

Study of Antibacterial Activity of *Ocimum basilicum* Against *Staphylococcus aureus* in Vitro

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ABSTRACT

The investigation of inhibitory effect of the alcoholic extract of *Ocimum basilicum* on the growth of *Staph. aureus* that was isolated from the skin infected in vitro have been studied. *Ocimum basilicum* was isolated using 95% ethanol. Out of which the percentage of extraction of 45% of weight of dried powder was prepared in ascending gradient concentrations of the alcoholic extract (10-100 mg/ml) and the effective one was selected by agar diffusion method using *Staphylococcus aureus*. The diameters of the inhibition zones of the bacterial growth were increased parallel with the concentrations of the alcoholic extract concentrations. Low efficiency detected post using 10-20mg/ml concentrations, medium efficiency post using 40-60 mg / ml, whereas concentrations of 80- 100 mg/ ml were highly effective and influential against growth of the *Staphylococcus aureus*.

1. Introduction

Medicinal plants are usually used for several years in various aspects of life such as food preservation, alternative medicine and the treatment of certain infectious diseases. Their derived compounds are considered normal and non industrial, thus they are more admissible from an environmental perspective. Currently, there are in great demand because there are used possibly as anti-viruses, anti-bacterial and anti-fungal substances in humans due to their minimum or lack side effects, compared with chemical medicines (1, 2). The medicinal plants are attracted to get new therapeutic alternatives anti-microbial substances (3-5).

Ocimum basilicum is well known as basil and it is grown and resides in different regions of Iraq. Their basil leaves are used in spices industries and its basil oil is used widely in the perfume industries and as anti-products of microbial and oxidative stress as well as mouth gargling industries. In addition to that their using in the packaging of antibiotics because they possess lower harmful effects to consumers (6).

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The flowers and leaves leaf of *Ocimum basilicum* are introduced in a large scale as it involved in manufacturing of acne, sinus treatments, insect stings and bites snakes in addition to skin infections (7). The latter includes *Listeria monocytogenes*, *Shigella*, *Salmonella* and *Proteus*, for the fungi *Trichophytonrubrum*, *Trichophytonmentagrophytes*, *Cryptococcus neoformans*, *Penicilliumislandicum* and *Candida albicans* (8-12). Recent records indicated that *O. gratissimum* useful in the medication people suffered Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome virus (AIDs) (13).

2. Materials and Methods

2.1 Culture media:

Culture media was prepared according to the manufacturer's instructions in which it must be sterilized well by autoclaving at 121 °C, under the pressure of 15 PSI after incubation at 37 °C for 24 hours. The method was also conducted in the cultivation and diagnosis of bacteria that were used in this study (14).

2.2 Methods:

2.2.1 Preparation of the plant:

Fresh *Ocimum basilicum* (voucher No. 1281) was collected from Baghdad province during the period

extended between July and August of 2015. These plants were air dried, examined and well identified at Ministry of Agriculture State Board for Seed Testing and Certification (S.B..T.C.).

2.2.2 Preparation of plant extract:

Dried leaves of the plant were mechanically grinded and subsequently the plant powder was extracted with ethanol. Aliquots of the extracts were washed for 24 hours at room temperature. After that the extracts were filtered by Whatman filter paper No. 1 and evaporated in the incubator at 30 °C (15). The resultant concentrates were stored in the refrigerator till their using.

2.2.3 Extraction methods:

Ocimum basilicum was skinned and sliced, and then 50 g sliced of pieces were cut and crushed in awarding blender for 10 minutes, subsequently, soaked with 450 ml of 95% ethanol. It was extracted for 3 months at room temperature, the mixture was kept and separated in the test tubes by centrifugation at 3000 rpm and the resulted filtrate was dried in oven at 37 °C for 24 hours. The final product was stored in a deep freeze at -20 °C (16).

2.2.4 Culture preparation:

The Bacteria was activated by re-culturing on nutrient agar and kept in the incubator for 24 hours at 37 °C and the transferred into sterilized tubes containing heart infusion broth, then incubated for 24-72 hours at 37 °C. The total bacterial count was estimated by the spectrophotometer in which the percentages of the light transmittance were up to 26% at a wav length of 580 nm, whereas the light transmittance for nutrient broth of the prepared bacteria was 100% (17).

2.2.5 Preparation standard dilutions of *Ocimum basilicum*:

The dilutions were prepared by using ethylene glycol which is inert solvent against microorganism (18). Serial concentrations from 10-100 mg prepared from the extract, and then diluted it with ethylene glycol and the volume was completed to 2 ml to get the final concentrations from 1 to 10 %.

2.2.6 Activity test of *Ocimum basilicum* extract in well diffusion method screening:

The screening of the antibacterial activity was performed according to well diffusion technique (19). In this technique, Mueller-Hinton agar plates were seeded with 0.1 ml of the standardized inoculums of bacteria. The inoculums were spread evenly over the plate with sterile

glass spreader. The seeded plates were allowed to dry in the incubator at 37 °C for 20 minutes. A standard crack border of 9 mm diameter was used to cut uniform wells on the surface of the plates and then 0.1 ml of each concentration was introduced into the well with ethylene glycol as a control. The inoculated plates were incubated at 37 °C for 24 hours and zones of inhibition diameters were recorded using nearest millimeter (mm).

3. Results

3.1. Identification of bacteria

- A. Bacteria grew well on the Mannitol salt agar(MSA).
- B. Microscopic examination: Bacteria was gram-positive and spherical in shape.
- C. Biochemical tests were confirmed the identification of *Staph aureus* bacteria which was catalase and gelatinase (+) ve, oxidase (- ve), blood agar (B-haemolysis and production of local golden pigment).

3.2. Inhibitory effect of *Ocimum basilicum* extract:

The sensitivity of the above mentioned bacteria found gradually increased with the increased concentration of the *Ocimum basilicum* extract. The zone of the inhibition was 10 mm recorded for the concentration of 10 mg/ml, and 25 mm for concentration 100 mg/ml. The current findings revealed that the concentrations of 10-20 mg/ml were less active in preventing the growth of *Staph aureus*, while the concentrations 40-60 mg/ml were moderately active, but the concentrations 80-100 mg/ml were highly active compared to effect of ethylene glycol which was considered as a control.

Records were listed in table 1, while the inhibitory effect of different concentrations of *Ocimum basilicum* extract on the growth of *Staph aureus* have been percent in Figure1. They revealed a relationship between the concentrations of extract and the diameters of inhibition zones of the growth of *Staph aureus*.

4. Discussion

In this study, the *O. basilicum* showed clearly the antibacterial effect against *Staph aureus*. The sensitivity of this microorganism was gradually increased with increased extract concentrations of *O. basilicum*.

In fact, the bacterial drug resistance is considered a big problem in the world, because numerous types of bacterial species become resistant toward antibacterial drugs (20). Therefore, it is highly required to evaluate the

efficacy of plant chemicals concerning with the growth of bacteria by using extracts of saving plants. Ethanolic extract of *O.basilicum* have been showed a strong antibacterial activity against *S. aureus*. This result was strongly agreement with previous findings (21) in which reported that gram negative bacteria are more resistant than gram positive bacteria to the essential oils which have antimicrobial activity.

One decade before, Nweze *et al* (22) reported antioxidant activities of *O. basilicum* because it possess alkaloids, tannins, glycoside, saponin, resins, cardiac glycoside, steroidal terpenes and flavonoids (23). These data have been agreement with result present elsewhere (24). Thus this can significantly affect the cellular wall of *S. aureus*, which invariably may lead to the collapse of the cell wall and subsequently affecting the entire metabolisms of the organism.

Different concentrations (20, 40, 60, 80, and 100 mg/ml) of the ethanol extract of *O. basilicum* showed positive proportional inhibitory effects toward *S. aureus*, growth with diameters of zones of inhibition of 10, 14, 16, 18, 23 and 25 mm, respectively. The increased concentration causes increased inhibition.

Antimicrobial activity of *O. basilicum* extract may be attributed to its structural composition out of one or more of active ingredients exhausted from the plant by the used ethanol (phenols, flavonoid and tannins). Similar finding were reported by other investigators that tested the antimicrobial activity of *O. basilicum* extract against *S. aureus* (25).

The inhibitory effected of *O. basilicum* on the growth of *Staph aureus* in the current study could be due to the presence of important compounds (phenols, flavonoid and tannins) in *O. basilicum* extract.

On the other hand, there are different species of the *Ocimum* plant, which have almost the same antibacterial effect particularly against *S. aureus* species with certainly some qualitative and quantitative differences in their activities that attributed to relative differences in their essential chemical constituents of *O. basilicum*, were found differences. These phenomena could be related to several cultivations such as, irrigation, fertilization, soil, environmental (temperature and humidity), technical (time of collection, process of extraction part of the plant used) (26-28).

Reference:

1. **Davis J. (1994).**Inactivation; of antibiotics and the dissemination of resistance genes. *Sci.*, 264:375–382.
2. **Service, R. F. (1995).** Antibiotics that resist resistance. *Sci.*, 270:724–727.
3. **Kalemba, D. &Kunicka, A. (2003).** Antimicrobial and antifungal properties of essential oils. *Cur. Med. Chem.*, 10:813–829.
4. **Juliani, H. R. & Simon, J. E. (2002).** Antioxidant activity of basil. In: Janick, J. and Whipkey, A. ed. *Trends in new crops and new uses.* Alexandria, V A: ASHS Press, PP. 575–579.
5. **Falerio, M. L.; Miguel, M. G.; Laderio, F.; Venâncio, F.; Tavares, R.; Brito, J. C.; Figueiredo, A. C.; Barroso, J. G. & Pedro, L. G. (2003).** Antimicrobial activity of essential oils isolated from Portuguese endemic species of *Thymus*. *Let. Appl. Microbiol.*, 36:35–40.
6. **Sappakul, P.; Miltz, J.; Sonneveld, K. & Bigger, S. W. (2003).**Antimicrobial properties of basil and its possible application in food packaging. *J. Agric. Food Chem.*, 51:3197–3207.
7. **Martin, K. W. & Ernst, E. (2004).** Herbal medicines for treatment of fungal infections: a systematic review of controlled clinical trials. *Mycoses.*, 47:87–92.
8. **Begum, J.; Yusuf, M.; Chowdhury, U. &Wahab, M. A. (1993).**Studies on essential oils for their antibacterial and antifungal properties. Part 1. preliminary screening of 35 essential oils. *J. Sci. Ind. Res.*, 28:3- 25.
9. **Nwosu, M. O. &Okafor, J. I. (1995).**Preliminary studies of the antifungal activities of some medicinal plants against *Basidiobolus* and some other pathogenic fungi. *Mycoses.* 38: 191-195.
10. **Akinyemi, K. O.; Mendie, U. E.; Smith, S. T.; Oyefolu, A. O. & Coker, A. O. (2004).** Screening of some medical plants for anti-salmonella activity. *J. Herb. Pharmacother.*, 5(1): 45-60.
11. **Janine de Aquino, L.; Xisto, S. P.; Orionaida de Fatima, L. F.; Realino de Paula, J.; Pedro, H. F.; Hasimoto de Suza, L. K.; Aline de Aquino, L. & Maria de Rosario, R. S. (2005).** Antifungal activity from *Ocimumgratissimum* L. towards *Cryptococcusneoformans*.*Mem. Inst. Oswaldo Cruz.*, 100(1): 55-58.

12. Lopez, P.; Sanchez, K.; Batlle, R. & Nerin, C. (2005). Solid and vapour phase anti-microbial activities of six essential oils susceptibility of selected food borne bacterial and fungal strains. *J. Agric. Food Chem.*, 53(17): 39-69.

13. Elujoba, A. A. (2000). Studies on the antidiarrhoea activity of *Ocimum gratissimum*. University of Ile Ife Press.

14-Forbes BA, Shan DF, & Weissfeld AS, Bailey and Scotts diagnostic microbiology, 12 th edition Elsevier . 2007 .

15. Ozdemir, G.; Karabay, N. U.; Dalay, M. C. & Pazarbasi, B. (2004). Antimicrobial activity of volatile component and various extracts of *Spirulina plantensis*. *Phytother Res.*, 18:754-757.

16-Krell R, Value-Added products from Bee keeping, *Agricultural Services Bulletin*, 1996; No. 124.

17-Jassim SJ, Effect of *Haloxylon articulatum* extract on some experimental bacterial and fungal skin infection in dogs . MSc. Thesis, Pharmacology and Toxicology Department / College of Veterinary Medicine, University of Baghdad, 2003 .

18-Charles OW, Ole G, & Robert FD, Textbook of organic medicinal and pharmaceutical chemistry, 5 th. Edition, J.B. Lippincott company, Philadelphia and Toronto, 1969 .

19-Saeed S, & Tariq P, Anti-bacterial activities of *Mentha piperita*, *Pisum sativum* and *Momordica charantia*, 2005; *Pak.J.Bot.*, 37:997-213 .

20-Garau J, “ B- lactamase : current situation and clinical importance “, *Intensive Care Med.* 1994;, 20(suppl.)3:55-59.

21. Agatemor, C. (2009). Antimicrobial activity of aqueous and ethanol extracts of nine Nigerian spices against four food borne bacteria. *Elect. J. Environ. Agric. Food Chem.*, 8(3): 195- 200.

22. Nweze, E. I.; Okafor, J. I. & Njoku, O. (2004). Antimicrobial activities of Methanolic Extracts of *Tremagaineensis* (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Medicinal Practice. *J. Biol. Res. Biotechnol.*, 2(1):39-46.

23. Ramanathan, R.; Lau, K. K. & Da, N. P. (1989). Antiperoxidative action of flavonoids and related products in ground pork (abstract) proceedings of III int. Symp. On: flavonoids in biology and medicine Singapore. P.56.

24. Robak, K.

& Gryglewski, R. J. (1988). Flavonoids are scavengers of super oxide anions. *Biochem. Pharmacol.*, 37: 837-841.

25. Adiguzel, A.; Gulluce, M.; Sengul, M.; Ogutcu, H.; Sahin, F. & Karaman, I. (2005). Antimicrobial effects of *Ocimum basilicum* (Labiatae) extract.

26. Izzo, A. A.; Di Carlo, G.; Bicardi, D.; De Fusco, R.; Mascolo, N.; Borrelli, F. & Capasso, F. (1995). Biological Screening of Italian Medicinal Plants for Antibacterial Activity. *Phytotherapy Res.*, 9:281-282.

27. Martinez, M. J.; Betancourt, J.; Alonso-Gonzales, N. & Jauregui, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. *J. Ethnopharmacol.*, 52:171-174. [PubMed]

28. Tunon, H.; Olavsdotter, C. & Bohlin, L. (1995). Evaluation of anti-inflammatory activity of some Swedish medicinal plants. Inhibition of prostaglandin biosynthesis and PAF induced exocytosis. *J. Ethnopharmacol.*, 48:61-76.

Table 1. Effect of different concentrations of *Ocimum basilicum* extract on the growth of *Staphylococcus aureus* measured by the diameter of zone of inhibition

Con. (mg/ml)	100	80	60	40	20	10
Zone of inhibition (mm)	25	23	18	16	14	10

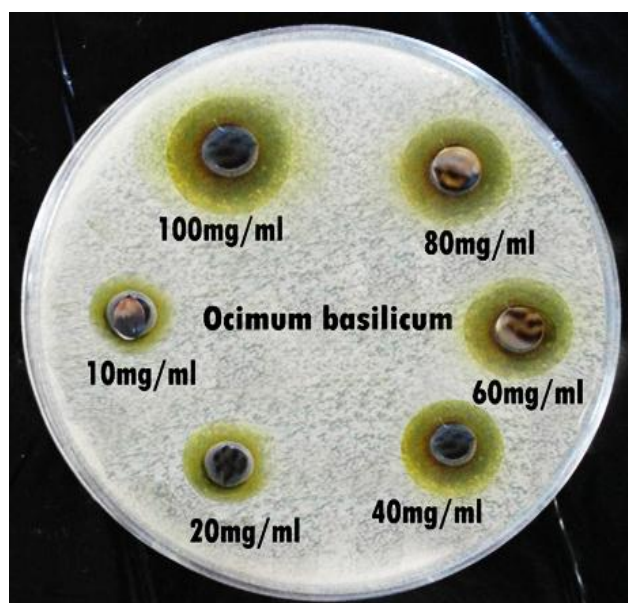


Fig. 1. The inhibitory effect of different concentrations of *Ocimum basilicum* extract on the growth of *Staph aureus*

دراسة الفعالية المضادة للبكتيريا لنبات الريحان ضد بكتيريا المكورات العنقودية الذهبية.

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الخلاصة

أجريت هذه الدراسة لمعرفة فعالية المستخلص الكحولي لنبات الريحان في تثبيط نمو جرثومة المكورات العنقودية المعزولة من الاصابات الجلدية في الزجاج. استخلص الريحان باستخدام ٩٥% من الكحول الايثيلي، حيث بلغت نسبة الاستخلاص ٤٥% من وزن المسحوق الجاف. حضرت تراكيز متدرجة من المستخلص الكحولي (١٠-١٠٠ ملغم/مل) واختبرت فعاليتها بطريقة الانتشار بالحفر باستخدام طبق الاكار المزروع بجرثومة المكورات العنقودية الذهبية مقارنة بطريقة استخدام الأتلين كلايول. اظهرت النتائج أن اقطار تثبيط نمو الجرثومة تزداد بازدياد تراكيز المستخلص الكحولي وكانت التراكيز ١٠-٢٠ ملغم/مل منخفضة الفعالية والتراكيز ٤٠-٦٠ ملغم/مل متوسطة الفعالية بينما تراكيز ٨٠-١٠٠ ملغم/مل كانت ذات فعالية عالية ومؤثرة ضد نمو المكورات العنقودية.