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ANTIBACTERIAL ACTIVITY OF CLOVES SEEDS OF *Syzygium aromaticum* and Cinnamon *Cinnamomum verum* BARK ESSENTIAL OILS

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ABSTRACT

The study was conducted between January and June 2017 at the Biotechnology Research Center/Al-Nahrain University, Baghdad, Iraq.

The antibacterial activity of the clove and cinnamon plant essential oils were evaluated by the disc diffusion method against five pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp*. and *Acinetobacter spp*. Sterile paper discs of 5 mm diameter were dipped aseptically in an appropriate concentration of essential oils and placed over Mueller–Hinton plates inoculated with pathogens. The best effect of the antibacterial activity of clove oil was observed on Acinetobacter spp. and the least was noted on Escherichia coli. Cinnamon essential oil showed the strongest antibacterial effect on all the five pathogens. On Escherichia coli, the zone of inhibition was 32mm at 1:2 dilution and minimum zone of inhibition was 28 mm at 1:8 dilution. On staphylococcus aureus as a representative of Gram-positive bacteria, the zone of inhibition was 30mm at 1:2 dilution and minimum zone of inhibition were 10 mm at 1:4 dilution.

The antibiotics TS (Trimethoprim), T (Tetracycline), E (Erythromycin), CIF (Ciprofloxacin) FOX (Cefoxitin)), SAM (Ampicillin) and NA (Nalidixic) were used in this study to get some results for comparing their effect with the clove and cinnamon oils. The effect of the clove essential oil is higher than the effect of antibiotics used against the Acinetobacter spp. The results of this study confirm that cinnamon is more active than clove and more potent than all antibiotics used against all tested bacteria.

Keywords: Essential oil, Clove, Cinnamon oil, Antibacterial.

1.INTRODUCTION

Various essential oils are stated to improve digestion, promote hormonal balance, and tone the nervous system in conditions including anxiety, depression, sexual dysfunction and exhaustion (Lehrner, et al., 2005). In addition to being used as an antiseptic, essential oils are used in flavoring food and pharmaceutical products (Ashurst, et al., 1990). Such oil, already brought from different plants possess antibacterial and antifungal activity (Bakkali, et al., 2008, Burt, 2004). The cloves are anti-inflammatory, antioxidant, antithrombotic, and they are used in dentistry as an anodyne (Pandey and Singh, 2011).

clove essential oil could have repressed microorganisms like *Lactobacillus* sp., *Fusarium*s p., *Clostridium* sp., *Bacillus* sp., *Salmonella* s., *and Penicillium* sp., (Soliman and Badeaa, 2002).

Besides, clove oil showed a strong antibacterial activity against *E. coli* followed by *Proteus mirabilis* even if to a lesser extent.

E. coli is the major cause of urinary tract infections (Anis and Arifa, 2013). It is suggested that the solvent extract of clove has a great potential for the inhibition of microbial load (Muhammad, et al., 2013).

Cinnamon additionally plays an important role in pharmacological effects such as anti-inflammation, antispasmodic, antimicrobial, antioxidant (Cui, et al., 2016). It is confirmed by many scholars that cinnamon oil has a broad range of antimicrobial activities (Tyagi, et al., 2011). The promising effect of cinnamon essential oils against several species of pathogenic bacteria have been reported (Brenes, et al., 2010).

2. MATERIALS AND METHODS

The antibacterial activity of the clove and cinnamon essential oils was evaluated by the disc diffusion method against five pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp*., and *Acinetobacter spp*.

2.1 Microbial Preparation: The pathogenic organisms used in this study were obtained from the laboratory of Al- Yarmouk Teaching Hospital.

McFarland standard was prepared to give an optical density comparable to the density of a bacterial suspension 1.5x 10^8 colony forming units (CFU/ml).

2.2 Agar Disk Diffusion Test: Discs diffusion method was used to determine the antimicrobial activity of the essential oils. Mueller–Hinton agar, were seeded with a test strain suspension (1.5 × 10^8cfu/mL) for obtaining the zone diameters of the antibacterial effect of the essential oil.

2.3 Preparation of essential oils: In order to extract the volatile oil from 50 gm of dried powdered plant material for each of the clove seeds, cinnamon bark, was done in this study by Hydro-distillation method using Clevenger apparatus according to British Pharmacopoeia procedure. After 8 hours of continuous distillation for each plant material, the following essential oils were obtainned

Cloves seeds ---------- 3.5 ml / 50 g dried seeds

Cinnamon bark ---- 1ml/50 g powdered dried bark

2.4 Preparation 5% DMSO (Dimethyl sulfoxide): It is prepared by dissolving 5ml of DMSO in 95ml of Mueller–Hinton broth which was used to make dilutions for essential oils from 1:2 to 1:8 and 5% DMSO used as a control.

2.5 Preparation of inoculum: When the inoculum has to be made from a pure culture in sterile normal saline, a loopful of the growth is suspended in saline. The tube was adjusted with the density of the standard. Then, the plates were inoculated; the excess inoculum could be removed by pressing the swab tightly against the side of the tube.

2.6 Antimicrobial sensitivity of clove and cinnamon plant essential oils: Sterile paper discs of 5 mm diameter were dipped aseptically in an appropriate concentration of essential oils and placed over Mueller–Hinton plates inoculated with pathogens. The plates were incubated at 37°C for 24 h. After overnight incubation, the diameter of each zone was measured in mm.

3. RESULTS AND DISCUSSION

The results of the study were recorded according to the size of the inhibition zone formed on the Mueller–Hinton plates by the disc diffusion method.

3.1 Effect of clove essential oil: Clove essential oil showed the maximum effect on *Acineto bacteria,* *Staphylococcus aureus***,** *Proteus spp* and minimum effect on *Pseudomonas aeruginosa* and *E. coli* (Table 1 and Fig 1)**,** on *Acinetobacter spp* the maximum zone of inhibition was 30mm at 1:2 dilution and minimum zone of inhibition was 14 mm at 1:8 dilution.

On staphylococcus aureus, the maximum zone of inhibition was 20mm at 1:2 dilution and minimum zone of inhibition were 12 mm at 1:8 dilution. On *Proteus spp* the maximum zone of inhibition was15 mm at 1:2 dilution and minimum zone of inhibition were 11 mm at 1:8 dilution. On Pseudomonas aeruginosa spp the maximum zone of inhibition was10 mm at 1:2 dilution and minimum zone of inhibition were 6 mm at 1:8 dilution. On Escherichia coli the maximum zone of inhibition was 8 mm at 1:2 dilution and minimum zone of inhibition was 5 mm at 1:8 dilution. Moreover, the best antibacterial activity of clove oil was shown on *Acinetobacter spp* and the least on *Escherichia coli*. This result was agreed with a study that found that clove oil was effective against Gram-positive bacteria *Staphylococcus aureus* (Yano, 2006, Matan, et al., 2006).

In this study, the clove oil shows the strongest antimicrobial activity against *Proteus mirabilis,* and also shows the least activity against *E. coli*. which does not agree with another study done on the clove oil; that showed: the strongest antimicrobial activity against *E. coli.* while the least activity was shown towards *Proteus mirabilis* (Ali, 2011).

Table1: Antibacterial effect of cloves essential oil on different pathogenic microorganisms

Organisms	Zone of inhibition in mm of Cloves at different dilutions using 5 mm disc diameter.			
	1:2	1:4	1:8	control
staphylococcus aureus	20mm	15mm	12mm	-ve
Escherichia coli	8mm	R	5mm	-ve
Pseudomonas aeruginosa	10mm	7mm	6mm	-ve
Proteus spp	15mm	13mm	11mm	-ve
Acinetobacterspp	30mm	24mm	14mm	-ve



Staphylococcus aureus, *Escherichia coli,* *Pseudomonas aeruginosa*

Proteus spp, *Acinetobacterspp*

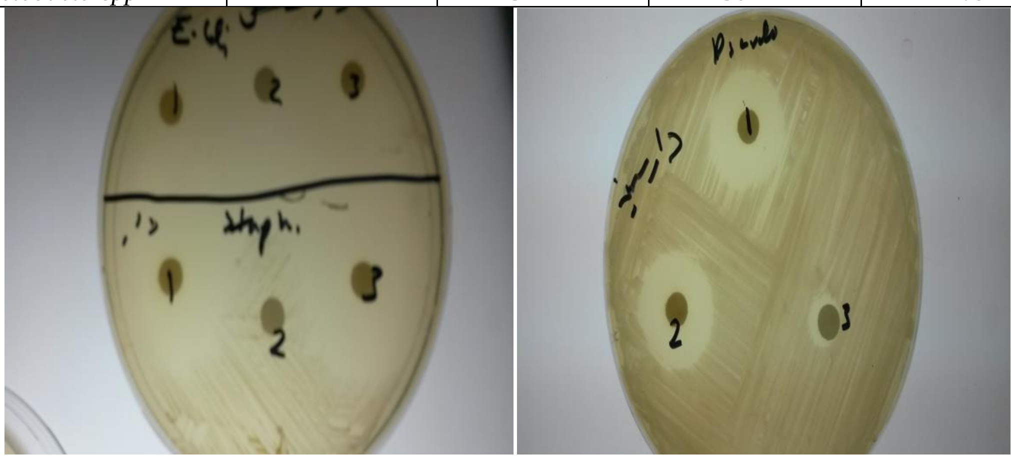
Figure 1: Inhibition zone diameter produced by Cloves essential oil against the test bacteria

3.2 Effect of cinnamon essential oil: Cinnamon essential oil showed the most effect on *Acinetobacter spp, E. coli, Staphylococcus aureus, Pseudomonas aeruginosaPseudomonas aeruginosa*, and *Proteus spp*. (table 2, Fig 2). On *Acinetobacter spp*, the maximum zone of inhibition was 44mm at 1:2 dilution and minimum zone of inhibition were 30 mm at 1:8 dilution. On *Escherichia coli*, the zone of inhibition was 32mm at 1:2 dilution and minimum zone of inhibition were 28 mm at 1:8 dilutions. On *staphylococcus aureus* as a representative of Gram-positive, the zone of inhibition was30 mm at 1:2 dilution and minimum zone of inhibition was 10 mm at 1:4 dilution. On *Proteus spp*, the zone of inhibition was30 mm at 1:2 dilution and minimum zone of inhibition 20mm at 1:8 dilution. On *Pseudomonas aeruginosa* the zone of inhibition was 30mm at 1:4 dilution and minimal area of inhibition become 20 mm at 1:8 dilution. Cinnamon essential oil showed a strong antibacterial effect on all the five pathogens, as it is added above respectively.

In this regard, the *Staphylococcus aureus* was found to be highly sensitive for the effect of cinnamon essential oil followed by *Escherichia coli, Pseudomonas aeruginosa and Proteus sp*, which were in concord with the results obtained in this study. Additionally, the results of this study are in agreement with the study (Cui, and Co, 2016, Mojtaba, 2015) who recorded that *Staphylococcus aureus* was highly sensitive to cinnamon oil (Mojtaba, et al., 2015).

Table 2: Antibacterial effect of cinnamon essential oil on different pathogenic microorganisms

Organisms	**Zone of inhibition in mm of cinnamon at different dilution using 5 mm disc diameter.**			
	1:2	1:4	1:8	control
staphylococcus aureus	30mm	10mm	34mm	-ve
Escherichia coli	32mm	30mm	28mm	-ve
Pseudomonas aeruginosa	28mm	30mm	20mm	-ve
Proteus spp	30mm	26mm	20mm	-ve
Acinetobacterspp	44mm	34mm	30mm	-ve



Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa

Proteus spp *Acinetobacter spp*

Figure2: Inhibition zone diameter produced by Cinnamon oil against the test bacteria.

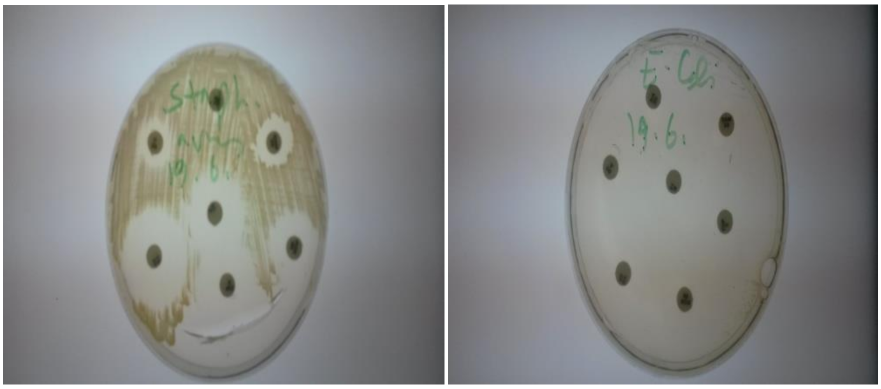
3.3 Comparison between the effect of antibiotics and the effect of essential oils on pathogenic organisms: The effect of the clove essential oil is higher than the effect of antibiotics used against the Acinetobacter spp. Besides, it is weaker than its effect on the rest of the path genes. The results of this study confirm that Cinnamon is more active than clove and more potent than all antibiotics used against all tested bacteria(Table 3, Fig 3). Similarly, Jagadeesh et al (2011), reported that the cinnamon essential oil was more active against all the bacterial pathogens examined than clove oil.

Table 3: Antibacterial activity of Antibiotic and essential oils against various tested bacterial pathogens.

Organisms	**Antibiotics**	**Zone of inhibition in mm**	**Essential oils**	**Zone of inhibition in mmat different dilution**		
				1:2	**1:4**	**1:8**
Staphylococcus aureus	TS	25	Clove	20	15	12
	T	14				
	E	22				
	CIF	12	Cinnamon	30	10	34
	FOX	28				
	Sam	20				
	Na	R*				
Escherichia coli	TS	30	Clove	8	R	5
	T	12				
	E	12				
	CIF	28				
	FOX	26	Cinnamon	28	30	32
	Sam	20				
	Na	R				
Pseudomonas aeruginosa	TS	R	Clove	10	7	6
	T	14				
	E	10				
	CIF	14				
	FOX	R	Cinnamon	20	30	28
	Sam	12				
	Na	R				
Proteus spp	TS	R	Clove	15	13	11
	T	R				
	E	R				
	CIF	14				
	FOX	20	Cinnamon	20	26	30
	Sam	30				
	Na	R				
Acinetobacterspp	TS	R	Clove	30	24	14
	T	27				
	E	18				
	CIF	12				

	FOX Sam Na	R 17 R	Cinnamon	30	34	44

Note: TS (TRIMETHOPRIM), T(TETRACYCLINE), E (ERYTHROMYCIN), CIF (CIPROFLOXACIN), FOX(CEFOXITIN), SAM (AMPICILLIN), NA (NALIDIXIX ACID). R: Resist



Staphylococcus aureus *Escherichia coli*

Proteus spp *Pseudomonas aeruginosa* *Acinetobacter spp*

Fig 3: Antibacterial activity of Antibiotic against the test bacteria

CONCLUSION

This study shows that the essential oil of cinnamon has a high antibacterial activity against both Gram-positive and Gram-negative bacteria. The results of this study were also confirmed that the cinnamon essential oil has the strongest antibacterial effect on all the five pathogens and can be used in food preservation, pharmaceutical, alternative medicine, and natural therapies.

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