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THE EFFECTS OF ETHANOLIC EXTRACT OF SEED SWEET BASIL (OCIMUM BASILICUM) AGAINST DIFFERENT GRAM NEGATIVE AND POSITIVE BACTERIA AND FUNGI

RAGHAD IBRAHIM AHMED[1]; ALHAN MOHAMMED[2]; ZAINAB AMER HATEM[3]

Abstract

Ocimum basilicum is a well-known medicinal plant which has received a great deal of attention over the past Extracts of *Ocimum basilicum* having strong antibacterial and antioxidant properties are widely used for medicinal purposes. From the above results, it is concluded that some medicinal plants showed antibacterial activity. Medicinal plants are important to human beings in preservations our health. The effects of ethanolic extract of seed sweet basil (*Ocimum basilicum*) against different bacteria such as *E.coli* ,*S.aureus* ,*S.epidermidis* ,*P.aerugionsa* and the fungi *Candida .albicans* were studied. At a concentration of 100 mg/ml, *Ocimum basilicum* caused a marked increase in zone of inhibition (mm) on this bacteria and fungi growth. Inhibition zones sizes were different and increased according to concentration of extract and again the growth was completely inhibited in the highest concentration. A similar outcome was observed using 24 hours incubation period of bacterial growth. Furthermore, *Ocimum basilicum* had dependent concentration effect on this bacteria inhibition; it extended the diameter of zone inhibition. Induction of zone inhibition was also time.

Introduction:

Some medical plants have been used for a wide variety of purposes such as food preservation, pharmaceutical, alternative medicine, and natural therapies for many thousands of years. It is generally considered that compounds produced naturally, rather than synthetically, will be biodegraded more easily andtherefore be more environmentally acceptable. Thus, natural antioxidants, antibacterial, cytotoxic, antiviral,fungicidal agents and nutrients have gained popularity in recent years, and their use and positive image amongconsumers are spreading. In recent years, multiple drug resistance in both human and plant pathogenicmicroorganisms have been developed due to the indiscriminate use of commercial antimicrobial drugscommonly used in the treatment of infectious diseases (Davis, 1994; Service, 1995).

During the last century, the practice of herbalism became mainstream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries.(shweash *et al*.,2014). The term of herbal drugs means that the part of plant used in medicines such as (stems ,flowers ,leaves ,barks, seeds) .(Anonymous,2007). And the example of this plant is *Ocimum basilicum* .

Basil (*Ocimum basilicum*) of the family *Lamiaceae*, typically called sweet basil, or Holy basil. The plant grows in several regions around the world. The genus *Ocimum* is ranked high among some of the astonishing herbs for having enormous medicinal potentialities. (Klima´nkova *et al*., 2008). In traditional medicine, *Ocimum basilicum* has been used as an antioxidant, antiseptic, preservative, digestive regulator and diuretic. It also has been used for the treatment of headaches, coughs, infections of upper respiratory tract, kidney malfunction and to eliminate toxins (Evans *et al*., 2006). Plants produce a high diversity of biologically active secondary metabolites .Some of these compounds are synthesized and stored during normal growth and development (e.g. phytoanticipins) while others are absent in healthy plants, accumulating only in response to pathogen attack or stress conditions .Elicitors are defined as molecules that stimulate defense or stress-induced responses in plants (van Etten *et al*.,1994). Following the advent of modern medicine, herbal medicine suffered a setback, but during the last two or three decades the advances in phytochemistry and in the identification of the plant compounds, providing effective against certain chronic diseases and emergence of multidrug resistant bacteria . This awakening has led to a sudden demand for herbal medicine. Worldwide as well as in the developing countries, the most humans died due to infectious bacterial diseases (Nathan,2004). The bacterial organisms including both Gram positive and Gram negative ones are the main cause of severe infections in humans, because they have the ability to survive in harsh conditions due to their multiple environmental habitats (Saha *et al*.,2013) .. the anti-microbial activity of *Ocimum basilicum* essential oil against microorganisms has been investigated by some researchers (Verma *et al* ., 2011)

Method of Plant Extraction and Antimicrobial activity: Plant Extraction: The following step are conducted based on Harbome (1973) for detecting the phenolic compounds for the studied species:First The seeds of the plants have been taken from *Ocimum basilicum* and have been grinned by using electric grinned, 50 (gm) from the specimen are weight, and added 500 ml of ethyl alcohol (70%), left in the room

temperature for 24- 48 hour,later filtration has been done by using ederol filter paper (medium pores filtering).The extract has been concentrated to a suitable volume to discard the ethyl alcohol by using air dryer in a moderate temperature.As many as the extract volume, petroleum ether (with boiling point 40-60°C) has been added to the extract, the mixture was shaken well, then has been put in separating funnel, and has been left till separate in to two obvious layer, in this point discarding take place from a large part of the chlorophyll that dissolved in the petroleum ether and floated above because it has less density than the aqueous extract of the phenolic compound and then withdrawn from funnel bottom.The extract of the phenolic compounds were concentrated to half of its volume by leaving it in dry air current .

Test microorganisms:In vitro antimicrobial studies were carried out on 4 bacteria strains *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*,and the fungi *Candida albicans*

Antimicrobial activity Sample collection: The samples were collected from patients found in Baquba hospital during period time from 20/11/2016 to 15/12/2016 and the samples that collected include different swabs from burns and wounds and vaginal .Bacterial identification was carried out by conventional biochemical methods according to the standard microbiological techniques.

Culture media for isolated microorganism Mannitol Salt Agar: This medium is used for the isolation and cultivation of bacteria and suitable for this medium suitable for growth of *Staphylococcus.aureus* and. *Staphylococcus epidermidis* The colonies appeared round, smooth, raised, mucoid and glistening.This medium was prepared according to the instruction of manufacture company, sterilized by autoclaving at 121ºC for 15min. after cooled to 50ºC, mixing well then poured in a sterile Petri-dish.

Eosin methylene blue agar (EMB): This medium is used for the growth *E.coli* bacteria because (Selective and Differential media) EMB media assists in visual distinction *Escherichia coli*, other nonpathogenic lactose-fermenting enteric gram-negative rods, and the *Salmonella* and *Shigella* genera. *Escherichia coli* colonies in Eosin Methylene Blue (EMB) Agar appear Greenish Metallic Sheen.

MacConkey agar: MacConkey Agar is selective for Gram negative organisms, and helps to differentiate lactose fermenting gram negative rods from Non lactose fermenting gram negative rods. It is primarily used for detection and isolation of members of family *enterobacteriaceae* and *Pseudomonas spp*

Sabouraud's Dextrose agar: The Sabouraud's Dextrose agar consists of Sabouraud's dextrose broth (pH, 5.6) and 1.5 % agar. This medium was autoclaved and cooled to 450 C. It was dispensed into sterile petri plates. The plates were incubated at 370 C for 24 – 48 hr. The suspected isolates of *Candida albicans* were sub cultured onto Sabouraud's agar plate.

Preparation of McFarland standard:Standard was prepared by mixing 0.5 ml of 1.75% (w/v) BaCl2·2H2O with 99.5 ml of 1% H2SO4·BaSO4 (v/v). The standard was put into screw cap test tube to compare the turbidity. The bacterial cultures of the selected strains were grown overnight and were subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 BaSO4 turbidity standard 108

Colony Forming Unit (CFU) per ml was achieved. These inoculum were used for seeding of the nutrient agar.

Maintenance of the Bacterial Culture

The isolates were maintained by sub-culturing them into a new prepared nutrient agar slant and stored in an incubator at 37oC.

Preparation of Inoculums

The Bacterial inoculums were prepared by sub-culturing of the test organisms from nutrient agar slants on another prepared nutrient agar plates and incubated at 37oC for 24 hours. The pure cultures on the nutrient agar plates were used as the inoculums.

Determination of antimicrobial activity by disc diffusion method

The antimicrobial sensitivity testing was conducted by the agar disc diffusion method. The sensitivity medium (Muller-Hinton agar) was prepared according to the manufacture company autoclaved at 121°C for 15 minutes at 15 lbs., and poured in sterile Petri plates .The bacterial isolates were suspended in peptone broth and incubated at 37° C for 3-4 hours . The turbidity of the broth culture must be adjusted to 0.5 McFarland tube. This gives a suspension containing 1-2 x 106 colony forming units (CFU)/ml. A sterile cotton swab was inserted into the bacterial suspension and rotated. The swab streaked on the surface of the sterile Muller-Hinton agar plate. the swab was streaked on the entire plate surface to ensure confluent growth . The antibacterial activity of *Ocimum basilicum* leaf extract the first dissolved in a ethanol solvent, and then varying concentrations of the extracts (100µg, 50µg, 25µg) were soaked on autoclaved discs of Whatmann filter paper. These filter paper discs were placed on a streaked Muller-Hinton agar plate surface. The plates incubated overnight at 37° C for 18-24 hours. The antimicrobial activity was detected by measuring of inhibition zones.

Antibacterial activity

To test antibacterial activity of the synthetic antibiotics, standardized discs of fusdic acid(15µg), erythromycin (15µg), ampicillin (10µg), , ceftriaxone (30µg) were tested by the agar disc diffusion method by placing on a streaked Muller-Hinton agar plate surface. The antimicrobial activity was also detected by measuring zones of inhibition according to NCCLS(2000).

Results: In our study the resutt showed the antimicrobial activities of *O. basilicum* (alcohol) extracts against the microorganisms, and their potency, were assessed by the presence orabsence of inhibition zones and zone diameter. The results are given in Table 1.

(Table-1) The antimicrobial activity of *O. basilicum* extracts against 5 microorganisms

Microorganisms	Concentrations of seed extract (in µg/disc)		
	100	50	25
Escherichia coli	2	1.5	1
Staphylococcus aureus	5	2	2
Staphylococcus epidermidis	2.5	1.6	1.5
Pseudomonas aeruginosa	5	2	2
Candida albicans	3	2	2

This study showed that the inhibition zones formed by positive control of antibiotic discs are presented in Table 2

Table 2. The inhibition zone formed by standard antibiotic disks at same conditions.

Micoorganisim	Antibiotics concentration in (µg/disc)			
	Fusdic acid (15 µg)	Erthromycin (15 µg)	Ampicillin (10 µg)	Ceftriaxone (30 µg)
Escherichia coli	2	1.5	2	3
Staphylococcus aureus	1.5	0.5	1	1
Staphylococcus epidermidis	2	2	1	2
Pseudomonas aeruginosa	-	-	-	-
Candida albicans	2	1	1	2

Discussion

The bacterial resistance to several different antibacterial agents constitute significant problem. Bacteria showing reduced susceptibility to an antibiotic imply that it should not be used on the patient Richard Wise .(1998) The antimicrobial activities of *Ocimum basilicum* ethanol extracts against the microorganisms examined in the present study .the presence or absence of inhibition zones and zone diameter. this study, as opposed to information in literature (Nakamura *et al*.,2004) This may be due to the absence of some secondary metabolites or the presence of some in low concentration; or it may be due to the type of strains used or a slight change in any of the factors mentioned earlier that are likely to affect rate of microbial growth or rate of diffusion of the test agent. Secondary metabolites of plants such as saponins, flavonoids, tannins, carbohydrates, cyanogenic glycosides, reducing sugar and all other active principles of plants have been shown to be responsible for the antimicrobial activities shown by these extracts (Nweze *et al*.,2004) .

Different concentrations of ethanolic extract of *Ocimum basilicum* were used in agar well diffusion assay, caused different degrees of zones of inhibition against different bacterial strains and the fungi *candida albicans.*The results of our study showed that the activity of *Ocimum basilicum* extract have effect for both Gram-negative and Gram-positive pathogens. This result agrees with the study of (Zahra *et al*., 2015). Different concentrations of ethanolic extract of *Ocimum* were used in agar well diffusion assay, caused different degrees of zones of inhibition against .The results showed that concentrations of ethanol extracts have different antimicrobial effects. Three different concentrations of ethanol extracts were used in our study. It was not surprising that more condensation of ethanol extracts resulted in more effective antimicrobial activity. If more concentrations of extracts are used, antimicrobial effect will increase. On the other hand .The sizes of inhibition zones were different according to concentration of extract table (1) . The inhibition zones were always different and had substantially increased with the concentration of *Ocimum basilicum* extract; and the growth was completely inhibited at the highest concentration. All the kinds of bacterial isolates and *candida albicans*. were highest inhibition at the concentration 100. *O. basilicum* has an inhibitory activity on these bacteria that is as effective as antibiotics. It would be possible to produce new effective antibiotics using the effective material to be refined from this plant. The results are given in table2.the highest activity was due to the action of Ceftriaxone against *E.coli* ; and the lowest activity was due to the action of erythromycin against *S.aureus* There was no activity against *Pseudomonas aeruginosa*.The results are given in table2.the highest activity was due to the action of Ceftriaxone against *E.coli* ; and the lowest activity was due to the action of erythromycin against *S.aureus* ,There was no activity against *Pseudomonas* .

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