ABSTRACT

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

Methods: The MIC of the extracts and isolated compounds was determined by broth dilution method, the Cinnamomum zeylanicumwas antibacterial activity on (Staphylococcus aureus, Streptococcus pyogenes and (Escherichia coli, Serratia marcescens, Enterobacter cloaceae, Klebsiella pneumonia) with inhibition zone (30 mm), while both (Pseudomonas aeruginosa, Proteus mirabilis) were with inhibition zone (28 mm). Clove oil showed significant inhibitory effect against Staphylococcus 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 cloaceae (25 mm), Klebsiella pneumonia (20 mm), the MIC results of Cinnamomum zeylanicum, Syzygium aromaticum, essential oil were indicated that antibacterial activities in lower concentrations 1/128 on Staphylococcus aureus, Streptococcus pyogenes, Proteus mirabilis Pseudomonas aeruginosa and 1/256 on serratia marcescens, enterobacter cloaceae, 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 pathogens 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 microorganisms [2]. The belief that herbal medicines might be of effec-tive 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 plants became the first medicines is a global 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. Clove buds and their essential oils have been known to possess various antimicrobial and antioxidant properties [8]. GC-MS analysis of the clove oil extract has shown eugenol acetate, eugenol and caryophyllene 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. It’s grown east and south east of Asia to Australia and it is covered with a smooth, pale bark [11]. Cinnamon mainly contains essential oils and important compounds like Cinnamaldehyde, eugenol, cinnamon acid and cinnamate. It has got good anti-inflammatory, anti-microbial, anti-oxidant, anti-atherosclerotic and antidiabetic [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 cloaceae, Klebsiella pneumonia and Staphylococcus aureus, Streptococcus pyogenes. Determination of minimum inhibitory concentration (MIC) of plant extracts by using micro-broth 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 Cleveger’s glass apparatus. 50 ml of diethyl ether was added into 20 gm of chopped spices and the mixture was kept 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.
pneumonia

was containing all solutions except extracts, the 10th material in serially descending concentrations. The 8th row of the same column and so that each well has 100 μl of test material from first row to the subsequent wells in the next final volume in each well was 200 μl. Two fold serial dilution CFU/ml of bacteria of 100 μl nutrient broth without extract. The activity of the essential oils were studied against Gram-positive microorganisms. 10 μl of resazurin solution as indicator was added containing 200 μl nutrient broth were adding for all 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 microtiter plates were used for Resazurin based Microtiter Dilution Assay. The rows of microtiter plate was filled with 100 μl of extract oils. All the wells of microtiter plates were filled with 100 μl of nutrient broth and microorganism suspension containing 5×10^8 CFU/ml of bacteria of 100 μl nutrient broth without extract. The final volume in each well was 200 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 8th column containing 100 μl of Cefotaxime (Ctx) positive control, the 9th column was containing all solutions except extracts, the 10th column containing 200 μl 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 microtiter 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 taken as positive result.

Cinnamon oil showed significant inhibitory effect against Staphylococcus 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 Staphylococcus 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 Staphylococcus 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 of Cinnamomum zeylanicum, Syzygium aromaroma-ticum, essential oil were indicated that antibacterial activities in lower concentrations 1/128 on Staphylococcus aureus, 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)** | **Cinnamomum zeylanicum** | **Syzygium aromaticum** | **Cefotaxime (Ctx)** |
| Staphylococcus 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 zeylanicum Syzygium aromaticum. |
|-----------------------------------------------|--|--|--|
| **Essential oil Bacteria** | **Cinnamomum zeylanicum** | **Syzygium aromaticum** |
| Staphylococcus 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 |
Fig. 1: Antibacterial activity of Cinnamomum zeylanicum Syzygium aromaticum, essential oil on bacterial isolates: 1. Syzygium aromaticum, 2. Cinnamomum zeylanicum, 3. control compared with antibiotics (Cefotaxime) in center.
DICUSION

The results of this study are showed the *Cinnamomum zeylanicum*, *Syzygium aromaticum* 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 inhibit 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 B0 and their fractions [23], and may allow them to partition the lipids of the bacterial cell membrane, turning them more permeable and leading to 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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