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Original Article

ANTIBACTERIAL ACTIVITIES OF CINNAMON ZELANICUM SYZYGIUM AROMATICUM ESSENTIAL OIL

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ABSTRACT

Objective: Evalution the in vitro antibacterial activity of the essential oil isolated from*Cinnamomum zeylanicum* bark*, Syzygium aromaticum* flowers, against Gram-positive (*Staphyllococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis, Serratia marcescens, Enterobacter cloacae, Klebsiella pneumonia*).

Methods: Extracts from the *Cinnamomum zeylanicum* bark*, Syzygium aromaticum* flowers were hydro distilled for 3 hours in Clevenger's glass apparatus. 50 ml of diethyl ether was added into 20 gm of chopped spices and the mixture was left for 6 hours, the ethereal layer was dried over anhydrous sodium sulphate, and ether distilled off on a gently heated water bath, after which the essential oils extracted were collected in amber closed vials and is used, antibacterial screening was carried out by disc diffusion method.

Results: The MIC of the extracts and isolated compounds was determined by broth dilution method, the *Cinnamomum zeylanicum*was antibacterial activity on(*Staphyllococcus aureus, Streptococcus pyogenes* and (*Escherichia coli,, Serratia marcescens, Enterobacter cloacae, Klebsiella pneumonia*)with inhibition zone (30 mm), while both (*Pseudomonas aeruginosa*, *Proteus mirabilis*)were with inhibition zone (28mm).Clove oil showed significant inhibitory effect against *Staphyllococcus aureus*(20 mm), *Streptococcus pyogenes*(23 mm) and (*Escherichia coli* (25 mm), *Pseudomonas aeruginosa*(16 mm), *Proteus mirabilis*(23 mm), *Serratia marcescens* (20 mm), *Enterobacter cloacae* (25 mm), *Klebsiella pneumonia*(20 mm).the MIC results of the *Cinnamomum zeylanicumSyzygium aromaticum,* essential oil were indicated that antibacterial activities in lower concentrations 1/128 on *Staphyllococcus aureus, Streptococcus pyogenes, Proteus mirabilis Pseudomonas aeruginosa and* 1/256 on *serratia marcescens, enterobacter cloacae, Escherichia coli, klebsiella pneumonia* .

Conclusion; This study was proved that both essential oil were important as herbal drug to used in pharmaceutical industries to treatment infectious diseases.

Keywords: *Cinnamomum zeylanicum Syzygium aromaticum,* MIC, Antimicrobial.

INTRODUCTION

The drug resistant pathogencs is one of the most serious to successful treatment of microbial diseases. Essential oils and other extracts of plants have evoked interest as sources of natural products, their potential uses as alternative remedies for the treatment of many infectious diseases [1]. Essential oils possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties against micoorganisms [2]. The belief that herbal medicines might be of effe-ctive benefit in the treatment of certain diseases, that are free from side effects.[3]. It is important to find out the particular micro-organisms for which the herbal extracts are active [4]. There is increasing acquaintance acceptability of the use of herbal drugs in today's medical practice.There is no effective machinery to regulate manufacturing practices and quality standards [5]. The use of medicinal plantsbecame the first medicines is aglobal phenomenon [6]. Plants have great possible against infectious agents and can be used for therapeutic purposes [7]. Clove (*Syzygium aromaticum*) constitutes one of the major spices. Cloves are dried unopened floral buds of an evergreen tree, *Syzygium aromaticum* belonging to the family *Myrtaceae* [8]. Clove is used as flavouring agent and as spice for scenting, chewing tobacco. It is aromatic, carminative &stimulantused for dyspepsia and gastric irritations. Clove buds and their essential oils have been known to possess various antimicrobial and antioxidant properties [9]. GC-MS analysis of the clove oil extract has shown eugenol acetate, eugenol and caryo-phyllene as the major constituents, the latter two are known to possess antibacterial and antifungal properties [10].

Cinnamomum is called true cinnamon belonging to the family Lauraceae . Its growin east and south east of Asia to Australia Cinnamon is an evergreen tree reachingabout nine meters in high and it is covered with a smooth, pale bark[11]. Cinnamon mainly contains essential oils and important compounds like Cinnamaldehyde, eugenol, cinnamic acid and cinnamate. It has got good anti-inflammatory, anti-microbial, anti-oxidant, anti-ulcer, anti-diabetic[12]. The objective of this study was to determine the antibacterial effect of extracts from the *Cinnamomum zeylanicum, Syzygium aromaticum,* essential oil against *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Serratia marcescens, Enterobacter cloacae, klebsiella pneumoniae*and *Staphylococcus aureus*, *Streptococcus pyogenes.*Determination of minimum inhibitory concentration (MIC) of plant extracts by using microbroth dilution assay.

MATERIALS AND METHODS

Extraction of essential oil

The plants samples were obtained from Herbal Medicine Center of Health Ministry of Iraq and identified by National Herbalism of Iraq.Extracts from the *Cinnamomum zeylanicum*bark*, Syzygium aromaticum*flowers were hydro distilled for 3 hours in Clevenger's glass apparatus. 50 ml of diethyl ether was added into 20 gm of chopped spices and the mixture was left for 6 hours, the ethereal layer was dried over anhydrous sodium sulphate, and ether distilled off on a gently heated water bath, after which the essential oils extracted were collected in amber closed vials and is used [13].

Antibacterial activity

The bacterial isolates were obtained from Department of Microbiology/Al- Hussein Hospital/Kerbala Province, morphological, cultural and biochemical methods were studied, these isolates identified with api 20E kit, api staph, api strept

(BioMerieux) [14]. Agar diffusion method by cutting wells from seeded agar and then filling with essential oil of extracts was used for determination of antibacterial activity [15]. The antibacterial activity of the essential oils were studied against Gram-positive (*Staphyllococcus aureus, Streptococcus pyogenes*) and Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis, Serratia marcescens, Enterobacter cloacae, Klebsiell, pneumonia*) pathogenic bacteria using inhibition zone method for the essential oils.

The microbial suspension was streaked over the surface of the medium using asterile cotton swab. 30 µL of essential oil of the *Cinnamomum zeylanicum*, *Syzygium aromaticum* were placed in wells(8mm). Cefotaxime(Ctx) Bioanalyse(Turkey) was used as positive control for Gram-negative and Gram –positive bacteria and inoculated plates incubated for 24 h in incubator at 37°C. The diameter of inhibition growth was measured after incubation [16].

Preparation of resazurin solution

The resazurin solution was prepared by dissolving a 270mg tablet in 40mL of distilled water. It was a well-dissolved with vortex mixer.and became homogenous solution.

Resazurin based Microtiter Dilution Assay (RMDA)

96 well microtitre plates were used for Resazurin based Microtitre Dilution Assay. The rows of microtiter plate was filled with 100 µl of extract oils. All the wells of microtitre plates were filled with 100 µl of nutrient broth and microorganism suspension containing 5×10^5 CFU/ml of bacteria.of 100 µl nutrient broth without extract .The final volume in each well was200 µl. . Two fold serial dilution (throughout the column) was achieved by starting transferring 100 µl test material from first row to the subsequent wells in the next row of the same column and so that each well has 100 µl of test material in serially descending concentrations. The 8^{th} column containing 100 µl of Cefotaxime(Ctx) positive control, the 9^{th} column was containing all solutions except extracts, the 10^{th}column containing 200µl nutrient broth were adding for all microorganisms.10 µl of resazurin solution as indicator was added in each well. To avoid the dehydration of bacterial culture, each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each microtitre plate had a set of 3 controls: (a) a column with Streptomycin as positive control, (b) a column with all solutions with the exception of the test extract and (c) a column with all solutions except bacterial solution replaced by 10 µl of nutrient broth. The plates were incubated in temperature controlled incubator at 37° C for 24 h.

The colour change in the well was then observed visually. Any colour change observed from purple to pink or colourless was taken as positive. The lowest concentration of plant leaf extract at which colour change occurred was recorded as the MIC value [17].

RESULTS

The anti-bacterial activity of the *Cinnamomum zeylanicum, Syzygium aromaticum* essential oils were studied against eight bacterial species is summarized in Table 1 and Figure 1. The results revealed that the selected essential oils showed antibacterial activity with varying values. The zone of inhibition above 7 mm in diameter was takenas positive result.

Cinnamon oil showed significant inhibitory effect against (*Staphyllococcus aureus, Streptococcus pyogenes* and (*Escherichia coli,, Serratia marcescens, Enterobacter cloacae, Klebsiella pneumonia*)with inhibition zone (30 mm), while both (*Pseudomonas aeruginosa*, *Proteus mirabilis*)were with inhibition zone (28mm).Clove oil showed .

Significant inhibitory effect against *Staphyllococcus aureus*(20 mm), *Streptococcus pyogenes*(23 mm) and (*Escherichia coli* (25 mm), *Pseudomonas aeruginosa*(16 mm), *Proteus mirabilis*(23 mm), *Serratia marcescens* (20 mm), *Enterobacter cloacae* (25 mm), *Klebsiella pneumonia*(20 mm), compared with positive control Cefotaxime *Staphyllococcus aureus*(10 mm), *Streptococcus pyogenes*(10 mm) and (*Escherichia coli* (8 mm), *Pseudomonas aeruginosa*(10 mm), *Proteus mirabilis*(8 mm), *Serratia marcescens* (10 mm), while *Enterobacter cloacae* was resist against Cephalothin .

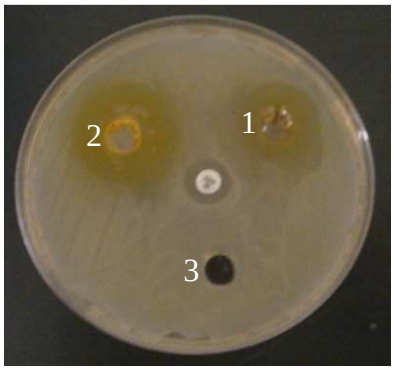
Table2 .was indicated that the MIC results ofthe *Cinnamomum zeylanicum*, *Syzygiumaroma-ticum,* essential oil were indicated that antibacterial activities in lower concentrations 1/128 on *Staph-yllococcusaureus, Streptococcus pyogenes, Proteus mirabilis, Pseudomonas aeruginosa and* 1/256 on *Serratia marcescens, enterobacter cloacae, Escherichia coli, klebsiella pneumonia*

Table 1: Inhibition zone of *Cinnamomum zeylanicum, Syzygium aromaticum* on bacterial isolates.

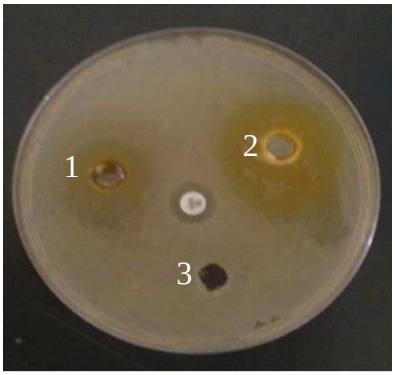
Inhibition zone(mm) Bacteria	*Cinnamomum zeylanicum*	*Syzygium aromaticum*	Cefotaxime(Ctx)
Staphyllococcus aureus	30	20	10
Streptococcus pyogenes	30	23	10
Escherichia coli	30	25	8
Pseudomonas aeruginosa	28	16	10
Proteus mirabilis	28	23	10
Serratia marcescens	30	20	8
Enterobacter cloacae	30	25	10
Klebsiella pneumonia	30	20	-

Table 2: The MIC results of the *Cinnamomum zeylanicumSyzygium aromaticum.*

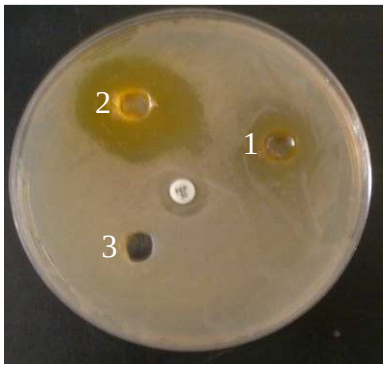
Essential oil Bacteria	*Cinnamomum zeylanicum*	*Syzygium aromaticum*
Staphyllococcus aureus	1/128	1/64
Streptococcus pyogenes	1/128	1/64
Proteus mirabilis	1/128	1/64
Pseudomonas aeruginosa	1/256	1/128
Serratia marcescens	1/256	1/128
Enterobacter cloacae	1/256	1/128
Escherichia coli	1/256	1/128
Klebsiella pneumonia	1/256	1/128



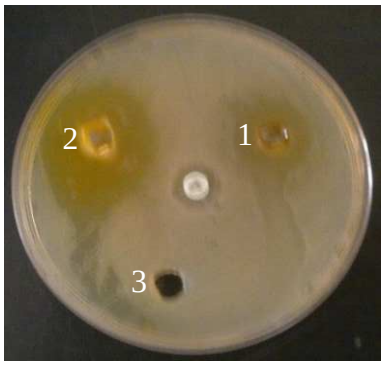
Streptococcus pyogenes



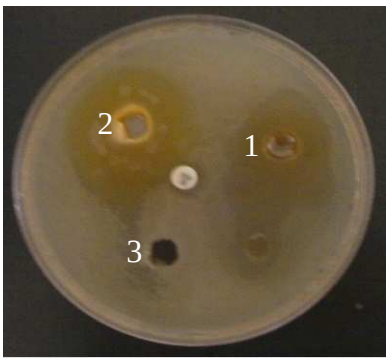
Staphyllococcusaureus



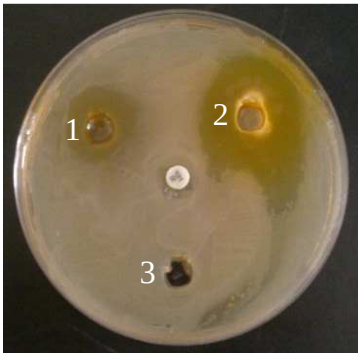
Pseudomonas aeruginosa



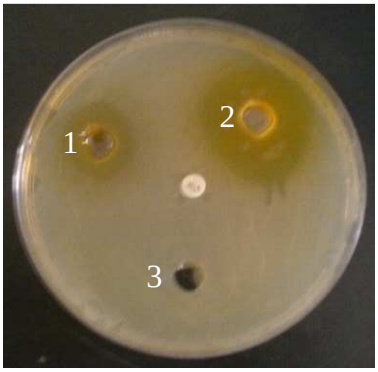
Enterobacter cloacae



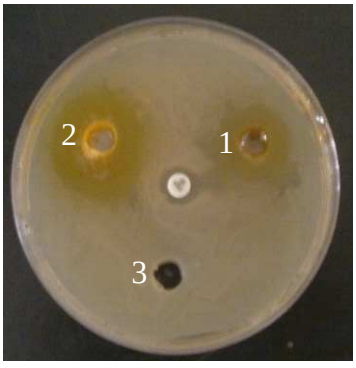
Proteus mirabilis



Serratiamarcescens



Klebsiellapneumonia



Escherichia coli

Fig. 1: Antibacterial activity of Cinnamomum zeylanicumSyzygium aromaticum, essential oil on bacterial isolates:1. Syzygium aromaticum, 2.Cinnamomum zeylanicum, 3.control compared with antibiotics(Cefotaxime) in center .

DICUSSION

The results of this study are showed the *Cinnamomumzeylanicum, Syzygium* aroma-ticum essential oils had higher inhibitory effect on gram positive and negative bacteria. Nine constituents representing 99.24% of the oil were identified by GC–MS techniques .The major compounds in the oil were (E)-cinnamaldehyde (68.95%), benzaldehyde (9.94%) and (E)-cinnamyl acetate (7.44%)[18]. The antibacterial activity has been attributed to the presence of some active constituents in the essential oils, the antibacterial activity of cinnamon was probably due to their major component, cinnamaldehyde and their constituents is also known to inhibits bacterial acetyl-CoA carboxylase and responsible for major antibacterial activity[19, 20]. Trans-cinnamaldehyde one of properties could be multiple. Cinnamaldehyde is a natural antioxidant and the animal studies suggest that an extract of cinnamon bark taken orally may help prevent stomach ulcer [21].

The anti-microbial action is considered to arise mainly from the potential of hydrophobic essential oils to obstruct the bacterial cell membrane and its structures which leads to ion leakage. Antibacterial assays of the column chromatography fractions were indicated that cinnamaldehyde is the primary compound .It has been proposed that eugenol and cinnamaldehyde inhibit production of an essential enzyme by the bacteria and/or cause damage to the cell wall of bacteria [22].

This could be explained by their hydrophobicity, an important characteristic that exists in EO and their fractions [23], and may allow them to partition the lipids of the bacterial cell membrane, turning them more permeable and leadingto leakage of ions and other cell constituents [24, 25].

CONCLUSION

This study was indicated importance of both essential oil in diseases treatment to reduce drug resistance in microorganisms .These herbs behave as antioxidant fight free radical in the body. In fact these herbs are very useful in pharmaceutical industries.

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