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Original Article

Antibacterial Activity of *Boswellia carterii* Aqueous Extract and Its Effect on Phagocytosis *in vitro*

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Abstract

Boswellia serrata has been traditionally used for the treatment of several inflammatory diseases, and bacterial resistance to antibiotics has recently increased the use of bioproducts. The present study aimed to assess the antibacterial effect and phagocytic ability of the aqueous extract of the *Boswellia serrata* in bacteria isolated in nosocomial infections. *Boswellia carterii* plant was collected and prepared from the aqueous extract in different concentrations. A total of 125 samples were collected from various clinical sources, including urine, sputum, wounds, otitis, and blood, from patients of both genders in different age groups. The results demonstrated that out of the 59 infected samples, urine samples had the highest infection (68%), followed by wounds, sputum, and otitis reported as (60%), (44%), and (40%), respectively. On the other hand, blood samples had the lowest percentage of infection (28%). Microscopic diagnostic results, biochemical tests, API Staph System, API 20E System, and Vitek 2 Compact pointed out that the highest infection rates were related to *Staphylococcus aureus* (32.20%), *Pseudomonas aeruginosa* (25.33%), and *Escherichia coli* (22.03%), while the lowest infection rate was detected in *Klebsiella pneumonia* (20.33%). The results indicated that aqueous extract of *Boswellia carterii* had an antibacterial activity for all bacterial isolates, 25 mg/ml of extract gave an inhibition zone of 10.8 mm,10.4 mm, 7mm, and 10mm for *S. aureus*, *E. coli*, *P aeruginosa*, and *K. pneumonia*, respectively, while 200 mg/ml of extract gave 24 mm, 22 mm, 18.4 mm, and 20 mm, respectively. The results pointed to a significant increase in the phagocytosis rate, with the phagocytosis of blood samples treated with *Boswellia carterii* extract (79.7%), as compared to control samples (57.75%). As evidenced by the results of this study, the aqueous extract of the *Boswellia carterii* plant showed antibacterial effects and a positive impact on the phagocytic ratio; nonetheless, it is recommended that further studies be conducted to characterize the compounds of this herb.

Keywords: Antibacterial activity, Aqueous extract, *Boswellia carterii*, *in vitro*, Phagocytosis

1. Introduction

Boswellia Carterii plant, from which frankincense is extracted, is found in Saudi Arabia, Somalia, Yemen, eastern Mediterranean countries, and Sudan (1). Other species of the genus *Boswellia sp*. also grow in Eritrea, Ethiopia, India, Kenya, Oman, and Nigeria (1). *Boswellia carterii is* a yellowish-white or yellowish-orange substance that is extracted from the cuttings of *Boswellia sp.* plant stems which drop collected after drying. There are about 25 species of the genus *Boswellia sp.* (1) which were used in Greece, Persia, and Romans (2). It is produced mainly from four species, including *Boswellia carterii* in East China and Africa, *Boswellia serrata* in India, *Boswellia sacra*in Arabia, and North Africa, and *Boswellia frereana* in Somalia, each of which produces various resins due to differences in soil and climate (1).

Boswellia carterii has a wide application in medicine; for instance, it showed effectiveness as a diuretic, disinfectant for the respiratory system, and

menstruation regulator. Moreover, it is used in cosmetics products, such as skin tonic, laxative, anti-wrinkle, and a fixative for perfumes (1). *Boswellia carteriial* alcoholic extract demonstrated analgesic, anti-inflammatory, and anti-arthritic effects, as well as anti-cancer effects, such as meningioma (3). A study on the effect of *Boswellia carteriial* on the respiratory system pointed out that 70% of people with bronchial asthma improved after using *Boswellia carterii*, compared to the control group. It is also used to treat colon ulcers and osteoporosis; moreover, it has been shown to induce cell death and exert positive effects on the treatment of diabetes (4).

Boswellia carterii consists of colloidal acids representing about (56%-65%), resins (20%-36%), and essential oils (4-8%), and its active substances belong to the colloidal acids, which are called boswellic acid. The immune effect of some of these compounds has been assessed in some studies which pointed to these positive effects on the treatment of tumors, cancer, and rheumatoid (5). *Boswellia chiechy* resins contain terpenoids, triterpenes, pentacyclic triterpenes, and tetracyclic triterpenes. Its pharmacological effects are based on hydroxyers-12-ene-23-oicacid which is a characteristic of all species of the genus *Boswellia sp*., the most notable of which is boswellic acid (6).

Boswellia carterii has efficacy against prostate cancer, and several studies have demonstrated its inhibitory effect on the growth of prostate cancer cells. In this regard, it has been demonstrated that Boswellic acid, Acetyl-11-keto-β-Boswellic (AKBA), has a special inhibitory impact on prostate cancer through the inhibition of blood vascular receptors and blood vascular endothelial growth factor (7). *Boswellia carterii* has distinct effects on diabetes by reducing the level of glucose in the blood in heavy and diabetic animals (8). *Boswellia carterii* extract removes redness and skin irritation. Frankincense was used in China as a treatment for skin bruises and infected sores (9).

Boswellia carterii has a high pharmacological safety since it has been used as a treatment for more than thousands of years. It has demonstrated no severe side effects and unlike many chemical anti-inflammatory medications, the dosage causes no harmful effects on blood pressure, heart rate, breathing, or other subjective responses with low toxicity. It is included in the list of safe substances and a permitted food additive by the United States Food and Drug Administration (FDA or USFDA) (10). Frankincense is considered a famous material sold and found in Arab medical herbalists and herbal shops. It has been utilized in folk medicine for the treatment of many diseases, the most important of which are tumors, inflamed ulcers, dysentery, and chest diseases, such as coughing, asthma, shortness of breath, and leprosy as a heart tonic (11). The biological activities as anti-bactericidal pathogenic bacteria *Escherichia coli* and *Salmonella sp*. were evaluated. It contains some antibacterial compounds, such as monoterpene, diterpene, sesquiterpene, incensole, boswellic acid, as well as phenolic compounds which are known to inhibit the growth of bacteria (12).

Recently, plant extracts have aroused great interest since they are considered a source of bioproducts that have protective properties when used as therapeutic alternatives in many pathogens, in addition to being an inhibitor of pathogenic bacteria. In light of the aforementioned issues, the present study aimed to assess the antibacterial and phagocytosis activity of aqueous extract *Boswellia carterii* against bacteria isolated from different clinical infections in Iraq (13).

2. Materials and Methods

2.1. *Boswellia carterii* Plant Collection and Preparation

An amount of 500 grams of *Boswellia carterii* which was obtained from local markets in Karbala, Iraq, was crushed and kept in sealed and opaque glass containers until the performance of necessary extraction and tests**.**

2.2. Preparation of the Aqueous Extract of *Boswellia carterii*

The extract was prepared according to the agar well diffusion method (Rios et al., 1988) in which 40 g of *Boswellia carterii* powder was mixed with 160 ml distilled water, and the sample was crushed using a

blender device; thereafter, the mixture was stirred by a magnetic stirrer for 60 min. Following that, the mixture was left in the refrigerator for 24 h for soaking. It was filtered, and the cooling centrifugation was then carried out at 2000 rpm /15 min. The filtrate was taken and lyophilized under vacuum pressure with a Lyophilizer supplied by Edwards Company-Germany. The samples were stored after drying in glass bottles with a tight lid in moisture-free conditions until use**.**

2.3. Preparation of Different Concentrations of Aqueous Extract of *Boswellia carterii*

Five concentrations of aqueous *Boswellia carterii* extract were prepared (25, 50, 75, 100, and 200 mg/ml, respectively) by dissolving sterile distilled water sterilized using 0.45 µm membrane filters**.**

2.4. Isolation and Identification of Bacterial

A total of 125 samples were collected from various clinical sources, including urine, sputum, wounds, otitis, and blood, collected from patients of both genders in different age groups with various infections. Bacterial isolates were diagnosed by studying the general culture characteristics of the colonies growing on the MacConkey's agar, Blood agar, Mannitol salt agar, and Eosin-Methylene blue agar. Where the samples were taken and cultured on these media to confirm their diagnosis and incubated at a temperature of 37°C for 24 h (13). In addition, phenotypic, microscopic, and biochemical tests, as well as a diagnosis system (Api 20E, API system, and Api Staph) and Vitek 2 Compact system, confirmed the diagnosis of bacteria. This study was approved by the Research Ethics Committee of the University of Warith Al-anbiyaa, Karbala, and all participants provided informed consent.

2.5. Antibacterial Activity Test

The bacterial suspension was prepared in the nutrient broth media at a concentration of 108 cells/ml to be equivalent to the 0.5 McFarland standard. Following that, 0.1 ml of the bacterial suspension was transferred and inoculated using a swab. Sterile cotton was placed on a plate containing Muller-Hinton agar (Oxoid), the plates were incubated at 37ºC for half an hour of impregnation. Thereafter, the discs of filter paper (Whatman No.1) with a diameter of 6 mm saturated with different concentrations of the extracts were placed by immersing them in these extracts. The discs were fixed with sterile forceps on the surface of inoculated dishes and incubated at 37ºC for 24 h. Upon the completion of incubation, the inhibition area diameters were observed and measured around the discs saturated with the extract, three dishes were used for each concentration and each isolate.

2.6. Phagocytosis Efficacy Test *in vitro*

Mackie and Mc-Cartney (14) method was followed to test the phagocytosis activity against *S. aureus* bacteria isolated from patients with different infections. A number of 20 blood samples were obtained from healthy people as control. Thereafter, 1 ml of drawn blood was transferred into a tube, 100 microliters of bacterial suspension were added, and 100 microliters of the aqueous extract of *Boswellia carterii* were then added with calm stirring at a rate of two repetitions for each tube. The blood, bacteria, and aqueous extract of *Boswellia carterii* were incubated at a temperature of 37°C for one hour and a half. The tubes were taken out from the incubator after the incubation period was completed and shaken well. A total of 2-3 drops of the blood, bacteria, and extract mixture were placed on a clean slide to make smears from the control samples. The glass slides were left to dry at room temperature and stained with Leishman stain. Slides were dried and investigated by light microscopy, and the number of phagocytic cells was calculated using the following equation:

$$\text{Phagocytosis rate} = \frac{\text{number of phagocytic cells}}{\text{number of phagocytic cells and non-phagocytes}} \times 100$$

3. Results and Discussion

Out of 125 examined samples, 59 samples infected with bacteria were isolated. As displayed in table 1, the

largest infection rate 17 (68%) was within the urine samples, followed by the infection rate in both wound swabs, sputum, and Otitis reported as 14 (60%), 11 (44%), and 10 (40%), respectively. On the contrary, blood samples had the lowest infection rate, amounting to only about 7 (28%) of the total.

Table 1. Numbers and percentages of pathological isolates according to different clinical infections

Sample source	Number of samples	The number of positive samples	Percentage %
urine	25	17	68%
wounds	25	14	60%
sputum	25	11	44%
Otitis media	25	10	40%
blood	25	7	28%
Total	125	59	47.2%

According to the results of biochemical tests and the use of the API Staph System, API 20E System, and Vitek 2 Compact System to confirm the diagnosis of bacteria, the highest rate of infection with *Staphylococcus aureus* was 19 (32.20%), followed by *Pseudomonas aeruginosa* bacteria 13 (25.33%), and *Escherichia Coli* bacteria 13 (22.03%), while the lowest percentage of infection was related to bacteria *Klebsiella pneumonia* which amounted to 12 (20.33 %) of the total as displayed in table 2.

The results revealed that *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most common ones among recovered gram-positive and negative bacteria, respectively. Based on the results, gram-positive and negative bacteria cause many infections, such as urinary tract infections (UTIs) and respiratory infections, including pneumoniae, wounds infections, otitis, as well as bacteremia .The results showed that *Staphylococcus aureus* isolates differed in isolation rates from different disease samples due to variations in the number of disease samples under the study.

Staphylococcus aureus bacteria is an important cause of the spread of numerous illnesses, ranging in severity from suppurating superficial skin infections to systemic infections. Sometimes it even leads to the death of the injured; moreover, it is a determining factor in hospital and wound infections, particularly surgical wounds, especially after the emergence of resistant strains of anti-methicillin (15). The high prevalence of *Pseudomonas aeruginosa* in hospitalized patients is due to its high ability to resist environmental conditions, in addition to the fact that the hospital environment is a suitable environment for the growth of this bacteria (16). The comparison of results revealed that the percentage of isolates varied based on the source of infection, the amount of attention to hygiene, as well as the type of sterilization and disinfection in hospitals (17).

Staphylococcus bacteria, especially *Staphylococcus aureus*, Coagulase-negative staphylococci (CoNS), are among the most important bacteria causing various infections in humans and animals. It represents an important health problem, especially in the so-called hospital-acquired infection. Epidemiological reports show the spread of *S.aureus* Methicillin-Resistant *S.aureus* (MRSA) clearly in these infections and the susceptibility of the bacteria to multiple resistance to treatments. Therefore, research projects are striving to find appropriate and effective treatments for such strains of bacteria (18).

Bacterial resistance to drugs, especially multidrug resistance, the side effects of some of these treatments, as well as the high cost of their preparation and production, have brought alternative medicine to the attention of researchers and scientists. Medicinal plants are one of the most significant alternative options that are constantly being studied (19). The results of the antibacterial activity of aqueous extract of the *Boswellia carterii* plant pointed to the antibacterial activity for all types of bacteria used in the study. This activity differed in targeting bacteria from one species to another; nonetheless, it was similar in its share of the increase in the used concentration of the extract. The rates of inhibition of growth diameters at a concentration of 25 mg/ml reached 10.8 mm for *S. aureus*, 10.4 mm for *E. coli*, 7 mm for *P. aeruginosa*, and 10 mm for *K. pneumonia*, while these rates reached the concentration of (200 mg/ml) 24 mm, 22 mm, 18.4 mm, and 20 mm for bacteria species, respectively, as illustrated in table 3.

Table 2. Numbers and percentages of bacteria isolated from different clinical infections

Sample source / Bacteria	urine	sputum	wounds	Otitis media	blood	Total	Percentage %
S. aureus	4	3	5	4	3	19	32.20%
E. coli	7	2	2	1	1	13	22.03%
P.aeruginosa	4	2	5	3	1	15	25.42%
K. pneumonia	2	4	2	2	2	12	20.33%
Total	17	11	14	10	7	59	100%

Table 3. Averages of inhibition diameters of the aqueous extract of *Boswellia carterii* (Inhibition diameter in mm)

Concentration / Bacteria	25mg/ml	50mg/ml	75mg/ml	100mg/ml	200mg/ml
S. aureus	10.8	12.8	14.6	18.8	24
E. coli	10.4	12.6	14.8	16.8	22
P. aeruginosa	7	10	12.4	14	18.4
K. pneumonia	10	12	14	16.2	20

A significant difference was observed at 25 mg/ml concentration ($P \leq 0.05$) since the lowest range of inhibition was against *P. aeruginosa* bacteria, while the highest range of inhibition was against *S. aureus* bacteria with a significant difference in concentration (200 mg/ml). They had the lowest inhibition against *P. aeruginosa*, as compared to other bacteria ($P \leq 0.05$). There has been a significant difference at the same level for the highest inhibition range, which was against bacteria *S. aureus*. The significant differences between the highest and lowest inhibition can be explained by the fact that the used bacteria are sensitive to the types of compounds found in the *Boswellia carterii* plant to different degrees from one type to another. Therefore, the response of the bacteria to these substances varies with different concentrations.

In pathological isolates, a marked increase was observed in antibacterial activity with elevated concentrations of aqueous extract of the *Boswellia carterii* plant. In this regard, *S. aureus* and *P. aeruginosa* were the most and least affected bacteria in all concentrations used in the study. This discrepancy can be attributed to different types of bacteria. Some bacteria may be sensitive to a particular substance of aqueous extract of the *Boswellia carterii*, and the sensitivity of bacteria to a specific substance varies according to the concentration of the substance (20). *Boswellia carterii* contains substances that may be effective in antibacterial activity, and among these substances, we can refer to boswellic acid which contains phenolic compounds that are known to prevent the growth of bacteria (21).

As indicated in a study by Mothana and Lindequist (22), the oils consisting of a monoterpene, Additrine, and sesquiterpene were responsible for this activity, while Basar (23) mentioned that the *Boswellia Carterii* contains substances, such as cembrene A, incensole acetate, and 24-12 dieneg, norursa-3, with antibacterial activity. Since these substances are included in the composition of essential oils, this explains the use of *Boswellia carterii* by fumigation to disinfect the infested areas. These results are consistent with those reported by Camarda, Dayton (12) who indicated the antibacterial activity of *Boswellia carterii*. The pathological and epidemiological significance of these methicillin-resistant strains is that about 40%-70% of staphylococci are spread in hospital intensive care units.

The evolution of *S.aureus* resistance to antibiotics highlights the importance of searching for alternatives, taking into consideration the importance of CoNS bacteria, which have recently become an important

health problem due to their increasing resistance to treatments and disinfectants. The increase in the inhibitory activity in elevated concentrations raises the possibility of the utilization of higher concentration levels of *Boswellia carterii* extract, especially since some studies had confirmed the absence of toxicity for this extract when used in laboratory experiments on animals. It is known that the chemical composition of *Boswellia carterii* generally consists of essential oils, Colloidal acids, and glue. Nevertheless, it is believed that the important effect of this substance results from the presence of Boswellic acids, which act as anticancer and anti-allergic agents, apart from their anti-inflammatory properties (24, 25).

Mikhaeil, Maatooq (26) indicated that *Boswellia carterii* contains Boswellic acid with phenolic substances which have antibacterial activities (27). The results of this research are in agreement with those reported by Umezu (27), Büchele, Zugmaier (21), and Mothana and Lindequist (22). Studies pointed to the effectiveness of *Boswellia carterii* extract in the incense method after the emergence of epidemics in antiquity (28). It is recommended to study other therapeutic activities (*in vivo*), conduct analytical studies to find the active compound *Boswellia carterii*, and use other solvents to extract the active substance to increase the therapeutic ability of this substance. As presented in table 4, the measurement of the phagocytic rate of blood samples treated with aqueous extract of *Boswellia carterii* showed a significant increase in phagocytic rate (79.7%), compared to control samples (57.75%).

The results of the current study pointed to a marked increase in phagocytosis rates of blood samples treated with aqueous extract of the *Boswellia carterii* plant. That is to say, *Boswellia carterii* activates phagocytic cells and increases their phagocytic ability. Based on the results, the aqueous extract of the *Boswellia carterii* plant had a positive effect on the phagocytic process by raising the phagocytic rate. *Boswellia carterii* was used to treat rheumatoid arthritis and other inflammatory diseases, such as Crohn's disease (29). The anti-inflammatory activity was a result of the capability of resin to regulate the production of immune cytokines. The main chemical component of *Boswellia carterii* is boswellic acid. Several studies have indicated that boswellic acid selectively reduces Leukotriene LTB_4, is a powerful chemoattractant and activator of macrophages, as well as granulocytes; moreover, it reduced leukocyte filtration to the site of inflammation (29). Some boswelli components have been found to affect the immune system in a different method; for instance, different boswellic acids, such as acetyl-11-keto-B-boswellic and boswellic acid keto, could also show actions in the immune system.

Table 4. Percentage of phagocytic cells in several blood samples treated and untreated with aqueous extract of *Boswellia carterii*

Number of samples	Percentage of phagocytes in blood samples (Control group)	Percentage of phagocytic cells of blood samples treated with aqueous extract of *Boswellia carterii* (Treatment group)
1	43	76
2	60	79
3	61	79
4	44	71
5	51	75
6	54	79
7	61	83
8	71	88
9	56	70
10	62	76
11	65	79
12	54	78
13	61	87
14	51	74
15	53	78
16	60	84
17	55	79
18	67	87
19	60	83
20	66	89
phagocytosis rate	57.75%	79.7%

Authors' Contribution

Study concept and design: B. S. A. A.
Acquisition of data: A. Sh. M. A.
Analysis and interpretation of data: T. A. H
Drafting of the manuscript: B. S. A. A

Critical revision of the manuscript for important intellectual content: A. Sh. M. A.
Statistical analysis: T. A. H.
Administrative, technical, and material support: A. Sh. M. A.

Conflict of Interest

The authors declare that they have no conflict of interest.

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