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Antimicrobial Activity of Ethanolic Extracts of *Ocimum basilicum* leaf from Saudi Arabia

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Abstract: Essential oils or volatile oils from medicinal plants have potentially very strong antimicrobial activities for control of pathogenic microorganisms. *Ocimum basilicum* is one of the medicinal plants which are widely used as sources of extracts with strong antibacterial and antioxidant properties. In this study, the leaf extract of *Ocimum basilicum* was tested for its antibacterial activity against some human pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*). Antimicrobial activity test was carried out by using the hole-plate diffusion method. Plant materials were dried and extracted with 95% ethanol. The tested extract showed very strong antimicrobial activity against both strains. The antimicrobial activity was evaluated by measuring the zone of inhibition. The strongest inhibition activity was observed against *E. coli* (21 mm zone) at 200 mg mL^{-1} of leaf extract followed by *Staphylococcus aureus* which showed 16 mm inhibition zone at 200 mg mL^{-1} of leaf extract. Physical properties of the extract of *Ocimum basilicum* were evaluated. The color of the extract is greenish, has intense scent, density is 0.937 g cm^{-3} at 25°C, refractive index is 1.489 and specific gravity is 0.887.

Key words: *Ocimum basilicum*, medicinal plant, ethanol leaf extract, antibacterial activity

INTRODUCTION

Ocimum basilicum or Al-Rehan (in Arabic) is a well-known medicinal plant which has received a great deal of attention over the past few decades around the world. It belongs to the Lamiaceae family of floral plants usually producing white-purple flowers (Daneshian *et al*., 2009). Extracts of *Ocimum basilicum* having strong antibacterial and antioxidant properties are widely used for medicinal purposes. Plant parts and essential oils from Ocimum plants are used as spices and flavors for many food products and effective drugs in folk medicine (Sacchetti *et al*., 2004; Jirovetz *et al*., 2003).

Many medicinal plants which produce antioxidants, antibacterial, antiviral and fungicidal agents have gained popularity in recent years. Extracts from *Ocimum basilicum* have shown strong inhibitory effects on HIV-1 reverse transcriptase and platelets aggregation (Yamasaki *et al*., 1998; Okazaki *et al*., 1998). These extracts are also a rich source of flavor compounds and volatile oils which possess compounds that contain antimicrobial activities (Yamasaki *et al*., 1998; Wannissorn *et al*., 2005). Some parts of sweet basil such as leaves and flowers are used as carminative, galactogogue, stomachic and antispasmodic medicinal plants in folk medicine (Marwat *et al*., 2011).

Due to random and wide usage of antibiotics in the treatment of infectious diseases, a multiple drug resistance against pathogenic microorganisms has been developed in recent years (Davies, 1994; Service, 1995). One of the best ways to solve this problem, is to look for new therapeutic agents from plants which contain antimicrobial activities against these pathogenic microorganisms (Kalemba and Kunicka, 2003; Faleiro *et al*., 2003).

Khaki *et al*. (2011) investigated the role of *O. basilicum* extracts as a source of natural antioxidants to assist the sperm parameters in rats. The results of their work showed that *O. basilicum* extracts significantly affect the process of spermatogenesis and might be a promising treatment for enhancing healthy sperm parameters in rats.

The purpose of this study was to evaluate the Saudi *Ocimum basilicum* leaf extract as antimicrobial agents against some human pathogens.

MATERIALS AND METHODS

Ocimum basilicum were cultivated and collected at the flowering stage from a home garden in the Eastern Province of Saudi Arabia. In this study, extracts from the leafs of *Ocimum basilicum* were tested for their antimicrobial activities.

Preparation of extracts: Plant materials were dried in shade at room temperature and ground by using a blender. Two hundred and fifty gram of plant powder was soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature. The mixture was mixed daily for regular infusion. After a five-day period, the extract was filtered by using Whatman filter paper No. 1. The filtrate was dried by using a rotary evaporator at 60°C. The dried extract was stored in sterile glass bottles at -20°C until use (Kandil *et al*., 1994).

Microorganisms: Two bacterial species, gram-positive *Staphylococcus aureus* and Gram-negative *E. coli* were used. These microorganisms were obtained from KFUPM clinic. We also tested *E. coli*-ATCC 25922 and *Staphylococcus aureus*-ATCC 33591 as standard strains.

Screening of antimicrobial activities: Inoculums containing 10^6 bacterial cells mL^{-1} were spread on nutrient agar medium. Antimicrobial activity test was carried out by using the hole-plate diffusion method (Khalil *et al*., 2009). Holes were made on the media by using 8 mm cork borer. The dried plant extracts were dissolved in dimethylsulfoxide (DMSO) to a final extract amount of 200, 150, 100 and 50 mg mL^{-1}. Each hole (diameter 8 mm) was filled with 50 μL of plant extract. The inoculated agar plates were incubated at 37°C for 24 h. After the incubation period, the diameter of inhibition zone to each hole was measured in mm (the inhibition zone is an average of 3 experiments). The inhibition zone is the area surrounding the hole and there is no growth of the inoculated microorganism. Negative controls of DMSO and 96% ethanol showed no antimicrobial activity against any of the tested microorganisms.

Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Penicillin-G (10 μg $disk^{-1}$) was used as a positive reference standard to determine the sensitivity of one strain from each bacterial species. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

RESULTS AND DISCUSSION

The antimicrobial activity of ethanol leaf extracts of *Ocimum basilicum* from Saudi Arabia was investigated against some human pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*). The strongest inhibition activity of the leaf extract was observed against *E. coli* followed by *Staphylococcus aureus*. Physical properties of the extract were investigated.

Physical properties: Table 1 summarizes the physical properties of the *Ocimum basilicum* extract; the colour of the extract is greenish, has intense scent, density is 0.937 g cm^{-3} at 25°C, refractive index is 1.489 and specific gravity is 0.887. These physical properties are in agreement with the physical properties reported in a previous study done by Hanif *et al*. (2011).

Antimicrobial activity: The antimicrobial activity of *Ocimum basilicum* leaf extract was tested against 2 pathogenic bacteria (*Staphylococcus aureus* and *E. coli*) shown in Table 2. The tested extract showed very strong antimicrobial activity against these 2 pathogenic bacteria. The antimicrobial activity was evaluated by measuring the zone of inhibition. The strongest inhibition activity of the leaf extract was observed against *E. coli* (21 mm zone) at 200 mg mL^{-1} of leaf extract followed by *Staphylococcus aureus* which showed 16 mm inhibition zone at 200 mg mL^{-1} of leaf extract (Table 2). The results showed that ethanol extract of *O. basilicum* has an antimicrobial effect against *E. coli* (Gram negative bacteria) and *Staphylococcus areous* (Gram positive bacteria) strains.

Table 1: Physical properties of leaf extract of *Ocimum basilicum*

Properties	Description
Color	Greenish
Smell	Intense minty scent
Density	0.937 g cm^{-3} at 25°C
Refractive index	1.489 at 25°C
Specific gravity	0.887
Solubility	Water insoluble, soluble in several organic solvents

Table 2: Antimicrobial activity of *Ocimum basilicum* leaf extracts against *E. coli* and *Staphylococcus areus*

Bacteria used	Conc. of extract (mg mL^{-1})	Inhibition zone (mm)
E. coli from KFUPM clinic	200	21
	150	14
	100	10
	50	4
Staphylococcus aureus from KFUPM clinic	200	16
	150	13
	100	9
	50	4
E. coli-ATCC 25922	200	20
	150	13
	100	9
	50	3
Staphylococcus aureus-ATCC 33591	200	16
	150	12
	100	9
	50	3

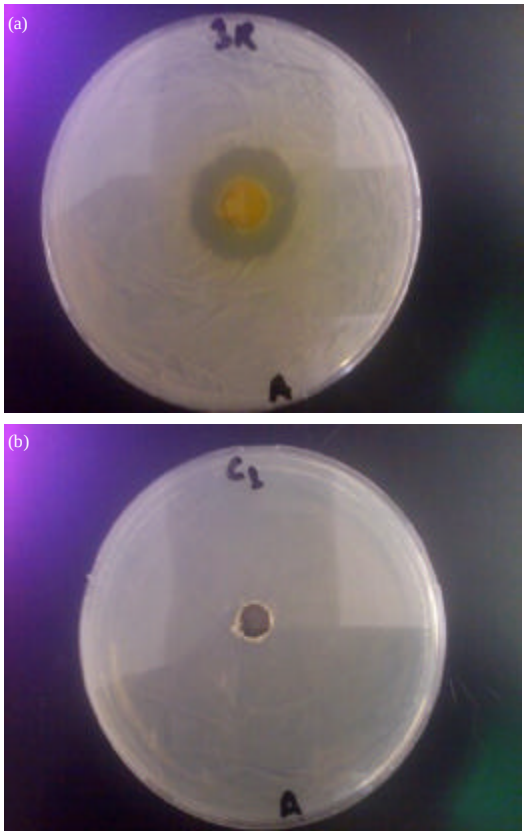

Fig. 1(a-b): Inhibitory effects of ethanol leaf extract of *Ocimum basilicum* against (a) *E. coli* and compare to (b) Control

Table 3: Antimicrobial activity of the positive control penicillin-G (10 μg disk^{-1})

Bacteria used	Inhibition zone (mm)
E. coli from KFUPM clinic	15
Staphylococcus aureus from KFUPM clinic	12
E. coli-ATCC-25922	17
Staphylococcus aureus-ATCC 33591	13

Based on these results, the ethanol extract has a stronger and broader spectrum of antimicrobial activities compared to positive control used in this study (Table 3) which is Penicillin-G (10 μg disk^{-1}).

Figure 1 shows the inhibitory effects of ethanol leaf extract of *Ocimum basilicum* against *E. coli* compare to the control (without extract). The inhibitory effects of ethanol leaf extract of *Ocimum basilicum* against *Staphylococcus areous* compare to the control was shown in Fig. 2. Similar results against *E. coli* and *Staphylococcus aureus* were obtained in a study done by Adebolu and Oladimeji (2005). The strong inhibitory activities against these microorganisms showed that

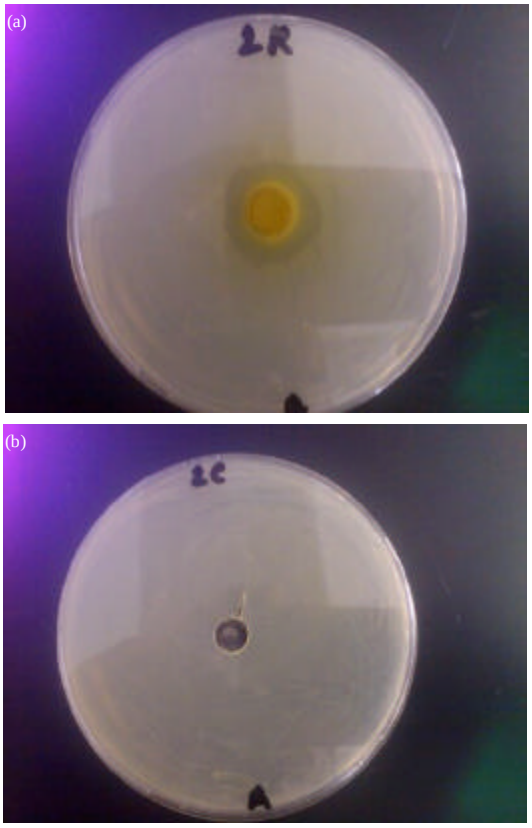

Fig. 2(a-b): Inhibitory effects of ethanol leaf extract of *Ocimum basilicum* against (a) *Staphylococcus areous* and compare to (b) Control

Ocimum basilicum can be used as a natural agent against some human pathogens. Many previous studies (Wannissorn *et al*., 2005; Bozin *et al*., 2006; Lopez *et al*., 2005; Sokovic *et al*., 2006) have reported the antimicrobial activities of *Ocimum basilicum* against various microbes.

CONCLUSION

The results of the present study suggest that *Ocimum basilicum* extracts possess compounds with potent antimicrobial properties against *E. coli* and *Staphylococcus aureus*as human pathogens.

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