

**Antimicrobial activity of ethanolic Leaf Extracts of
Ocimum basilicum some bacteria**

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Abstract

This study was designed to determine the in-vitro antimicrobial effects of ethanolic leaf extract of *Ocimum basilicum*, by Agar well diffusion antimicrobial bioassay. Results showed a broad spectrum of activity since the extracts inhibit the growth of both gram positive (*Staphylococcus aureus*, *Streptococcus pneumonia* and *Enterococcus faecalis*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) . Antimicrobial assay of 400%, 200% and 100% mg/ml ethanolic extracts shows very potent antimicrobial agent on both gram positive *S.aureus* (highest inhibition zone 13 mm) and gram negative bacterial isolates.

Key words: *Ocimum basilicum* Antimicrobial activity, Inhibition zone,

Microbiology Classification QR- (75-99.5)

Introduction

In the recent years, the antioxidant and antimicrobial potential of plants have attracted the attention of scientific community. The antioxidants may be useful in retarding oxidative deterioration of food materials especially those with high lipid contents (1) and also protect the living cells from oxidative damage that occur due to formation of free radicals and reactive oxygen species during metabolic activity. This oxidative damage of cellular constituents lead to cell injury leading to cell death which is associated with pathogenesis of various chronic diseases like carcinomas, coronary heart disease and many other health problems related to advance age. (2) There is a growing interest in natural substances exhibiting antimicrobial and antioxidant properties that are supplied to human and animal organisms as food components or as specific pharmaceuticals. (3) Plants are the primary sources of naturally occurring antioxidants for humans

The use of traditional medicines holds a great promise as an easily available source as effective medicinal agents to cure a wide range of ailments among the people particularly in tropical developing countries like India. Medicinal plants and herbs are of great importance to the health of individual and communities. Despite the existence of herbal medicines over many centuries, only relatively small number of plant species has been studied for their application. However, in the recent past, increasing research evidence is getting accumulated, which clearly indicate the positive role of traditional medicinal plants in the prevention or control of some metabolic disorders like diabetes, heart diseases and certain types of cancers(1).The steadily

increasing bacterial resistance to existing drugs is a serious problem in Antimicrobial therapy. One way to prevent antibiotic resistance of pathogenic species is to use new compounds that are not based on existing synthetic antimicrobial agents(4). It is anticipated that phytochemicals with adequate antimicrobial efficacy could be used for the treatment of bacterial infections(5). One of the great advantages of these medicinal plants is that they are easily available and have moderate side effects(6) There are several reports of antibiotic resistance of human pathogens to Biomolecules of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human and plant pathogens and hence in the present investigation, leaves of *Ocimum basilicum*, *Cymbopogon citrates*, *Catharanthus roseus* and *Andrographis paniculata* was tested for its efficacy to inhibit against human pathogens. These plants are well known for its medicinal value as antibiotic antidiabetic, antioxidants and antimicrobial properties. In order to elucidate such a phenomenon, as well as seek highly effective plants, a number of plant extracts and isolated compounds have been tested of with crude plant extracts against different pathogens bacterial infections has led to the emergence and spread including *S. aureus*, *P. aeruginosa*, *E. coli*, Extended of resistant strains. Methicillin-resistant *Staphylococcus* Spectrum β -lactamases-producing multidrug-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections. *E. coli* and vancomycin-resistant enterococci infections are very difficult to cure (*Enterococcus faecalis*) (6). Some Palestinian plant strains are resistance against almost all clinically available exhibit significant potency against

human bacterial antibiotics. For most MRSA strains, glycopeptide-type (9). But plant extracts as antimicrobials are rarely used drugs such as vancomycin are the only effective as systemic antibiotics at present, this may be due to their antimicrobial agents (14).

Many plant-derivate antimicrobial compounds have a wide spectrum of activity against food borne pathogens and this has led to suggestions that they could be used as natural preservatives in foods (7). The safest way to look for natural food preservative is to search for activity against classes of standard microorganisms. These include *Escherichia coli* and *Pseudomonas aeruginosa* (Gram positive), *Staphylococcus aureus* and *Bacillus cereus* (Gram negative), *Candida albicans* (yeast), *Aspergillus flavus* and *A. niger* (moulds) (11).

Since the use of medicinal plants have seconded a wide range in treating diseases, the present study concentrates on commonly available medicinal plants like *Ocimum basilicum* and their effects on human pathogens like, *Escherichia coli*, *S pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* the family. The leaf can be used for the treatment of throat infections and wound healing, and also used as anti-venom and migraine pain relief. *O. basilicum* belongs to Lamiaceae, distributed throughout the Southern part of Tamil subjected to hydro-distillation using reverse. The steam distillate was removed, dried over anhydrous sodium sulphate and stored at 4 °C.

2- Plant ethanolic Extracts:

Plant, leaves of *Ocimum basilicum* which identified by Dr. Abdul Kareem Al bermany philosophy of botany in Babylobn university were washed separately under running tap water and

Nadu. It is used for various applications as poultice or salve for insect bites, acne and ringworms, as a gargle for mouth or thrush, as a bath herb for increased energy and eyewash for tired eyes. The essential oils of the basil are added to massage for sore muscles. The dried herb used as antiseptic incense and the juice can be applied to fungal infections (10).

O. basilicum commonly grows semi wild and is cultivated in vegetable gardens. Current domestic uses are only for food and folk medicine 7. Nevertheless, a large scale production of the basil essential for well-known value-added applications 8, 9 is quite viable under local circumstances. The chemical composition of *O. basilicum* essential oils has been intensively investigated throughout the world At the same time, very little work has been done on the chemical composition and the antimicrobial activities of *O. basilicum* In addition, there are very few data available either for practical use or for basic research needs about antimicrobial properties of the essential materials of *O. basilicum* growing in Iraq .

The aim of the present work was to investigate the activity of ethanolic leaves extract of *O. basilicum*.. Specifically, extract percentage composition was determined along with their bacteriostatic and activity using bacterial strains known.

Material and methods:

1-Collecting the Plant samples :

Plants material were collected in March and June, 2013, *Ocimum basilicum* leaves were purchased from the local market, , The aerial part of *Ocimum basilicum* was harvested and

and 400 μ l prepared with Whatmann No.1 paper and used for the test(8).

RESULTS:

The results of the conducted experiments using *O.basicum* ethanol extract with disc -diffusion method demonstrate that 3 strains of gram positive bacteria and 2 strains of gram negative bacteria were tested. This change was noticed in contain bioactive compounds of this plant extract.

The antibacterial activity of the *O. basilicum*, are assessed using the disc diffusion method at different concentration showed the maximum activity against three pathogens. The effect of different concentrations of the ethanolic extracts are presented in Table 1 and Fig. 1,2 reveals that ethanol extract of leaves were successful in inhibiting the bacterial growth.

Table 1 summarizes the results of Anti-bacterial activity and inhibition zone of three concentrates of ethanolic extracts of *O. basilicum* against human pathogens and results obtained were: the maximum zone of inhibition zone was (13mm ,9 and 6)for *S.aureus* in concentrations 400,200 and 100 respectively also there was effected

shade-dried at room temperature, powdered, and individually extracted sequentially, the powder material was soaked in 50 ml of ethanol ethanol for 48hr at room temperature,. After that the extract was filtrated and collected, the extracts were concentrated under low pressure at 40°C. The resulting crude extracts were stored at – 20 °C until they were tested(15).

3- Bacterial Isolation and Identification:

The samples were inoculated on nutrient agar and MacConkey agar media incubated at 37°C for 24 hr. on nutrient agar plate, Mannitol Salt agar, Blood and and Chocolate agar plated and further identified by biochemical tests for confirmation of bacteria by specific biochemical tests such as IMViC test, catalase test, coagulase test, urease test, oxidase test, bile solubility test, and carbohydrate fermentation test were performed. Nitrate reduction. (11, 12)).

4-Antibacterial Assay (Disc Diffusion Assay):

Antimicrobial activity of leaf extracts was tested against bacterial strains using Disc Diffusion method. of. 6mm diameter discs were located in 100,200

Table 1: Minimum inhibitory concentration of ethanolic extract of *Ocimum basilicum*

Nam of plant	Ethanolic exteact/ μ l	Inhibition zone/ mm				
		<i>S.aureus</i>	<i>S.feacalis</i>	<i>E.coli</i>	<i>P.aeruginosa</i>	<i>S.pnumoniae</i>
<i>Ocimum basilicum</i>	100	6	4	0	0	4
	200	9	8	0	0	7
	400	13	12	1	0	12

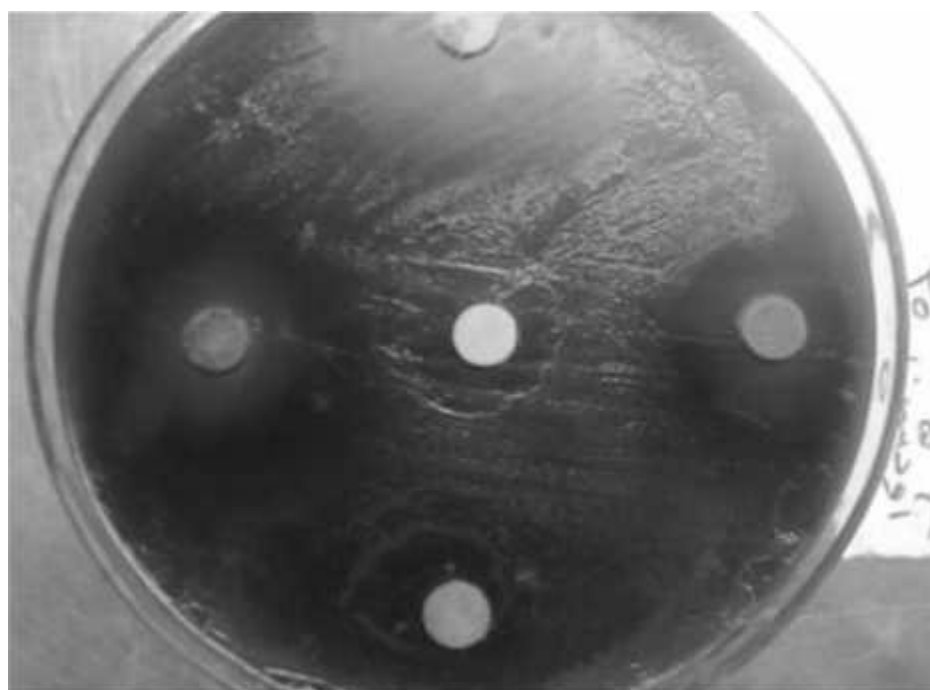


Figure 1. Anti-microbial activity of selected ethanol plant extracts against *S. pneumoniae* by the disc diffusion method.

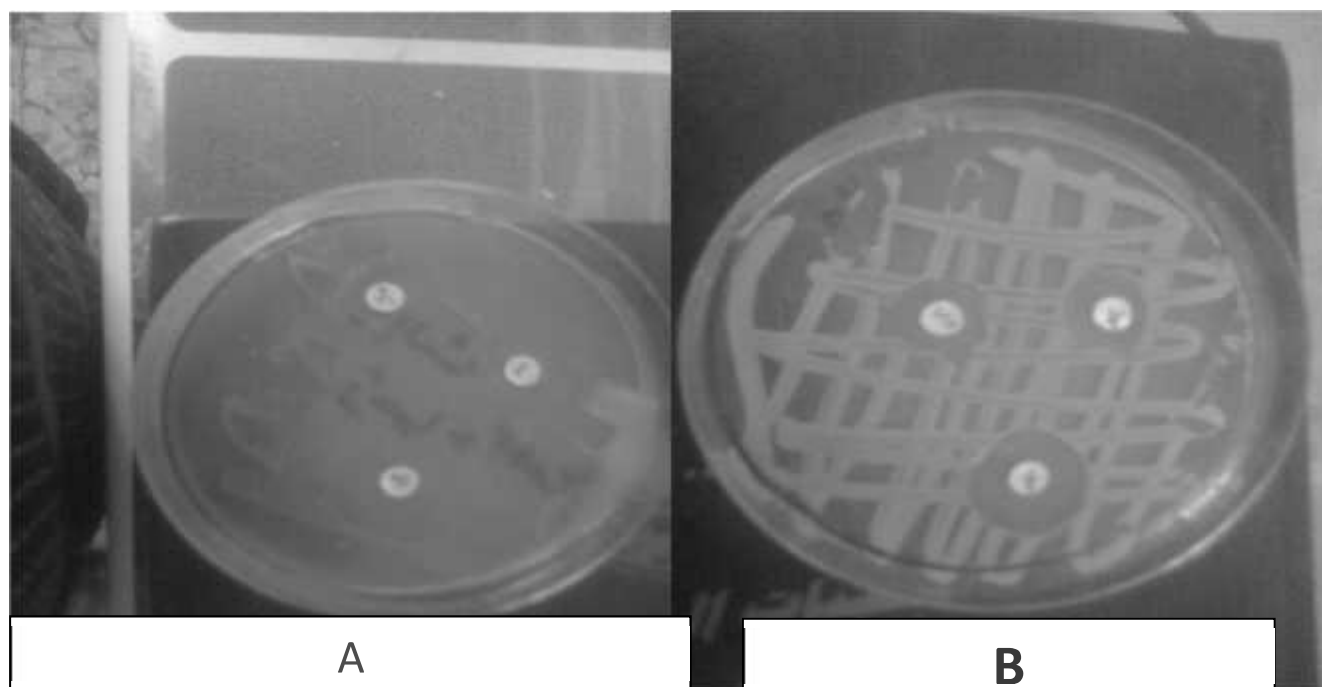


Figure 2. Anti-microbial activity of selected ethanol plant extracts against (A) *S.aureus*,(B) *E.faecalis* : by the disc diffusion method

Discussion

The in vitro antimicrobial activity of *O. basilicum*, against Gram positive *S. aureus* and *E. faecalis* and efficient results were observed against *S. aureus*. Very little or no activity was observed against Gram-negative bacteria (*E. coli* and *P. aeruginosa*). This can be explained because the outer membrane of Gram-negative bacteria is known to present a barrier to the penetration of numerous antibiotic molecules and the periplasmic space contains enzymes which are able of breaking down foreign molecules introduced from outside. There were no literature reports of antimicrobial activity for most species studied(11).

The extracts showed activity on bacterial strains. Further more, the bacteria studied were *S.aureus*, *S.peumoniae* and *E.feacalis* was susceptible to all extracts of zone inhibition, ranging between 12 and

13mm. This may indicate that the *O. basilicum* leaves extracts have broad inhibitory activity to pathogenic microorganisms and are promising to act as potential antibacterial and agents from natural plant sources. It is not surprising that these are differences in the antimicrobial activities of plant groups, due to the phytochemical differences. Negative results do not indicate the absence bioactive constituents, nor is that the plant inactive(6).

In general agreement with previously published qualitative analysis of Lamiaceae species(, the phytochemical screening of the phytoconstituents of the plant studied showed that leaves were found to have flavonoids, saponins, cardiac glycosides, alkaloids, phenols and tannins. They were known to show medicinal activities well as exhibiting physiological activity (3). This is also in reement with Javanmardi and his co others, who reported that rosmarinic acid is the most abundant component in *O.*

basilicum (5). *Ocimum* species has been extensively reported for its essential oil content (1). Phenolic compounds are an important group of secondary metabolites, which are synthesized by plants due to plant adaptation in response to biotic and abiotic stresses (Infection, water stress, cold stress, high visible light) (2). So far, in the *Ocimum* species the *O. basilicum* and *O. sanctum* leaves have been reported for their secondary metabolite content (8).

this study shows the antibacterial activities ethanol extracts of *O. basilicum* against tested organisms. In this present investigation, when the ethanol extract of the plants, it recorded significant zone of inhibition

activities(exhibited significant result) against *S. aureus* and ranging from 4 to 13 mm, respectively. The plants are the vital source of innumerable number of antimicrobial compounds. Several phytoconstituents like flavonoids, phenolics and polyphenols, tannins, terpenoids, sesquiterpenes, etc., are effective antimicrobial substances against a wide range of microorganisms(9). It can be seen from the above results that the leaf extract contains alkaloids and other phytochemicals. These compounds could be used as substitutes for synthetic antibiotics for the treatment of chronic kidney infection, bacterial endocarditis and carrier conditions of typhoid(13).

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الفعالية المضادة للمكروبات للمستخلص الكحولي لنبات الريحان ضد بعض البكتريا

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الخلاصة:

صممت هذه الدراسة من اجل تحديد الأهمية الطبية لخلاصة الكحول لنبات الريحان في الزجاج وخصائصه المضادة للمكروبات والتي درست بواسطة طريقة الانتشار بالأقراص . اظهرت النتائج طيف واسع من الفعالية لخلاصة الريحان لبعض الانواع البكتيرية الموجبة والسالبة لصبغة غرام والتي حضرت بتراكيز 200,100 و400 ملغم لكل مل لمعرفة مدى تأثيرها على الأجناس المعزولة من خلال استخدام طريقة الانتشار بالأقراص من خلال قياس قطر منطقة التثبيط حول القرص المشبع بخلاصة الريحان الكحولية حيث أظهرت النتائج ان المستخلص ذو تأثير واضح على البكتريا الموجبة لصبغة غرام وصل الى 13 ملميترا .

كلمات مفتاحية: نبات الريحان, الفعالية المضادة للمكروبات, قطر منطقة التثبيط