extracts obtained from A.arabica and T.chebula had a better anti-bacterial property when compared to the other two medicinal plants and the extracts obtained from A.indica and V.vinifera had a better anti-fungal property when compared to the other two medicinal plants.[19]

Study conducted by Prakasam et al in 2014 demonstrated that, Acinetobacter strains were inhibited from 0.06 to 0.25%, 0.25-1% and 0.125-1% for clove,0.06 to 0.25% ,0.25-1% and 0.125-1% for eucalyptus oil and 0.06 to 0.25%,0.25-1%, and 0.125-1% for peppermint oil. In clove oil, 14/50 (28%) isolates were inhibited at 0.06%, 25/50 (50%) isolates were inhibited at 0.125% and 11/50 (22%) isolates were inhibited at 0.25% of clove oil. In peppermint oil, 34/50 (68%) isolates were inhibited at 0.25%, 12/50 (24%) isolates were inhibited at 0.5% and 4/50 (8%) were inhibited at 1% concentrations of peppermint oil . In eucalyptus oils, 10/50 (20%) isolates were inhibited at 0.125%, 18/50 (36%) isolates were inhibited at 0.25%, 16/50 (32%) isolates were inhibited at 0.5% and 6/50 (12%) were inhibited at 1% . Thus, the Minimal inhibitory concentration of clove oil was found to be 0.06%, Minimal inhibitory concentration for peppermint oil was 0.25% and minimal inhibitory concentration for eucalyptus oil was 0.125%.[20]

In contrast, in our study, we used peppermint oil against Pseudomonas aeruginosa isolates. 40% of isolates were inhibited at 0.06%, 25% of isolates were inhibited at 0.125% of essential oil, 20% of isolates was inhibited at 0.25% of essential oil and 5% of isolates were inhibited at 0.5% of essential oil. Thus, the Minimal inhibitory concentration of peppermint oil against P. aeruginosa was found to be 0.06%.

5. Conclusion

The peppermint oil is found to have antibacterial activity against P. aeruginosa.Theminimum inhibitory concentration was 0.06% which shows that peppermint oil is essential against the bacteria. However, the studies on toxic and irritant properties of essential oils are imperative, especially when considering any new products for human administration.Thus is cannot be given as a first choice of drugs in human against the bacteria.This can be used as alternativeand complementary antibacterial agents for controlling the Pseudomonas infections.

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