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ANTIBACTERIAL ACTIVITY OF SPICES AGAINST MULTI DRUG RESISTANT BACTERIA ISOLATED FROM URINARY TRACT INFECTION

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ABSTRACT

India has been recognised as home of spices since long gap of times. Spices and herbs are used generally to impart flavour. Present study has been carried out with a view to analyses the ability of some spices as antibacterial agents. Antimicrobial activity of extracts of clove, cinnamon and pepper has been evaluated against *Escherichia coli*, *Staphylococcus aureus* , *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* In agar well diffusion method, clove extracts showed excellent antimicrobial activity against all test organisms.The cinnamon extracts, however, showed only moderate antimicrobial activity whereas the pepper showed the least activity against the test organisms.

KEYWORDS: Antimicrobial activity, Spices, Multi-drug resistant organisms, Eugenol, Dimethyl sulphoxide, Antibiotics.

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INTRODUCTION

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multi drug resistant bacteria has become a major cause of failure of the treatment of infectious diseases[1]. The use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains of microbial pathogens, potentially complicating treatment for plants, animals and human [2]. Urinary tract infection is the second most common infection. *E. coli* is the commonest cause of UTI. In recent year, drug resistance to human pathogenic bacteria and fungi has been commonly reported from all over the world. Therefore, the increasing prevalence of multi drug resistant strains of microorganisms and the recent appearance of strains with reduced susceptibility to antibiotics raises an urgent need to search for new sources of antimicrobial agents[3]. The spices have evoked an interest as sources of natural products for their potential use as an alternative remedy to heal many infectious diseases[4]. Spices are the common dietary adjuncts that contribute to the taste and flavor of foods as well as are recognized to stabilize the foods from the microbial deterioration[5]. Spices are rich sources of biologically active antimicrobial compounds. Spices are used as substances that increase the taste and variation of food[6]. The inhibitory effects of spices are mostly due to the volatile oils present in their composition[7]. In the present study, we have evaluated the antimicrobial effect of the extracts of four spices in India such as clove, pepper, cinnamon and cardamom against multi drug resistant strains isolated from Urinary tract infection.

MATERIALS AND METHODS

Test micro organims

The four test microorganisms used in this study are multidrug resistant uro- pathogens. They are *E. coli*, *S. aureus, P.aeruginosa* and *K.pneumoniae.* The urine samples were procured from Perundurai Medical College, cultured and identified by conventional methods.

Collection and preparation of spices extract

The fresh spices were collected from local market. The spices were cleaned and washed in sterile distilled water and air dried at room temperature. The dried spices were powdered using blender. 10 gram of powdered spices were weighed and mixed with 100 ml of five different solvents (methanol, ethanol, acetone, chloroform and distilled water) in conical flasks and kept in a rotatory shaker at 150 rpm for 24 hours. After 24 hours it was filtered with Whatman No.1 filter paper. The filtrates were evaporated in a hot air oven at 40^{o}C until dry. One gram dried extracts were resuspended in 10 ml of Dimethyl Sulphoxide (DMSO) individually. The extracts were stored in sample bottles at 4^{o} C prior to use.

Antibiotic sensitivity testing

The microorganisms were tested for their sensitivity against antibiotics Norfloxacin, Amikacin, Ciprofloxacin, Penicillin G, Amoxycillin, Methicillin, Oxacillin, Chloramphenicol, Clindamycin, Erythromycin, Gentamycin, Ampicillin, Kanamycin, Cloxacillin, Nitrofurantoin, Vancomycin, and Pefloxacin by the disc diffusion method. The cultures were enriched in sterile nutrient broth for 6-8 hours at 37^{o}C, using sterile cotton swabs, the cultures were aseptically swabbed on the surface of sterile Mueller-Hinton Agar plates. Using an ethanol dipped and flamed forceps, the antibiotic discs were aseptically placed over the seeded MHA plates. Plates were incubated at 37^{o}C for 24 hours and the diameter of the inhibition zones was measured in mm.

Antibacterial activity testing using agar well method

The selected strains of bacteria were inoculated into 10 ml of sterile nutrient broth, and incubated at 37^{o}C for 16-18 hours. Using a sterile cotton swab, the nutrient broth cultures were swabbed on the surface of sterile Mueller- Hinton Agar plates and left to dry for few minutes at room temperature. Agar wells were prepared with the help of sterilized cork borer. Different volume of spices extracts (50µl, 100µl, 150µl, 200µl) were added to different wells in the plate. The plate was incubated in upright position at 37^{o}C for 24

hours. The diameter of inhibition zone was measured in mm and the result was recorded.

Figure 1
Antimicrobial activity of clove cinnamon and pepper against E.coil

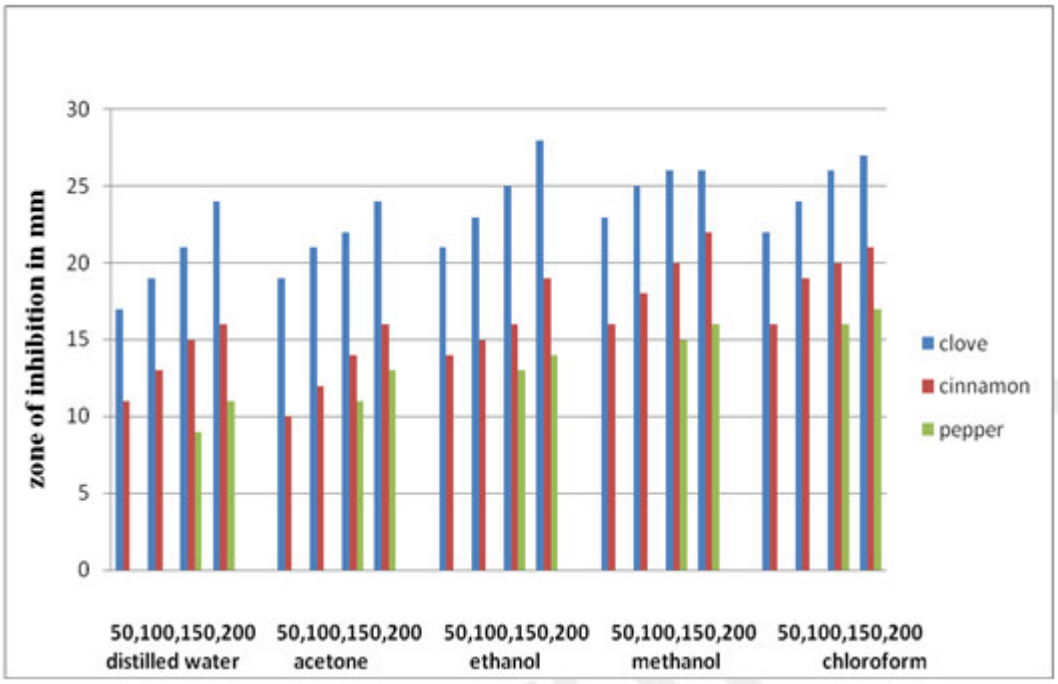


Figure 2
Antimicrobial activity of clove ,cinnamon and pepper against Staphylococcus aureus

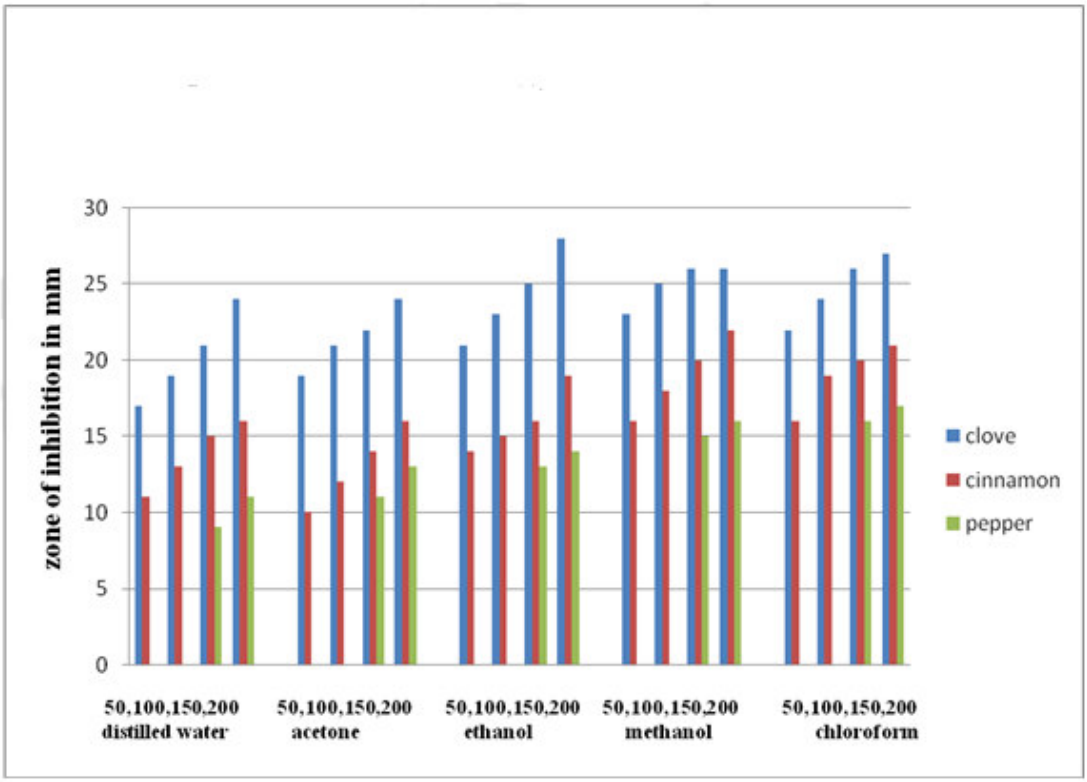


Figure 3
Antimicrobial activity of clove ,cinnamon and pepper against Klebsiella pneumoniae

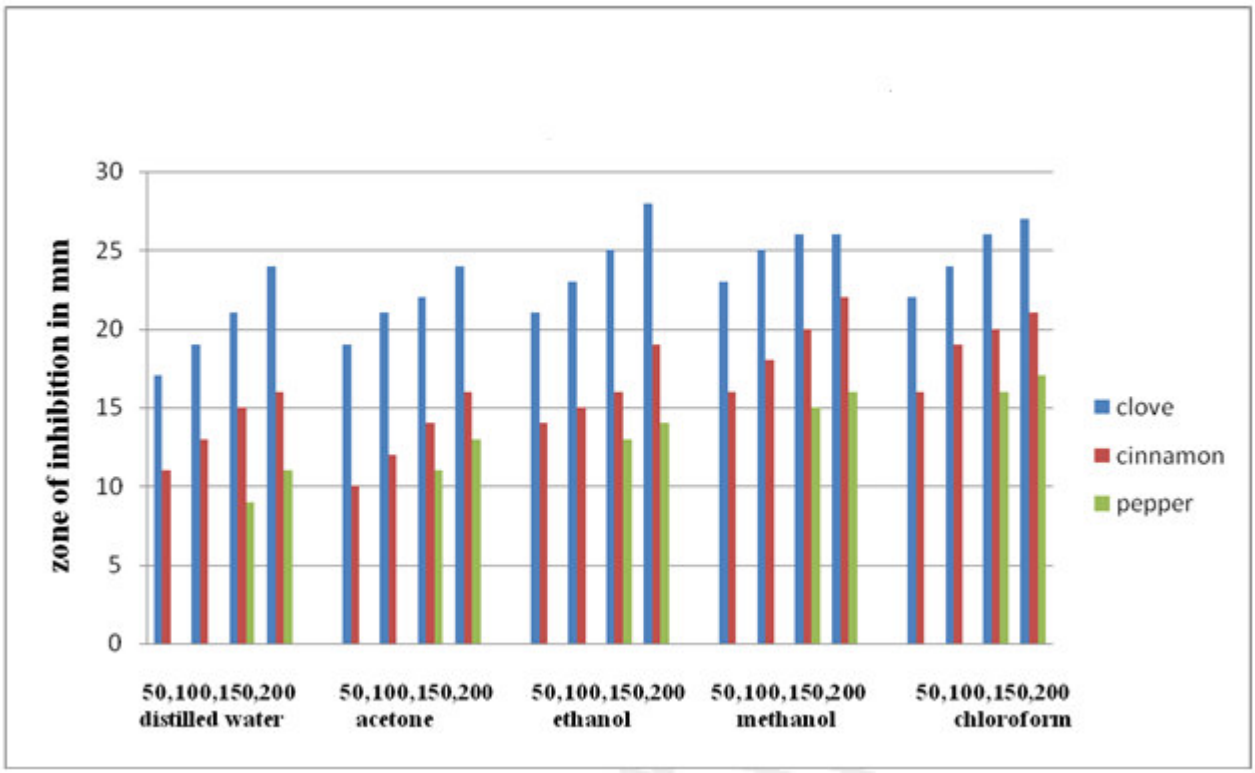
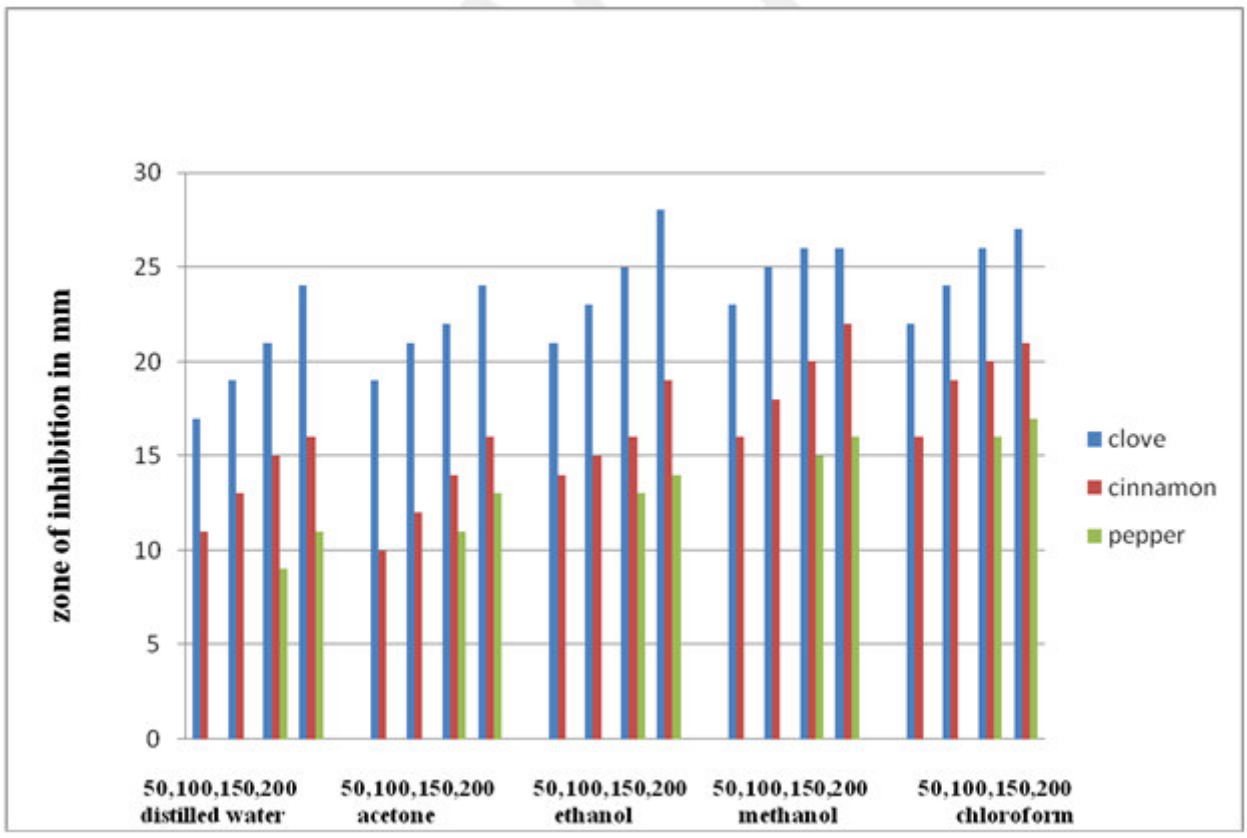


Figure 4
Antimicrobial activity of clove ,cinnamon and pepper against Pseudomonas aeruginosa



RESULTS AND DISCUSSION

50 urine samples were procured from Perundurai Medical College Hospital for the isolation of bacterial pathogens. The samples were analyzed by calibration loop technique for isolation of bacterial pathogens present in the urine sample. Among the 50 urine samples *Staphylococcus aureus* and *Escherichia coli* were found in only eight samples. *Klebsiella pneumoniae* was found in four samples. *Pseudomonas aeruginosa* was found in three samples. From this study it was observed that 16% isolates were *Staphylococcus aureus* and *Escherichia coli*, 8% isolates were *Klebsiella pneumonia*e and 6% isolates were *Pseudomonas aeruginosa.* Isolated colonies obtained from calibration loop technique were taken for identification. The organisms are identified based on their biochemical reactions given in Bergey's manual of systematic classification. The strains of *E.coli* was resistant to Amikacin ,Penicillin G, Amoxycillin, Methicillin, Oxacillin, Clindamycin, Erythromycin, Ampicillin, Kanamycin, Cloxacillin and Vancomycin. The strains of *S.aureus*was resistant to Norfloxacin, Ciprofloxacin, Penicillin G, Methicillin, Oxacillin, Clindamycin, Gentamycin, Ampicillin, Kanamycin, Cloxacillin, Nitrofurantoin, Vancomycin and Pefloxacin. The strains of *K.pneumoniae* was resistant to Norfloxacin, Amikacin, Ciprofloxacin, Penicillin G, Amoxycillin, Methicillin, Oxacillin, Clindamycin, Erythromycin, Gentamycin, Ampicillin, Kanamycin, Vancomycin and Pefloxacin. The strains of *P.aeruginosa* was resistant toNorfloxacin, Amikacin, Ciprofloxacin, Penicillin G, Amoxycillin, Methicillin, Oxacillin, Chloramphenicol, Clindamycin, Erythromycin, Gentamycin, Ampicillin, Kanamycin and Vancomycin. Among the four spices tested, three spices showed antimicrobial activity. The antimicrobial test results of the spices samples are shown in (Figure 1 to 4) Cardamom did not have any antibacterial activity against these four uropathogens. At the end of the analysis, only clove and cinnamon was found to have an inhibitory effect against all the test strains. The clove showed excellent antibacterial activity at all concentrations that are (50µl, 100µl, 150µl, and 200µl). Cinnamon exhibited moderate antibacterial activity against all the test strains. The pepper showed the least activity against the test organisms. At 200 µl concentration of clove, cinnamon and pepper when tested. The ethanol clove extracts showed the highest antibacterial activity against *E.coli* (28 mm), *S.aureus* (26 mm), *K.pneumoniae* (29 mm) and *P.aeruginosa* (28 mm). In cinnamon, the methanol cinnamon extracts showed the highest antibacterial activity against *E.coli* (22 mm), *S.aureus* (21 mm), *K.pneumoniae* (23 mm) and *P.aeruginosa* (22 mm). In pepper, the chloroform extracts showed the highest antibacterial activity against *E.coli* (18 mm), *S.aureus* (13 mm), *K.pneumoniae* (15 mm) and *P.aeruginosa* (17 mm). According to the result, clove was found to be in varying degrees, the most effective spices against tested microorganisms. This spice was effective against *E. coli*, *S. aureus, P.aeruginosa* and *K.pneumonia*e. The antimicrobial effect of cloves may be explained by the action of eugenol and eugenol acetate contained in its volatile oil[8]. Where as, in the case of cinnamon, it showed moderate effect against *E. coli*, *S. aureus, P.aeruginosa* and *K.pneumonia*e. Cinnamon was detected to exhibit a similar inhibitory effect against *P.aeruginosa* and *E. faecalis*, and its weakest activity was against *E.coli*and *M.luteus*[9]. The eugenol shown to have a stronger bactericidal activity against *E.coli* and *K.pneumoniae*[10]. Previously,cinnamon and clove had a strong inhibitory activity against microorganisms[11]. In the present study, pepper showed least activity against all the test microorganisms. The Pepper exhibited moderate antimicrobial activity against *E.coli* and *S.aureus* and no activity against *M. morgani* and *P.vulgaris*[12]. Pepper can actively prevent bacteria such as *E.coli*[13]. In conclusion clove, cinnamon and pepper were found to have considerable antimicrobial activity against the test strains. The result of the present study are quite encouraging as almost all spices except cardamom exhibited antimicrobial activity against most of the pathogens, but the antimicrobial activity varies widely depending on the type of spices, test medium and microorganisms. This study opens up the possibility for the search of new spices as antimicrobial agents as an alternative to the antibiotics.

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