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Antibacterial Activity of Ocimum basilicum Leaves Extracts Against Staphylococcus aureus ATCC 25923

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In memory of the late Huda Mohammed Albasheer.

Abstract: *Medicinal plants have been used in a wide variety of purposes such as pharmaceutical purposes, in alternative medicine, and as natural therapies for many thousands of years. They are generally considered as natural products, few are produced synthetically, and they have antioxidant, antibacterial, cytotoxic, antiviral and fungicidal activity. Also, they can be biodegraded more easily. Ocimum basilicum (OB) contains many active ingredients that can be used as antibiotics. In addition, it is inexpensive. This experimental study was conducted at University of Gezira in the period from January 2023 to March 2023. The study aimed to detect the antimicrobial effect of several OB leaves extracts against Staphylococcus aureus ATCC 25923. Extraction of OB leaves was prepared by maceration method, with different concentrations and then were tested using agar well diffusion technique. The ethanolic extract of Ocimum basilicum showed the highest inhibitory activity against examined bacteria with inhibition zones mean ranged from 22–28 mm, followed by the hexane extract of Ocimum basilicum with inhibition zone mean ranged from 19–25 mm and methanolic extract of Ocimum basilicum with the lowest inhibition zone mean ranged from 18–24 mm. This study verified the use of ethanolic extract of Ocimum basilicum, hexane extract of Ocimum basilicum and methanolic extract of Ocimum basilicum as antimicrobial agents against Staphylococcus aureus infections. Further studies are recommended to detect the antimicrobial activity against other microorganisms.*

Keywords: Ocimum basilicum; Staphylococcus aureus; antibacterial activity; ethanolic extract; methanolic extract; hexane extract; agar well diffusion

Introduction

Traditional medicine is defined as the sum total of the knowledge, skills and practices based on the theories and experiences used in the maintenance of health, as well as in the prevention, diagnosis, improvement and treatment of physical and mental illness [1]. Half a million species of plants have been used in medicine; the trial and error method was used to treat illnesses or even simply to feel better, and in this way, to distinguish useful plants with beneficial effects [1].

Medicinal plants have been used for a wide variety of purposes such as food preservation, pharmacy, alternative medicine, and as natural therapies for many thousands of years [2]. It is generally considered that products produced naturally, rather than synthetically, have antioxidants, antibacterial, cytotoxic, antiviral and fungicidal activity, biodegraded more easily, environmentally acceptable and have gained popularity with their positive image among consumers [2].

The therapeutic properties of plants gave rise to medicinal drugs made from certain plants with these benefits. Until the 18th century, the therapeutic properties of many plants, their effects on the human and their method of treatment were known, but the active compound was unknown [1].

Ocimum basilicum "Rehan" commonly known as basil, is widely distributed in tropical and warm temperate regions of the world [3]. Basil is naturally distributed in the East Anatolia region in Turkey and the leaves of these plants are used in traditional cuisine as spices and it is a popular culinary herb. Its essential oils have been used extensively for many years in flavoring of meats and sausages, perfumery, as well as in dental and oral products [4].

Ocimum basilicum has been used as an antiseptic, preservative, sedative, digestive regulator and diuretic; it also has been recommended for the treatment of headache, cough, infections of upper respiratory tract, and kidney dysfunction [3]. Ocimum extracts were shown to exhibit antibacterial activities against gram positive and gram negative bacteria [3]. Leaves and flowering parts of basil are traditionally used externally, when applied for the treatment of acne, disguising of smell, insect stings, snake bites, and skin infections [3].

The Ocimum basilicum has been described to be active against several species of bacteria and fungi; they have been also used as a folk remedy to treat various ailments such as feverish illnesses, poor digestion, nausea, abdominal cramps, gastro-enteritis, migraine, insomnia, depression, gonorrhea, dysentery, and chronic diarrhea exhaustion [3].

In recent years, antimicrobial agents have several problems and side effects such as nausea, indigestion, loss of appetite, feeling full and stomach pain [5]. Excessive use of antibiotics may cause natural bacterial flora imbalance which can lead to opportunistic infections [5]. Some antibiotics may interact with other medicines and supplements which may enhance antibiotic side effects and drug interaction consequences [5].

The development of new antibiotics has become useless; due to their higher cost and time consuming process, these lead to an increasing awareness in searching for valuable alternative antibiotics with different mode of action on pathogens. Hence, medicinal plants appeared to be the best alternative source for new antimicrobial drugs [6].

Diseases caused by Staphylococcus aureus may be largely or wholly the result of actual invasive infection, overcoming host defense mechanisms, and the production of extracellular substances which facilitate invasion; a result of toxins in the absence of invasive infection ("pure" toxinoses); or a combination of invasive infection and intoxication [7]. Localized skin infections, subcutaneous abscesses called furuncles (boils) often form around foreign bodies such as splinters. Carbuncles are larger, deeper, multiloculated skin infections. Impetigo is usually seen in children and deep infections are metastatic from superficial infections or skin carriage or may result from trauma [7].

Methicillin resistant Staphylococcus aureus (MRSA), classified as one of the most nosocomial pathogens of major worldwide importance, causes infections due to its ability to spread from patient to patient; also it is an increasingly frequent cause of community acquired infections that cause considerable morbidity and mortality [8].

In recent years, multiple drug resistance in bacteria has developed due to the suboptimum use of antimicrobial drugs, and the subsequent spread of resistant bacteria have made many currently available antibiotics ineffective, and bacteria from different parts of the world produce drug resistance [4]. Excessive use of antibiotics is the commonest cause of life threatening immune mediated drug reactions that are including anaphylaxis, organ specific and severe cutaneous adverse reactions [9]. In Sudan, antibiotics are under threat of self-medication which led to emergence of antibiotic resistant strains of bacteria [10]. On the other hand, basil extracts have no toxicity, with no side effects, and are available [2]. For these above mentioned reasons, the study evaluated the antimicrobial activity of the basil extracts.

Materials and Methods

Study Design

This study is an experimental study conducted at University of Gezira, Faculty of Medical Laboratory Science, from January 2023 to March 2023.

Study Variables

Dependent Variables

Standard bacterial strain Staphylococcus aureus ATCC 25923.

Independent Variables

Different concentrations of ethanolic leaves extract of Ocimum basilicum.
Different concentrations of methanolic leaves extract of Ocimum basilicum.
Different concentrations of hexane leaves extract of Ocimum basilicum.

Hypothesis

Alternative Hypothesis

Ocimum basilicum leaves extracts will inhibit the growth of Staphylococcus aureus ATCC 25923.

Null Hypothesis

Staphylococcus aureus ATCC 25923 colonies grow on the plate and resist the activity of Ocimum basilicum leaves extracts.

Study Criteria

Inclusion Criteria

Basil leaves with age not more than 65 to 75 days [11].
Basil leaves with height not more than 15 to 20 cm [11].

Exclusion Criteria

Basil age more than 75 days.

Ethical Considerations

The ethical approval for this study was obtained from the Council of Faculty of Medical Laboratory Sciences, Department of Microbiology, University of Gezira.

Data Collection and Analysis

Data was collected by observation of the inhibition zones; the diameters of inhibition zones of each concentration of different extracts were measured and registered on research sheets. Data was analyzed using means of the measured diameters of inhibition zones for each extract. Also, data was analyzed by comparing with control antibiotics (Amikacin and Vancomycin).

Materials

Methanol, ethanol, hexane, dimethylsulphoxide (DMSO)
Rotary evaporator
Crystal violet, iodine, safranin, carbol fuchsin
Petri dishes, conical flasks (1000 ml), glass funnel, sterile test tubes
0.5 McFarland standard, sterile normal saline, distilled water
Automatic pipettes (1000 µl, 100 µl), yellow tips, blue tips
Filter papers, sterile cork borer (10 mm), forceps, sensitive balance
Amikacin, vancomycin
Staphylococcus aureus ATCC 25923 (Gram positive bacteria)
Mannitol salt agar (MSA), Mueller Hinton agar

Methods

Cultivation of Ocimum basilicum

The seeds were collected from Gasim educational plantation, Khartoum state. The seeds were mixed with sand and were sown to a depth of 2 cm because they were very small. Soil was spread in a thin layer over the seeds and was irrigated. The seeds germinated in 15 days and were transplanted at 6 weeks old at 3–5 leaf stage. The soil was irrigated immediately after transplantation.

Collection of Ocimum basilicum Leaves

Leaves of Ocimum basilicum were collected using scissors, then exposed to air for drying away from dust and sunlight.

Preparation of Ocimum basilicum Extracts

Extraction was carried out according to the method described by Handa et al. [12].

Preparation of Methanol Extract

100 g of plant sample was coarsely powdered using mortar and pestle; the powder was soaked in 1000 ml absolute methanol for 24 hours. On the second day, the mixture was filtered by Buchner filtration. On the third day, the solvent was allowed to evaporate under reduced pressure using rotary evaporator. The sample extract was allowed to air dry in an evaporating dish till complete dryness. The yield percentage was calculated as follows: Weight of extract obtained / Weight of plant sample × 100. The yield of methanol extract was 7%.

Preparation of Ethanol Extract

100 g of plant sample was coarsely powdered using mortar and pestle; the powder was soaked in 1000 ml absolute ethanol for 24 hours. On the second day, the mixture was filtered by Buchner filtration. On the third day, the solvent was allowed to evaporate under reduced pressure using rotary evaporator. The sample extract was allowed to air dry in an evaporating dish till complete dryness. The yield of ethanol extract was 9%.

Preparation of Hexane Extract

150 g of plant sample was coarsely powdered using mortar and pestle; the powder was soaked in 1000 ml dimethyl sulfoxide (DMSO) solution for 24 hours. On the second day, the mixture was filtered by Buchner filtration. On the third day, the solvent was allowed to evaporate under reduced pressure using rotary evaporator. The sample extract was allowed to air dry in an evaporating dish till complete dryness. The yield of hexane extract was 1.3%.

Cultivation of Bacteria on Mannitol Salt Agar

The tested organism was streaked on mannitol salt agar media; after overnight incubation at 37 °C, the colour change of media to yellow was observed (mannitol fermenter colonies) [13].

Preparation of Mueller Hinton Agar

38 g of Mueller Hinton agar powder was suspended in one liter of distilled water, then dissolved completely. The mixture was sterilized by autoclaving at 121 °C for 15 minutes. The medium was cooled to 40–45 °C and poured into sterile Petri dishes and allowed to solidify.

Preparation of Bacterial Suspension

Using a sterile wire loop, 3–5 well-isolated colonies of similar appearance of the tested organism were emulsified in 3–4 ml of sterile physiological saline. In a good light, the turbidity of the suspension was matched to the turbidity standard (0.5 McFarland).

Preparation of Different Concentrations of Each Basil Extract

Solution of known concentration was prepared by dissolving a known quantity of a solute in a known quantity of a solvent and then diluted to desired concentrations.

Ethanolic Extract Concentrations

Stock solution was prepared by mixing 2 g of ethanolic extract with 10 ml of diluted methanol to a final concentration of 200 mg/ml. First concentration of 100 mg/ml was prepared by adding 2 ml of ethanol extract to 2 ml of diluted methanol. Second concentration of 50 mg/ml was prepared by adding 1 ml of ethanol extract to 3 ml of diluted methanol. Third concentration of 25 mg/ml was prepared by adding 0.5 ml of ethanol extract to 3.5 ml of diluted methanol.

Methanolic Extract Concentrations

Stock solution was prepared by mixing 2 g of methanolic extract with 10 ml of diluted methanol to a final concentration of 200 mg/ml. First concentration of 100 mg/ml was prepared by adding 2 ml of methanol extract to 2 ml of diluted methanol. Second concentration of 50 mg/ml was prepared by adding 1 ml of methanol extract to 3 ml of diluted methanol. Third concentration of 25 mg/ml was prepared by adding 0.5 ml of methanol extract to 3.5 ml of diluted methanol.

Hexane Extract Concentrations

Stock solution was prepared by mixing 2 g of hexane extract with 10 ml of DMSO solution to a final concentration of 200 mg/ml. First concentration of 100 mg/ml was prepared by adding 2 ml of hexane extract to 2 ml of DMSO solution. Second concentration of 50 mg/ml was prepared by adding 1 ml of hexane extract to 3 ml of DMSO solution. Third concentration of 25 mg/ml was prepared by adding 0.5 ml of hexane extract to 3.5 ml of DMSO solution.

Antibacterial Activity Testing

By using a sterile swab, a plate of Mueller Hinton agar was inoculated; the swab was streaked evenly over the surface of the medium in three directions, and the plate was rotated approximately to ensure even distribution. The agar was allowed to set, then one well of 7 mm in diameter was cut under aseptic conditions using a sterile cork borer and the agar disc was removed. The wells were filled

with 100 µl of each extract by using automatic micropipettes and were allowed to diffuse at room temperature for two hours [14]. The plates were then incubated in the upright position at 37 °C for 18 hours. After incubation, the growth was detected and the presence of zone of inhibition was tested and measured [14]. Each extract concentration was tested three times.

Results

The zones of inhibition were 19–25 mm using hexane extract, 22–28 mm using ethanol extract and 18–24 mm using methanol extract.

Table 1.

Inhibition zones diameter and meaninmm of different concentrations of HEOB against *Staphylococcus aureus* ATCC 25923

		HEOB **concentration (mg/ml)**			**Positive control**		**Negative control**
		100	**50**	**25**	**V**	**AMK**	**D.W**
Zone Of Inhibition Diameter (mm)	**First test**	26	22	21	18	23	0
	Second test	25	21	18	18	23	0
	Third test	25	21	20	17	23	0
	Mean	25	21	19	18	23	0

HEOB:hexan extract of *Ocimum basilicum*, **mm:** millimeter,**Van:**Vancomycin, **AMK:**

Amikacin,**D.W:** Distilled (Negative Control), **IZ:** Inhibition Zone.

Table.2

Inhibition zones diameter and meaninmm of different concentrations of EEOB against *Staphylococcus aureus* ATCC 25923

		EEOB **concentration (mg/ml)**			**Positive control**		**Negative control**
		100	**50**	**25**	**V**	**AMK**	**D.W**
Zone Of	**First test**	30	26	22	18	23	0

Inhibition Diameter (mm)	**Second test**	28	26	22	18	23	0
	Third test	27	25	21	17	23	0
	Mean	28	25	22	18	23	0

EEOB:ethanol extract of *Ocimum basilicum*, **mm:** millimeter, **Van:**Vancomycin, **AMK:**

Amikacin, **D.W:** Distilled (NegativeControl), **IZ:** Inhibition Zone.

Table 3

Inhibition zones diameter and meaninmm of different concentrations of MEOB against *Staphylococcus aureus* ATCC 25923

		MEOB **concentration (mg/ml)**			**Positive control**		**Negative control**
		100	**50**	**25**	**V**	**AMK**	**D.W**
Zone Of Inhibition Diameter (mm)	**First test**	25	23	19	18	23	0
	Second test	24	22	18	18	23	0
	Third test	23	21	17	17	23	0
	Mean	24	22	18	18	23	0

MEOB:methanol extract of *Ocimum basilicum*, **mm:** millimeter, **Van:** Vancomycin, **AMK:**

Amikacin,**D.W:**Distilled (NegativeControl), **IZ:** InhibitionZone.

Table 4.

Identification of Chemical composition of ethanolic extract of *Ocimum basilicum*by chromatography-mass spectroscopy

	Chemicalcompound	**Molecular formula**	**Commonname**

2	1H-thieno(3,4)imidazole-2-(3H)one,tetrahydro-,5,5-dioxide.	C5H8N2O3S	n-Nitrosoproline
3	2,2-Dihydroxy-3,5-dihydroxy-6-methyl-4H-Pyran-4-one	C6H8O4	3-Hexenedioic acid
5	**n-Hexadecanoicacid**	**C16H32O2**	**Palmiticacid**
6	3Methyl-1-phenylthio-2-prop-2-enyl-2,5-dihydrothiophene1,1dioxide	C14H16O2S2	Naphthalene
7	4-Methylphenylisothiocyanato-4-methyl pentane	C7H13NS	Hexyl isothiocyanate
8	**1-Butaneboronic acid,butylboronicacid,n boronic acid**	**C4H11BO2**	**Butylboronic acid**
9	Clycooctane,1-(diethylboryl)-cyclooctyl(diethyl)borane.	C12H25B	Clycooctane,1-(diethylboryl)
10	2H-Tetrazole-2-ethanole,5-phenyl-ethanol,2-(4-phenyltetrazole)2-9pheny-2H-tetrazol.	C9H10N4O	Benzamide
11	2-Methoxy-4-vinylphenol	C9H10O2	Ethylbenzoate
12	**1-H-Tetrazol-5-amine,N-(phenylmethyl)-Benzylaminotetrazol,NBenzyl-1H-tetrazol-5amine**	**C8H9N5**	**Guanidinobenzimidazole**
13	**4(Methoxy-2,6-dimethylphenyl)boric acid.**	**C9H13BO3**	**Boricacid**
14	2,2-Dihydro-benzofuran	C6H8O	Cyclohexan
15	**4-Allyl-2-methoxyphenol**	**C10H12O2**	**Eugenol**
16	**Tetrazolo(1,5)pyridazin-6-amine,N-tetrahydrofurfuryl-(tetrahydro-2-furanylmethyl)-1,5pyridazin-6-amine.**	**C9H12N6O**	**Phenolmelamine**
18	Cyclohexane,isocianato-isocyanic,acid,cyclohexyl ester , cyclohexylisocyanate	C7H11NO	Cyclohexyl isothiocyanate
19	Decanoic acid,silver(1+) salt,silver decanoate	C10H19AgO2	Silver neodecanoate
21	4-Methyl-1,2,3-dioxathiane2-oxide	C4H8O3S	Methytrimethylen esulfite

Discussion

Recently, drug resistance to human pathogenic bacteria has been commonly reported from all over the world; however, the situation is alarming in developing as well as developed countries. Due to indiscriminate use of antibiotics, alternative antimicrobial strategies are urgently needed such as medicinal plants which could be a good source for a variety of drugs [15].

In this study, crude hexane, ethanol and methanol leaves extracts of Ocimum basilicum were screened for antibacterial activity against standard isolate of Staphylococcus aureus ATCC 25923, using agar well diffusion technique. The most important finding is that the Ocimum basilicum extracts possessed broad-spectrum antimicrobial properties (as containing different components with antibacterial activity) against Staphylococcus aureus ATCC 25923, and this was reported also by Adiguzel et al. in Turkey, 2005 [16]. Considering these results, Ocimum basilicum leaves could be tested for the management of bacterial infections such as diarrhea, wound and skin infections, and respiratory infections which are caused by Staphylococcus aureus.

S. aureus was inhibited by the Ocimum basilicum extracts at different concentrations of 25 mg/ml, 50 mg/ml and 100 mg/ml. The maximum diameter of inhibition zones was measured with ethanolic extract. In general, alcoholic extracts (ethanolic and methanolic) of Ocimum basilicum leaves produce the best activity against Staphylococcus aureus ATCC 25923 when compared to other solvents such as chloroform and acetone [17], and this finding could be helpful in studying and isolating the possible active compounds.

Results of methanol extract of Ocimum basilicum showed antibacterial activity; this result is similar to a study that was reported in Bangladesh by Hossain et al. (2010), that methanol extract of Ocimum basilicum showed antimicrobial activity against the tested microorganisms and caused degradation of the cell walls of sensitive bacteria [18].

Results of oil extract of Ocimum basilicum were in agreement with the study that was reported in Sudan by Abbas (2018); the oil extract of Ocimum basilicum showed antimicrobial activity against gram positive bacteria including Staphylococcus aureus [19].

Results of ethanol extract of Ocimum basilicum also agreed with the study reported in Saudi Arabia by Khalid (2013), that ethanol extract of Ocimum basilicum showed high inhibitory activity against Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 33591 [20]. In contrast, our result disagreed with the study done in Turkey by Kaya et al. (2008), that methanol extract of Ocimum basilicum showed no antimicrobial activity against Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa, and Saccharomyces cerevisiae [21].

Ethanolic leaves extract of Ocimum basilicum was injected into the injection port and was mixed with the carrier gas stream (Helium), then it was moved through the column with the carrier gas at high temperature; the molecules present in EEOB were separated into volatile and non-volatile molecules. Volatile molecules were burned with hydrogen and oxygen from the cylinder and produced ions which conducted an electric current that was amplified and detected by computer and was recorded as peaks on a chromatograph that demonstrated the amount of current passing through a unit of time (retention time).

The retention time of different volatile molecules was compared using standards from the library. Sixteen volatile molecules were identified that had antibacterial activity as mentioned in Table 4.

Eugenol is one of many chemical compounds of Ocimum basilicum that have antibacterial susceptibility [22]. Eugenol ($C_{10}H_{12}O_2$: 4-allyl-2-methoxyphenol) is an aromatic compound belonging to the group of phenols, which is a pale-yellow liquid with a strong basil aroma. It naturally exists in the leaves [22]. Eugenol exhibits a number of potentially beneficial biological properties, including antimicrobial, antioxidant, anti-inflammatory, anticarminative, and antispasmodic activities [22]. Eugenol inhibited biofilm formation, disrupted the cell-to-cell connections, detached the existing biofilms, and killed the bacteria in biofilms of both MRSA and MSSA with equal effectiveness; it decreased the gene expression of biofilm and enterotoxin genes, and decreased bacterial colonization [22].

Conclusion:

- Ethanolic extract of Ocimum basilicum demonstrated the strongest antibacterial activity against Staphylococcus aureus ATCC 25923, with inhibition zones of 28, 25, and 22 mm at concentrations of 100, 50, and 25 mg/mL, respectively.
- Hexane extract exhibited moderate antibacterial activity, producing inhibition zones of 25, 21, and 19 mm at the same concentrations.
- Methanolic extract also showed antibacterial activity, with inhibition zones of 24, 22, and 18 mm at 100, 50, and 25 mg/mL, respectively.
- Overall, the antibacterial efficacy followed the order: ethanol > hexane > methanol.

Recommendations:

- Evaluate O. basilicum extracts against clinical isolates of S. aureus, including multidrug-resistant strains.
- Determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by testing concentrations below 25 mg/mL.
- Investigate the antimicrobial activity of O. basilicum against a broader range of clinically relevant microorganisms.
- Conduct phytochemical, pharmacological, toxicological, and in silico studies to identify active compounds and assess their safety and therapeutic potential.
- Explore the potential of O. basilicum-derived bioactive compounds as candidates for the development of novel antimicrobial agents.

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