



ANTIBACTERIAL ACTIVITY OF CARAWAY SEEDS AND INDIAN COSTUS ROOT ESSENTIAL OILS

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ABSTRACT

The disc diffusion method was used to examine the antimicrobial activity of the caraway seeds and Indian costus root essential oil. This was done against five pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp.*, and *Acinetobacter spp.* The antimicrobial effect of Caraway essential oil was shown to be weak on *Staphylococcus aureus*, *Proteus spp.*, *Escherichia coli*, *Pseudomonas species* but its efficacy was high on *Acinetobacter spp.* Diameter zone of inhibition (mm) of 1:2, 1:4, 1:8 dilutions of essential oil of Costus against *Staphylococcus aureus* were 50, 12, zero; and against Gram negative organisms according to its strength respectively were: *Acinetobacter spp.* 50, 36, 14; *Proteus spp.* 50, 24, 14; *Escherichia coli* 34, 15, 11; *Pseudomonas species* 20, 10, zero. Therefore, this indicates the strong effect of Costus essential oil against all tested pathogens. The researcher of present study used the antibiotics TS (Trimethoprim), T (Tetracycline), E (Erythromycin), CIF (Ciprofloxacin), fox (Cefoxitin), SAM (Ampicillin), and NA (Nalidixic Acid), so as to reach to some results that can help in comparing their effect with the caraway and costus essential oils. The effect of such oils could be higher than the effect of antibiotics. Moreover costus is more active than caraway and all antibiotics used against the tested bacteria.

KEY WORDS: Essential oil, Caraway, Costus, Antibacterial.

INTRODUCTION

It is reported that the antimicrobial properties of medicinal plants and many biologically active compounds found in plants are studied from different parts of the world. Researchers state that the world health organization estimated that nearly 80% of the world's population is based on traditional medicinal system for some aspect of primary health care (Samy and Ignacimuthu, 1998, Samy *et al.*, 1999, Hasan and Qari, 2010). Besides many scholars identified many mechanisms to give an explanation for the activity of an Essential oil on bacterial cells. This oil can affect both the external envelope of the cell and the cytoplasm. In this regard, one can say that Gram-negative bacteria are more resistant to EOs than Gram-positive bacteria (Filomena *et al.*, 2013). A great deal of research discovered a potential antimicrobial action of caraway. This action was assessed against gram-positive and gram-negative bacteria (Deepak, 2013, Yousef and Hammad, 2010, Anupam *et al.*, 2016). This is in on the one hand. Antifungal activity of caraway oil, on the other hand, is recorded towards soil, food, animal and human pathogens (De Martino *et al.*, 2009). Diameter zone of inhibition (mm) of essential oil of *Carum carvi* seeds against *Staphylococcus aureus* were: 29, 34, 38; and against Gram -negative organisms *Escherichia coli* 31, 33, 36; *Pseudomonas species* 29, 32, 36 respectively (Ali, 2015). Low impact of caraway essential oils was observed toward bacteria belonging to the genus *Pseudomonas* (Iacobellis *et al.*, 2005). Another point to emphasize here is that Costus is an important medicinal plant used to treat different diseases. This plant is found to own many pharmacological activities such as antibacterial, antifungal, antioxidant, antiinflammatory, antipyretic,

antidiuretic, antistress and estrogenic activity (Pawar and Pawar, 2014). Moreover the methanolic extract of costus was found to have important activity against gram-positive-*Staphylococcus aureus* and *Bacillus subtilis* and gram-negative bacteria- *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimrium* (Yogita *et al.*, 2014). Sulakshana *et al.* (2013) stated that the rhizome extract of *Costus* species has a potent antibacterial property against *S. aureus* followed by *P.aeruginosa* and *E.coli*. The concentrations 5, 10, 15 and 20% of *Costus* species were shown to have a high inhibitory rate on the growth of *E. coli*, *P. aeruginosa* and *S. aureus* (AL-Kattan and Manal, 2013).

MATERIALS & METHODS

The antimicrobial activity of Caraway seeds and Indian Costus root essential oils by using the disc diffusion method against five pathogens including *Staphylococcus aureus*, *Escherichia coli*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp.*, and *Acinetobacter spp.*

Microbial preparation

A number of pathogenic organisms were used in this study including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Acinetobacter spp.* They were obtained from the laboratory of Al-Yarmouk Teaching Hospital. McFaland standard was prepared to give an optical density comparable to the density of a bacterial suspension 1.5×10^8 colony forming units (CFU/ml).

Agar disk diffusion test

In order to determine the antimicrobial activity of the essential oils. The discs diffusion method was used. Mueller-Hinton agar was seeded with a test strain

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

Preparation of essential oils

Indian costus roots and Caraway seeds were done by hydrodistillation method by using Clevenger apparatus according to birth pharmacopoeia procedure, so as to extract the volatiles from 50 gm of dried powdered plant for each plant material. When distillation continued for 8 hours the following were obtained.

Caraway seed ----- 1ml / 50g

Indian costus root-----0.3 ml /50 mg

Preparation 5% DMSO (Dimethylsulfoxide)

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 control.

Preparation of inoculum

When the inoculum was intended to prepare from a pure culture in sterile normal saline, a loopful of the growth was suspended in saline. In this case, the tube was compared to the standard and the density was adjusted to that of the standard by adding more bacteria or more sterile saline. Then, the plates were inoculated by dipping a sterile swab into the inoculum. Excess inoculum was removed by pressing the swab firmly against the side of the tube.

Antimicrobial activity of Caraway seeds and Indian Costus root essential oils

An important aspect of the antimicrobial activity of the essential oil in this study is that the sterile paper discs of 5 mm diameter were dipped aseptically in an 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.

RESULTS & DISCUSSION

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

Effect of Caraway essential oil

The results of this study indicated that the Caraway essential oil showed the maximum effect on *Acinetobacter spp.*, *E. coli*, *staphylococcus aureus*, *Proteus spp.* and minimum effect on *Pseudomonas aeruginosa*.

On *Acinetobacter spp.* the maximum zone of inhibition was 34mm at 1:2 dilution and minimum zone of inhibition was 30 mm at 1:8 dilutions.

On *E. coli* the maximum zone of inhibition was 16 mm at 1:2 dilution and no zone of inhibition at 1:8 dilution.

On *staphylococcus aureus* the maximum zone of inhibition was 12 mm at 1:2 dilution and no zone of inhibition at 1:8 dilution. On *Proteus spp.* the maximum zone of inhibition was 11 mm at 1:2 dilution and minimum zone of inhibition was 8 mm at 1:8 dilutions. On *Pseudomonas aeruginosa* the maximum zone of inhibition was 10 mm at 1:2 dilution and minimum zone of inhibition was 6 mm at 1:8 dilutions (Table 1 and Figure 1).

The best effect of antibacterial activity of Caraway oil in this study was shown against *Acinetobacter spp.* and the least on *Pseudomonas aeruginosa*. Such results do not agree with the findings of (Ali, 2015) but agree with the findings of (Iacobellis *et al.*, 2005).

TABLE 1: Antibacterial effect of Caraway essential oil on different pathogenic microorganisms

Organisms	Zone of inhibition of Caraway at different dilutions using 5 mm disc diameter			
	1:2	1:4	1:8	control
<i>staphylococcus aureus</i>	12	R	R	-ve
<i>Escherichia coli</i>	16	R	R	-ve
<i>Pseudomonas aeruginosa</i>	10	7	6	-ve
<i>Proteus spp</i>	11	10	8	-ve
<i>Acinetobacter spp</i>	34	30	30	-ve



FIGURE 1: Inhibition zone diameter produced by Caraway essential oil against the test bacteria

Effect of Costus essential oil

Costus essential oil showed a great effect on *Acinetobacter spp.*, *E. coli*, *staphylococcus aureus*, *Pseudomonas aeruginosa* and *Proteus spp.* (table 2 and Figure 2).

Diameters zone of inhibition (mm) of 1:2, 1:4, 1:8 dilutions of essential oil of Costus against *Staphylococcus aureus* were 50, 12, zero and against Gram negative organisms according to its strength were: *Acinetobacter*

spp 50, 36, 14; *Proteus spp* 50, 24, 14; *Escheriachia coli* 34, 15, 11; *Pseudomonas species* 20, 10, zero; respectively. The results of this study seem to agree with (Yogita *et al.*, 2014, Sulakshana *et al.*, 2013, AL-Kattan and Manal, 2013). Table -3 below makes it clear the effect of the Costus essential oil is greater than the Caraway essential oil effect on all the five pathogenic bacteria used in this study.

TABLE 2: Antibacterial effect of Costus essential oil on different pathogenic microorganisms

Organisms	Zone of inhibition of Costus at different dilutions using 5 mm disc diameter			
	1:2	1:4	1:8	control
<i>staphylococcus aureus</i>	50	12	R	-ve
<i>Escherichia coli</i>	34	15	11	-ve
<i>Pseudomonas aeruginosa</i>	20	10	R	-ve
<i>Proteus spp</i>	50	24	14	-ve
<i>Acinetobacter spp</i>	50	36	14	-ve

**FIGURE 2:** Inhibition zone diameter produced by Costus essential oil against the test bacteria**Comparison between the effect of antibiotics and the effect of Caraway seeds and Indian Costus root essential oils on pathogenic organisms**

The antibiotics used against the tested pathogens with the exception of *Acinetobacter spp.*, seem to be stronger than

the effect Caraway essential oil. Moreover, the effect of Costus on all tested bacteria was significantly higher than all the antibiotics used. It is also more effective than Caraway essential oil (Table3 and Figure 3).

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	
<i>Staphylococcus aureus</i>	TS	25	Caraway	12	R	R	
	T	14					
	E	22					
		CIF	12	Costus	50	12	R
		FOX	28				
		Sam	20				
		Na	R*				
	TS	30	Caraway	16	R	R	
	T	12					
	E	12					
<i>Escherichia coli</i>		CIF	Costus	34	15	11	
		FOX		26			
		Sam		20			
		Na		R			
		TS		R			
<i>Pseudomonas aeruginosa</i>		T	Caraway	10	7	6	
		E		14			
		E		10			
		CIF	14	Costus	20	10	R
		FOX	R				
		Sam	12				
		Na	R				
<i>Proteus spp</i>		TS	Caraway	11	10	8	
		T		R			
		E		R			
		CIF	14	Costus	50	24	14
		FOX	20				
	Sam	30					
	Na	R					

Antibacterial activity of caraway seeds and Indian costus root

<i>Acinetobacter spp</i>	TS	R	Caraway	34	30	30
	T	27				
	E	18				
	CIF	12	Costus	50	36	14
	FOX	R				
	Sam	17				
Na	R					

TS (Trimethoprim), T (Tetracycline), E (Erythromycin), CIF (Ciprofloxacin), FOX (Cefoxitin), SAM (Ampicillin), NA (Nalidixic Acid).R: Resist

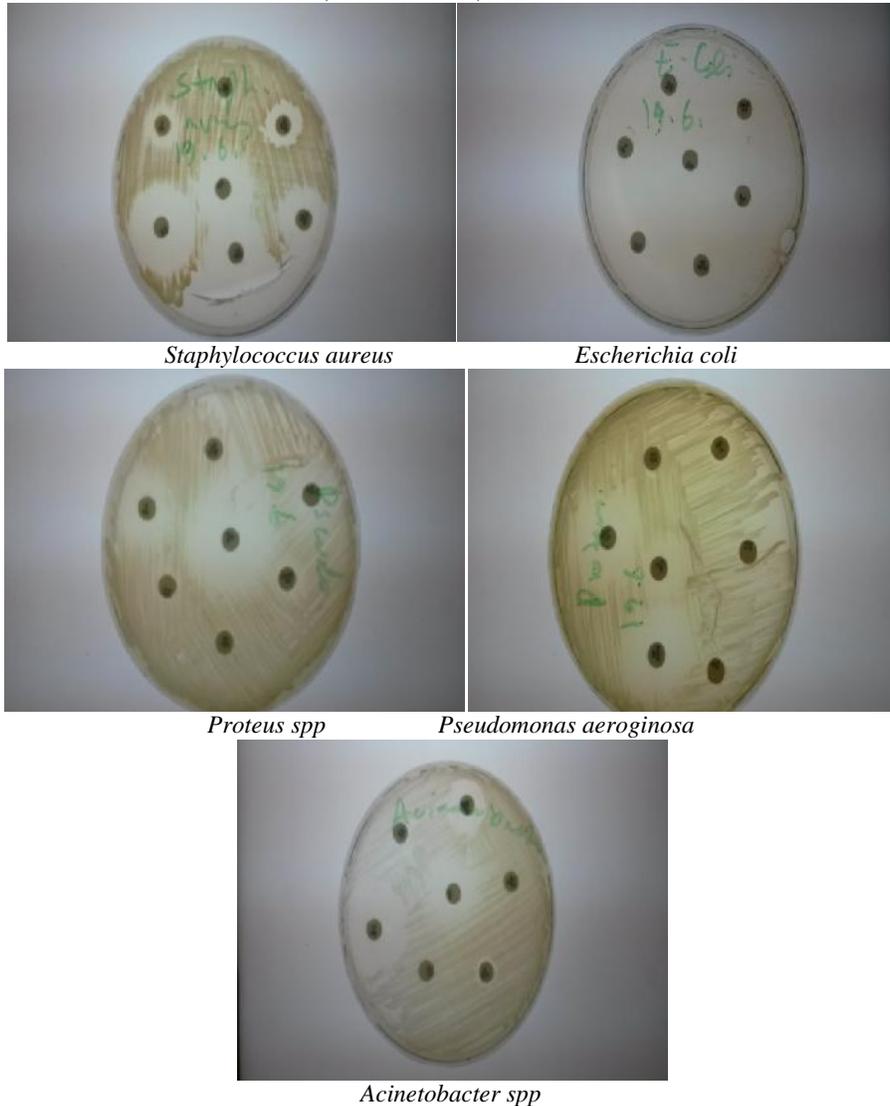


FIGURE 3: Antibacterial activity of Antibiotic against the test bacteria

CONCLUSION

The present study showed that the five examined bacterial strains, being continuously isolated from many disease conditions, are resistant to many available antibiotics used to treat their diseases.

This tells that there is a need for alternatives. Besides it is possible to use the costus essential oil as one of the alternative medicines to treat the locally antibiotic - resistant bacteria.

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