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Antibacterial Activity Of Cloves Seeds (Syzygium Aromaticum) And Cinnamon Bark Essential Oils

Mohammed Al-Janabi , jaleel Ibrahim Asaad

Biotechnology Research Center/Al-Nahrain University, Iraq.

ABSTRACT

The antimicrobial activity of the Clove and cinnamon plant essential oils was evaluated by the disc diffusion method against five pathogens including Staphylococcus aureus, Escherichia, Escherichia coli, Pseudomonas aeroginosa, Proteus spp., and Acinetobacter spp.

5% solution of DMSO was used to make dilutions for essential oils from 1:2 to 1:8.

Sterile filter paper discs of 5 mm diameter were dipped aseptically in appropriate concentration of essential oils

and placed over Mueller–Hinton plates seeded with respective pathogens. The plates were incubated at 37°C for 24 h. After overnight incubation, the diameter of each zone was measured in mm, and the results were recorded according to the size of inhibition zone. The best effect of antibacterial activity of clove oil was on Acinetobacter spp, the maximum zone of inhibition was 30mm at 1:2 dilution and minimum zone of inhibition was14mm at 1:8 dilution , and the least on Escherichia coli.

Cinnamon essential oil show strongest antibacterial effect than clove on all the five pathogens. On Acinetobacter spp the maximum zone of inhibition was 44mm at 1:2 dilution and minimum zone of inhibition was 30 mm at 1:8 dilution.

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 was30 mm at 1:2 dilution and minimum zone of inhibition was 10 mm at 1:4 dilution.

The antibiotics (TS (TRIMETHOPRIM),T(TETRACYCLINE), E (ERYTHROMYCIN) ,CIF (CIPROFLOXACIN), FOX (CEFOXITIN), SAM (AMPICILLIN), and NA (NALIDIXIc ACID), were used in this study to get a results for comparing their effect with the clove and cinnamon oils.

The effect of the clove essential oil higher than the effect of antibiotics used against the Acinetobacter spp. But 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

KEYWORD : Essential oil, Clove , Cinnamon, Antibacterial.

INTRODUCTION

Various essential oils are used to improve digestion, promote hormonal balance, and tone the nervous system in conditions including anxiety, depression, sexual dysfunction and exhaustion (Lehrner et al ., 2005). . It is used as an antiseptic and essential oils are also used in flavouring food and pharmaceutical products (Ashurst, 1990).
The essential oils (EOs) from many plants are known to possess antibacterial and antifungal activity Hence, there
is a great demand for novel antimicrobial drugs agents, justifying the intense search for new drugs that are more
effective and less toxic than those already in use (Bakkali,2008; Burt , 2004).The cloves are anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic , It is also used in dentistry where the essential oil of clove is used as anodyne for dental emergencies. (Pandey and Singh, 2011).

Micro-organisms like Alternaria sp., Aspergillus sp., Canninghamella sp., Lactobacillus sp. Fusarium sp., Clostridium sp; Mucor sp., Salmonella sp. Penicillium sp. Bacillus sp. could be repressed by using clove essential oil (Soliman and Badeaa, 2002). clove oil showed strong antibacterial activity against E.coli followed by Proteus mirabilis although to a lesser extent.

E.coli is the major causes of urinary tract infections (Anis and Arifa , 2013). Antimicrobial activity of extract revealed that the solvent extract of clove has a great potential for the inhibition of microbial load. The value of antimicrobial activities of solvent extract ranged from 12 to 17 mm in the disc diffusion method as compared to aqueous extract i.e ranged from 12 to 16 mm. Minimum Inhibitory Concentrations were found from 17 to 23 mm for solvent extract and 13 to 17 mm for aqueous extract (Muhammad and Muhammad, N. 2013).

cinnamon also plays an important role in pharmacological effects such as : ant- inflammation , antimicrobial , antioxidant , antidiabetes type 2, antispasmodic , antiulcer, and cytotoxic properties (Cui.et al 2016). In recent years, some studies have reported that cinnamon oil had a broad range of antimicrobial activities against gram-positive and gram-negative bacteria (Tyagi and Malik , 2011). Several reports have shown the promising effect of cinnamon essential oils against several species of bacteria such as Staphylococcus aureus, Listeria monocytogenes , Escherichia coli, Bacillus cereus, and Salmonella typhimurium (Burt, 2004; Brenes and Roura ,2010).

MATERIALS AND METHODS

The antimicrobial activity of the Clove and cinnamon plant essential oils was evaluated by the disc diffusion method against five pathogens including Staphylococcus aureus, Escherichia, Escherichia coli, Pseudomonas aeroginosa, Proteus spp., and Acinetobacter spp.

Microbial Preparation

The pathogenic organisms used in this study (staphylococcus aureus , Escherichia coli, Pseudomonas aeroginosa, Proteus spp., Acinetobacter spp.) were obtained from the laboratory of Al- Yarmouk Teaching Hospital .

McFarland opacity standards was prepared, which provides an optical density comparable to the density of a bacterial suspension 1.5x 10^8 colony forming units (CFU/ml) (McFarland and Nephelometer, 1907).

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 (1.5×10^8 CFU/mL) for obtaining the zone diameters of antibacterial effect of essential oil.

Preparation of essential oils

In order to extract the volatiles from 50 gm of dried powdered plant material for each of (clove seeds, cinnamon bark) were done in this study by Hydrodistillation method using Clevenger apparatus according to birth pharmacopoeia procedure . After 8 hours of continuous distillation for each plant material , the following essential oils were obtained

cloves seeds ---------- 3.5 ml / 50mg

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

(British pharmacopoeia volume **I V a**ppendices**- 2014**.

Appendix **X I** E . volatile oil in drugs (Ph.Eur.method 2.8.12)

Preparation 5% DMSO (Dimethy losulfoxide)

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 control.

Preparation of inoculum

When the inoculum has to be made from a pure culture in sterile normal saline , a loopful of the confluent growth is similarly suspended in saline. Compare the tube with the turbidity standard and adjust the density of the test suspension to that of the standard by adding more bacteria or more sterile saline. Inoculate the plates by dipping a sterile swab into the inoculum . Remove excess inoculum by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.

Antimicrobial sensitivity of Clove and cinnamon plant essential oils

Sterile filter paper discs of 5 mm diameter were dipped aseptically in appropriate concentration of essential oils

and placed over Mueller–Hinton plates seeded with respective pathogens.

The plates were incubated in an upright position at 37°C for 24 h. After overnight incubation, the diameter of each zone was measured in mm. Discs with 7 mm diameter were considered as having no antibacterial activity. Diameter between 7 and 12 were considered as moderatively active and those with > 12 mm were considered as highly active.

RESULTS & DISCUSSION

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

Effect of clove essential oil

Clove essential oil showed maximum effect on Acinetobacter spp**,** staphylococcus aureus**,** Proteus spp and minimum effect on Pseudomonas aeroginosa 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 was20 mm at 1:2 dilution and minimum zone of inhibition was 12 mm at 1:8 dilution.

0n Proteus spp the maximum zone of inhibition was15 mm at 1:2 dilution and minimum zone of inhibition was 11 mm at 1:8 dilution.

0n Pseudomonas aeroginosa spp the maximum zone of inhibition was10 mm at 1:2 dilution and minimum zone of inhibition was 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.

The best effect of antibacterial activity of clove oil was shown on Acinetobacter spp and the least on Escherichia coli .

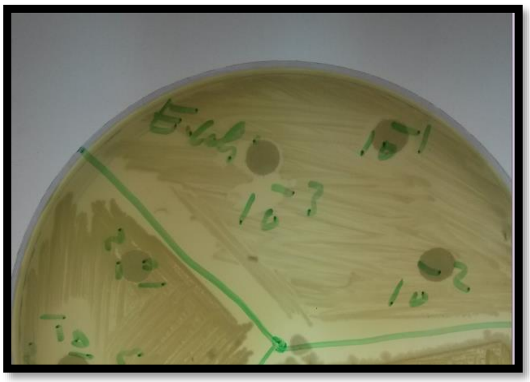
Also the results are agree with another study that found clove oil was effective against Gram positive bacteria staphylococcus aureus (Muhammad and Muhammad, 2013).

In this study clove oil show strongest antimicrobial activity against Proteus mirabilis, and it shows least activity against E.coli . which is not agree with that clove oil show strongest antimicrobial activity against E.coli, and it shows least activity towards Proteusn mirabilis .(Anis and Arifa , 2013).

Table 1: Antibacterial effect of Cloves essential oil on different pathogenic microorganisms

Organisms	Zone of inhibition of Cloves at different dilution using 5 mm disc diameter.			
	1:2	1:4	1:8	control
staphylococcus aureus	20	15	12	-ve
Escherichia coli	8	R	5	-ve
Pseudomonas aeroginosa	10	7	6	-ve

Proteus spp	15	13	11	-ve
Acinetobacter spp	30	24	14	-ve

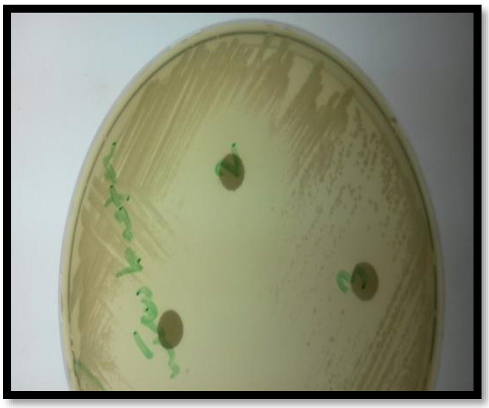


Staphylococcus aureus

Escherichia coli

Pseudomonas eroginosa

Proteus spp



Acinetobacter spp

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

Effect of cinnamon essential oil

Cinnamon essential oil showed most effect on Acinetobacter spp, E.coli ,staphylococcus aureus, Pseudomonas aeroginosa 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 was 30 mm at 1:8 dilution.

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 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 inhibition20 mm at 1:8 dilution.

On Pseudomonas aeroginosa spp the zone of inhibition was30 mm at 1:4 dilution and minimal area of inhibition become 20 mm at 1:8 dilution.

Cinnamon essential oil show strong antibacterial effect on all the five pathogens. as it is mentioned above respectively.

The results obtained by Ali (2011) concluded that 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 in this study are in agreement with Cui, et al. (2016) who recorded that Staphylococcus aureus was highly sensitive to cinnamon oil.

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

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

Staphylococcus aureus & Escherichia coli Pseudomonas aeroginosa

Proteus spp Acinetobacter spp

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

comparison between the effect of antibiotics and the effect of essential oils on pathogenic organisms

The effect of the clove essential oil higher than the effect of antibiotics used against the Acinetobacter spp. But 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 essential oil of cinnamon was found to be active against all the bacterial pathogens tested, when compared to clove oils.

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

organisms	Antibiotics	Zone of inhibition	Essential oils	Zone of inhibition		
				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	Cinnamon	28	30	32
	FOX	26				
	Sam	20				
	Na	R				
Pseudomonas aeroginosa	TS	R	Clove	10	7	6
	T	14				
	E	10				
	CIF	14	Cinnamon	20	30	28
	FOX	R				
	Sam	12				
	Na	R				
Proteus spp	TS	R	Clove	15	13	11
	T	R				
	E	R				
	CIF	14	Cinnamon	20	26	30
	FOX	20				
	Sam	30				
	Na	R				
Acinetobacter spp	TS	R	Clove	30	24	14
	T	27				
	E	18				
	CIF	12	Cinnamon	30	34	44
	FOX	R				
	Sam	17				
	Na	R				

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

R: Resist

Staphylococcus aureus Escherichia coli

Proteus spp Pseudomonas aeroginosa

Acinetobacter spp

Fig 3 : Antibacterial activity of Antibiotic against the test bacteria

CONCLUSION

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

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Appendix XI E. Volatile Oil in Drugs(Ph. Eur. method 2.8.12)

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