

ANTIBACTERIAL ACTIVITIES OF CINNAMON ZELANICUM SYZYGIUM AROMATICUM ESSENTIAL OIL

HASSAN A.HUSSEIN¹ IBRAHIM S.ABAAS² RAGHAD HUSSAIN ALI³

¹Department of Clinical Laboratory Sciences, College of Pharmacy, University of Kerbala, Iraq, ²Department of pharmacognosy and Medicinal Plants, College of Pharmacy, University of Kerbela, Iraq, ³Emergency Operations, Medicalmam Al-Hussein Medical City, Kerbala, Iraq.

Email: Raghadhussain35@yahoo.com

Received: 26 Feb 2014 Revised and Accepted: 21 Mar 2014

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 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 (*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). the MIC results of the *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 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 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 effective 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. 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 & stimulant used 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 grown east and south east of Asia to Australia. Cinnamon is an evergreen tree reaching about 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 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 (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Serratia marcescens*, *Enterobacter cloacae*, *Klebsiella pneumoniae*) pathogenic bacteria using inhibition zone method for the essential oils.

The microbial suspension was streaked over the surface of the medium using sterile 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 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 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 taken as positive result.

Cinnamon oil showed significant inhibitory effect against (*Staphylococcus aureus*, *Streptococcus pyogenes* and (*Escherichia coli*, *Serratia marcescens*, *Enterobacter cloacae*, *Klebsiella pneumoniae*) 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 pneumoniae* (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.

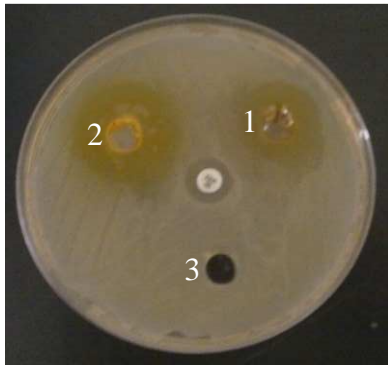
Table 2 was indicated that the MIC results of the *Cinnamomum zeylanicum*, *Syzygium aromaticum*, essential oil were indicated that antibacterial activities in lower concentrations 1/128 on *Staphylococcus aureus*, *Streptococcus pyogenes*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and 1/256 on *Serratia marcescens*, *enterobacter cloacae*, *Escherichia coli*, *klebsiella pneumoniae*

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

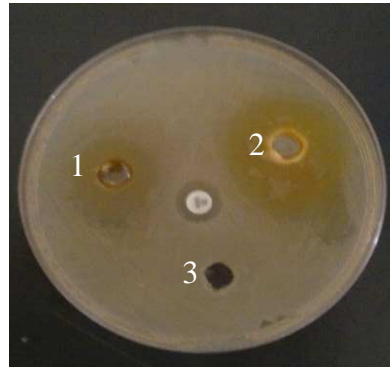
Inhibition zone (mm) Bacteria	<i>Cinnamomum zeylanicum</i>	<i>Syzygium aromaticum</i>	Cefotaxime (Ctx)
<i>Staphylococcus aureus</i>	30	20	10
<i>Streptococcus pyogenes</i>	30	23	10
<i>Escherichia coli</i>	30	25	8
<i>Pseudomonas aeruginosa</i>	28	16	10
<i>Proteus mirabilis</i>	28	23	10
<i>Serratia marcescens</i>	30	20	8
<i>Enterobacter cloacae</i>	30	25	10
<i>Klebsiella pneumoniae</i>	30	20	-

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

Essential oil Bacteria	<i>Cinnamomum zeylanicum</i>	<i>Syzygium aromaticum</i>
<i>Staphylococcus aureus</i>	1/128	1/64
<i>Streptococcus pyogenes</i>	1/128	1/64
<i>Proteus mirabilis</i>	1/128	1/64
<i>Pseudomonas aeruginosa</i>	1/256	1/128
<i>Serratia marcescens</i>	1/256	1/128
<i>Enterobacter cloacae</i>	1/256	1/128
<i>Escherichia coli</i>	1/256	1/128
<i>Klebsiella pneumoniae</i>	1/256	1/128



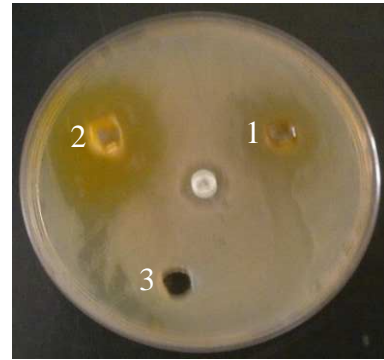
Streptococcus pyogenes



Staphylococcus aureus



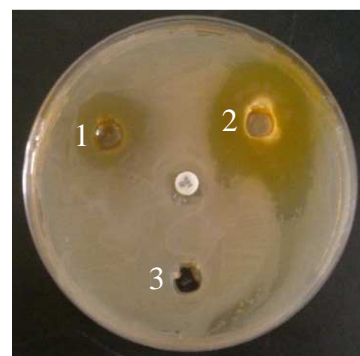
Pseudomonas aeruginosa



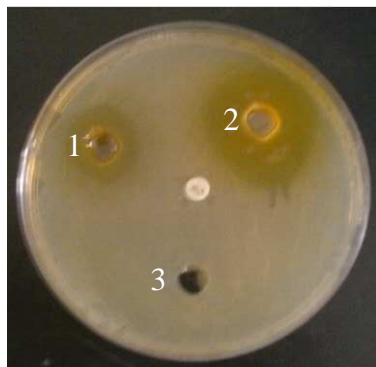
Enterobacter cloacae



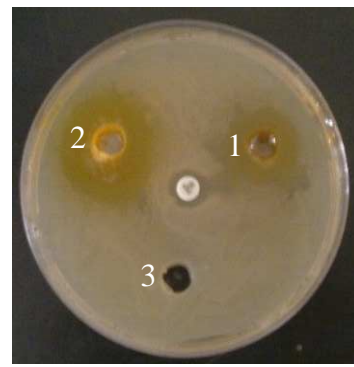
Proteus mirabilis



Serratia marcescens



Klebsiella pneumoniae



Escherichia coli

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.

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 cinnamom 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.

REFERENCES

1. Tepe B, Daferera D, Sokmen M, Polissiou M, Sokmen A: In vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigi* M. Zohary et P.H. Davis. *J Agric Food Chem* 2004; 52:1132-1137.
2. Burt SA: Essential oils: their antibacterial properties and potential applications in foods: a review. *Inter J Food Microbiol* 2004; 94:223-253.
3. Ravikumar, S., G.P. Selvan and A.A. Gracelin, Antimicrobial Activity of Medicinal Plants along Kanyakumari Coast, Tamil Nadu, India. *African Journal of Basic & Applied Sciences*, 2010;2(5-6): 153-157
4. Ravikumar, S., M. Gnanadesigan1, P. Suganthi and A. Ramalakshmi, . Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens. *Int. J. Med. and Med. Sci.*, 2010;2(3): 94-99.
5. Kunle OF, Egharevba HO, Ahmadu PO . Standardization of herbal medicines. *Int. J. Biodiv. Conserv.* 2012;Vol. 4(3):101-112
6. Seyydneyad S.M., M. Niknejad, I. Darabpoor and H. Motamedi, . Antibacterial activity ofhydroalcoholic extract of *Callistemon citrinus* and *Albizia lebbbeck*. *Am. J. Applied Sci.* 2010; 7: 13-16.
7. Upadhyay, R.K., S. Ahmed, R. Tripathi, L. Rohtagi and S.C. Jain, Screening of antimicrobial potential of extracts and pure compounds isolated from *Capparis decidua*. *J. Med. Plants Res.*, 2010; 439-445.
8. Shyamala, M. P., Venukumar, M.R. and Latha, M.S.. Antioxidant potential of this *Syzygiumaromaticum* (Gaertn.) Linn. (Cloves) in rats fed with high fat diet. *Ind. J. Pharmacol* 2003;35: 99-103.
9. Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z. Sun, S. and Efferth, T.. Antimicrobial activity of clove and rosemary essential oils alone and in combination. *Phytother. Res.* 2007;21: 989-994.
10. Nassar MI, Gaara AH, Ghorab AH, Farrag AR, Shen H, Huq E, et al. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. *Rev Latinoameri de Quim* 2007;35: 47-57.
11. Meena Vangalapati, Sree Satya N, Surya Prakash DV, Sumanjali Avanigadda.A Review on Pharmacological Activities and Clinical effects of Cinnamon Species. *RJPBCS* 2012; (3)1 : 653-663
12. Hanafy MSM, Hatem ME.. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J. Ethnopharmacol.* 1991;34: 275-278.
13. Betty A.Forbes, Daniel F.Sahm, Alice S.Weissfeld.. Bailey and Scott's Diagnostics Microbiology, 12.ed.Mosby Company, Missouri. 2007.pp116,
14. Acar, J.F. and Goldstein, F.W. Disk susceptibility test. In: V. Lorian (ed.), *Antibiotics in Laboratory Medicine*, Williams & Wilkins, Maryland. 1996, pp1-23.
15. Yongabi KA .Studies on the potential uses of medicinal plants and macrofungi (lower plants) in water and waste water purification FMENV/ZERI Research centre, Abubakar Tafawa Balewa University Bauchi, Nigeria.2003 pp.1-4.
16. Anjum Gahlaut and Anil K.Chhillar..Evaluation of antibacterial potential of plant extracts using Resazurin based microtiter dilution assay. *Int J Pharm Pharm Sci* 2013; Vol 5, Issue 2, 372-376
17. Mehmet Unlu a, Emel Ergene b, Gulhan Vardar Unlu a, Hulya Sivas Zeytinoglu b, fl, Nilufer Vural.. Composition, antimicrobial activity and in vitro cytotoxicity of essential oil from *Cinnamomum zeylanicum* Blume (Lauraceae). *Food and Chemical Toxicology* 2010;48 :3274-3280
18. Rana IS, Singh A, Gwal R: In vitro study of antibacterial activity ofaromatic and medicinal plants essential oils with special reference to cinnamon oil. *Int J Pharm Pharm Sci* 2011; 3:376-380.
19. Meades G Jr, Henken RL, Waldrop GL, Rahman MM, Gilman SD, Kamatou GP, Viljoen AM, Gibbons S: Constituents of cinnamon inhibit bacterial acetyl CoA carboxylase. *Planta Med* 2011; 76:1570-1575.
20. Blumenthal M: The Complete Commission E Monographs, Therapeutic Guide Herbal Medicines. Boston, Mass: Integrative Medicine Communications; 1998, pp110,
21. Helander, I.M., Alakomi, H-L., Latva-Kala, K., Mattila-Sandholm, T., Pol, I., Smid, E.J., Gorris, L.G.M., . Characterization of the action of selectedessential oil components on Gram-negative bacteria. *Journal of Agriculturaland Food Chemistry* 1995;46, 3590- 3595.
22. S. Burt, "Essential oils: their antibacterial properties and potential applications in foods—a review," *International Journalof Food Microbiology*, 2004;vol. 94, no. 3, pp. 223-253.
23. K. Knobloch, H. Weigand, N. Weis, H. M. Schwarm, and H. Vigerschow, "Action of terpenoids on energy metabolism,"in *Proceedings of the Progress in essential oil research: 16th International Symposium onEssential, E. J. B. Oils, Ed., De Gruyter*, 1986.pp.429-445.
24. Ultee, M. H. J. Bennik, and R. Moezelaar, "The phenolic hydroxyl group of carvacrol is essential for action against thefood-borne pathogen *Bacillus cereus*," *Applied and Environmental Microbiology*, 2002;vol. 68, no. 4, pp. 1561-1568