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# PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITIES OF FRANKINCENSE OF *BOSWELLIA SERRATA*

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## Abstract

This research was interested in studying the anti-bacterial activity by the natural products separated from the crude frankincense resin of *Boswellia serrata*, as a number of fatty acids have been separated (Myristate, Palmatic, Oleic, Linolic, Arachidate, Arachidatenate and Lignocerate) were separated by the GLC device from chloroformic extract. The following phenolic compounds were also separated in their free form Kaempferol, Qurcetine, Epigene, Gallic acid, Catechin by HPLC device from methanol extract. Four different concentrations of separated active compounds were prepared (100, 75, 50, 25 mg/ml) and then tested their anti-bacterial efficacy for two types of bacteria that gram positive *Bacillus subtilis, Streptococcus pneumoniae* and a one type of gram negative *Proteus vulgaris*. The inhibition zoon was measured in comparison with the ciprofloxacin antibiotic as positive control and at a concentration (5µg/ml). The test results showed a different inhibitory efficacy of the phenols and fatty acids separated from the plant against the bacteria used in the study and by using the Disc Diffusion Method and the result Show that for phenols had a higher inhibitory effect than the fatty acids against the bacteria used and compared with the standard antibiotic.

***Key words:*** *Boswellia serrata*, Natural Products, GLC and HPLC analysis, Antibacterial Activity, Ciprofloxacin.

## Introduction

Medicinal plants contain active compounds that are natural products of the metabolism process within the plant and are of medical importance for treating many diseases that affect humans, especially infections caused by bacterial infections. (Dalirsani, *et al*., 2011 and Al-Dulayymi, 2014). Plant antimicrobials are an important factor in eliminating pathogenic bacterial infections (Gibbons, 2008). Many researchers stressed the need to support the chemical treatment system with active substances separated from plants to treat diseases (Archana and Abraham, 2011). Researchers continued their efforts to interest in separating and diagnosing active compounds from medicinal plants and studying their antibacterial activities (Stavri, *et al*., 2007; Mahalingam, *et al*., 2011 and Ismail, *et al*., 2014). Frankincense resin is extracted from the *Boswellia serrata* tree scattered in the Arabian Peninsula, northern Somalia, Ethiopia and Iraq and is used as an incense due to its sweet aroma (Banno, 2006).

Frankincense resin is extracted from the tree by making a wound or an incision in its bark that appears like milk or resin and which dries out and appears in a brown olibanum (Ismail, *et al*., 2014). *Boswellia serrata* tree gum and resin extract is used to treat many bacterial and fungal infections (Weckesser, 2007). It is also used to treat cancerous diseases, as many studies have proven its effectiveness against human leukemia (Ernst, 2008 and Mohammed Aman, *et al*., 2010).

Frankincense resin is used in the treatment of many diseases as it strengthens the heart and brain and treats forgetfulness and blood diarrhea as it treats arthritis and other infections due to its work to regulate the production of immune cytokines and also contains cortisone inhibiting inflammation, which does not have side effects such as that caused by the industrial cortisone (Banno, 2006; Langmead, 2006 and Chevrier, 2005).

Several research studies have confirmed that the *Boswellia serrata* tree resin contains fatty acids, especially what has been separated in this current study, Such as Myristate, Palmatic, Oleic, Linolic, Arachidate, Arachidatenate, and Lignocerate. It was diagnosed with GLC-MAS device and which has proven its anti-bacterial effectiveness (Ahmed, *et al*., 2015 and Yuan, *et al*., 2006). And also demonstrated that *Boswellia serrata*

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tree resin contains phenolic compounds such as Thujene, Camphene, β-Pinene, Myrcene, Limonene, M-cymene and cis-verbenol and that has proven its effective antibacterial and this was confirmed by researchers (Ayub, *et al*., 2018).

## Materials and Methods

### The Preparation of Plant Extracts Using Continuous Soxhlet Apparatus

Using the Soxhlet system, the chloroform and methanol extract were prepared and extracted according to the boiling point of each solvent as 100g of frankincense resin was weighed and placed in the device at a rate of 20 hours extraction until the solvent became colorless and then the second solvent was applied in the same way and then concentrated extracted with a rotary evaporator at 45°C. Samples are then kept in the refrigerator in dark conditions until use (Harbone, 1984 and Al-Dulayymi, 2014).

### Saponification

In the saponification process, the fatty acids are separated and in basic media (Arthur, 1972), as 5g of chloroform extract to frankincense resin was added and 50 ml of KOH was added and left for 90 min by refluxed and at a temperature not exceeding 100°C. After cooling, add distilled water by 50ml and then separate the solution aqueous about fat from non-adhesion by adding 25ml ether by suppurating funnel. Then we took the extract that contains the fatty acids and added concentrated sulfuric acid to reach PH = 2. Then we get the free fatty acids with the ethereal, The product is then esterified from fatty acids to decrease the polarity and increase its volatility in the GLC diagnostic apparatus (Loury, 1967 and Sultan, *et al*., 2020a).

### Acid hydrolysis

The free phenols were separated from the crud methanol extract and by the acid hydrolysis process, as 50 ml of HCL was added to 10g of the methanol extract of the frankincense resin and by refluxed the phenols were obtained and after the solution cooled, the ethyl acetate was used in suppurating funnel to isolate the organic layer from the layer Aqueous, then the ethyl acetate is evaporated with a rotary evaporator to obtain free phenols, and then kept in a cooled dark condition until diagnosis (Harborne 1998 and Sultan, *et al*., 2020a).

### Diagnosis of fatty acids

Gas Liquid Chromatography (GLC) was diagnosed with fatty acids and by comparing Retention time (Rt) of the extract sample with standard fatty acids, the measurement was performed at the Ministry of Science and Technology in Baghdad, with a device of Japanese origin. This technique was used to diagnose fatty acids based on the retention time (Rt) values for the extracted samples compared to the standard retention time.

### High Performance Liquid Chromatography (HPLC)

Use the HPLC device to diagnose free separated phenols with purified by filters with a diameter of 0.1 Micrometer and the column used C18-ODS wavelength of 360 nm, measured at the Ministry of Science and Technology in Baghdad.

### Antimicrobial activity

Isolates were taken from the bacterial bank at the University of Mosul, as bacterial pathogens were selected that were pathogenic to humans, gram positive such as *Bacillus subtilis*, *Streptococcus pneumoniae*, and one gram negative such as *Proteus vulgaris*, the bacterial isolates were kept at 4°C in the center of the nutrient broth.

### Determination of antimicrobial activity

The bacterial suspension was prepared under sterile conditions and to obtain young and newly developed colonies and incubated at 37°C for 24 hours, then the bacterial suspension was diluted by normal slain and compared with McFarland solution 108 cells/$cm^3$, spread the bacterial suspension uniformly with a sterile (L) penis in the Hinton agar medium and while the spread the bacterial is impregnated in the medium, filter disc saturated with active compounds separated from crude gum are prepared as the filter paper disc are of 6 mm diameter and Watmann No. 1 as four concentrations of separated active compounds were prepared, Which 25, 50, 75, 100 mg/$cm^3$, the filter paper disc were saturated at a rate of 0.1$cm^3$ (Miladinovic, 2000 and Sultan, 2018).

The experiment was carried out with three replications for each extract and each bacterium, as the saturated disc were installed in the medium and under complete sterilization conditions and incubated for 16 hours at a temperature of 37°C. The damping inhibition zoon measured by millimeters and by means of a titration instrument. DMSO solution was used without using the extract as a negative control sample. (Djipa, *et al*., 2000; Nascimento, *et al*., 2000 and Sultan, *et al*., 2020b).

Inhibition results were compared with the ciprofloxacin (5µg/ml) antibiotic as a control sample. The data were analyzed statistically using the Duncan test with a $p \leq 0.01$ level and by applying the SAS program between the averages and knowledge of the differences between them.

## Results and Discussion

### Diagnosis of some fatty acids by Gas-liquid chromatography GLC

The fatty acids of the frankincense resin extract were diagnosed with GLC technique. GLC-chromatograms and extractor retention time were obtained within the apparatus and compared with standard compounds. The concentration of each fatty acid inside the extract was also known.

The following fatty acids were diagnosed: (Myristate, Palmatic, Oleic, Linolic, Arachidate, Arachidatenate, Lignocerate), as table 1 and Fig. 1 indicated the rotation time of each of them.

As shown in table 1 and Fig. 2 the ratios of the fatty acids diagnosed with the GLC device separated from the chloroformic extract after its saponification are from the highest concentration to the lowest concentration (Palmatic 0.129, Myristate 0.119, Lignocerate 0.114, Lenolic 0.026, Arachidatenate 0.008, Oleic 0.007). It also showed the retention time and concentration of each fatty acid separated from Olibanum.

The results of the current study showed that Olic acid is present with the lowest percentage of 0.007 and that Palmatic acid is present with the highest percentage of 0.129 and then the rest of the separated fatty acids follow (Yuan, *et al*., 2006).

Several researchers have confirmed that the *Boswellia serrata* plant contains fatty acids, among which Oleic acid, Linoleic acid, Arachidic acid, Arachidonic acid, Palmitic acid and Lauric acid (Ahmed, *et al*., 2015; Ayub, *et al*., 2018 and Yuan, *et al*., 2006).

Chloroforme extract also contained Omega of type 6 and 9, which has a role in antibacterial activity. It also has a high effectiveness in strengthening the immune system, reducing cholesterol and repairing body tissues (Ikuyma, *et al*., 2007 and Sultan, *et al*., 2020a).

### Diagnosis of some free phenols with high performance liquid chromatography HPLC

The acid hydrolysis process was performed on the methanol extract of frankincense resin and some free phenols were separated and diagnosed with HPLC, which showed a difference in the content of methanol

**Table 1:** The Ret. time of standard and extract fatty acid by GLC analysis.

| Stan-dard | Fatty acid | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | yristate | | Palmatic | | Oleic | | Lenolic | | Arachidate | | Arachidatenate | | Lignocerate | |
| | $R_t$ min 15.578 | Conc. | $R_t$ min 17.785 | Conc. | $R_t$ min 20.731 | Conc. | $R_t$ min 21.959 | Conc. | $R_t$ min 23.755 | Conc. | $R_t$ min 24.407 | Conc. | $R_t$ min 29.456 | Conc. |
| Extract | 15.566 | 0.119 | 17.655 | 0.129 | 20.759 | 0.007 | 21.921 | 0.026 | 23.772 | 0.107 | 24.330 | 0.008 | 29.430 | 0.114 |

**Table 2:** The Ret. time of standard and extract phenolic compounds by HPLC analysis.

| Stan-dard | Phenolic | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Gallic acid | | Catechin | | Epigene | | Quercetin | | Kaempferol | |
| | $R_t$ min 4.79 | Conc. | $R_t$ min 6.04 | Conc. | $R_t$ min 8.07 | Conc. | $R_t$ min 11.90 | Conc. | $R_t$ min 13.75 | Conc. |
| Extract | 4.736 | 41.2 | 6.044 | 5.2 | 8.080 | 11.3 | 11.996 | 4.7 | 13.772 | 8.6 |

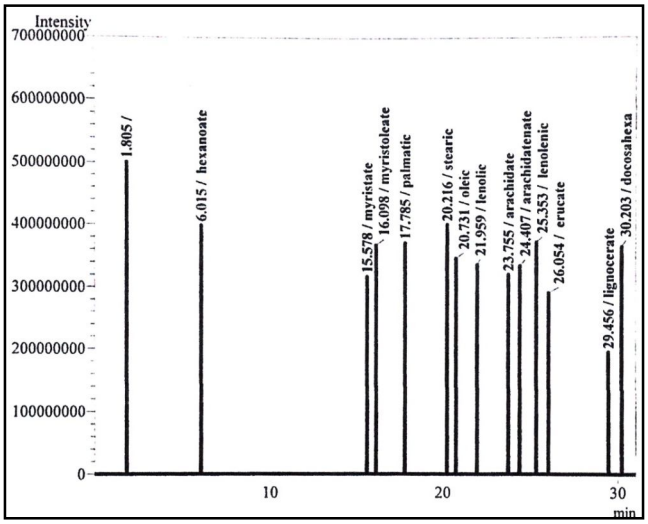



**Fig. 1:** GLC analyses of standard fatty acid.

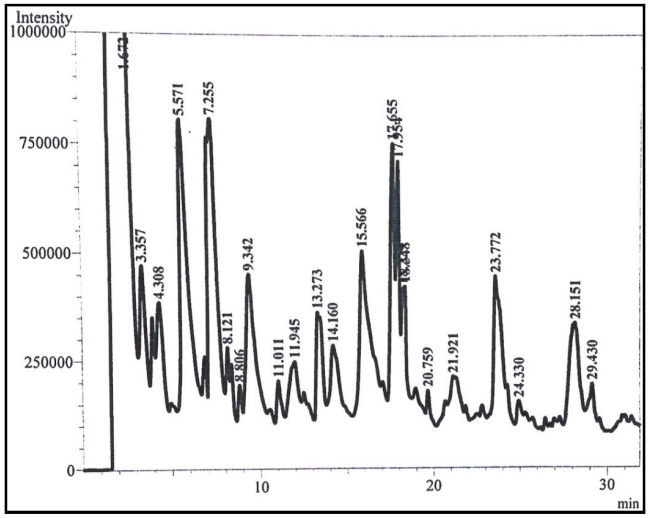



**Fig. 2:** GLC analyses of extract fatty acid.

extract of phenols, with concentrations, retention time and sub-curve space different from each other, as follows: (Gallic acid 4.736, Epigene 8.080, Kaempferol 13.772, Catechin 6.044, Quercetin 4.7) as shown in table 2 and Fig. from 3 and 4.

The results of the current study agreed with the results of the researcher Ayub, *et al*., 2018 in the frankincense resin *Boswellia serrata* plant containing free phenols

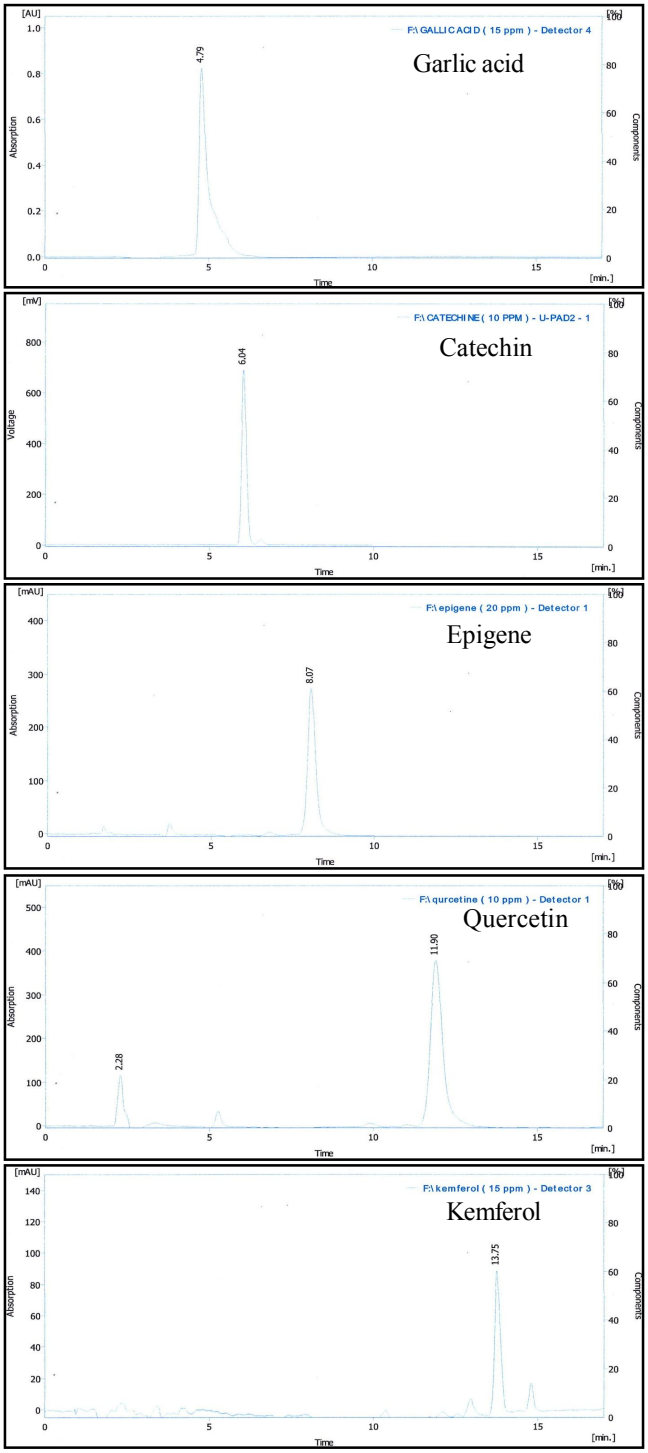



**Fig. 3:** HPLC analyses of standard phenolic compounds.

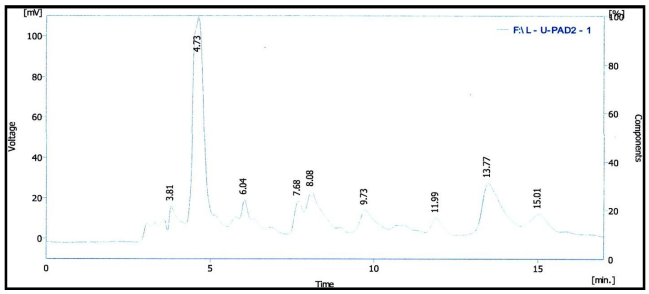


**Fig. 4:** HPLC analyses of extract phenolic compounds.

used in the treatment of many diseases that affect humans, especially alleviating the symptoms of arthritis, and this is due to its anti-inflammatory properties. The researcher Ismail, *et al*., 2014 also confirmed that the *Boswellia serrata* plant contributes to treating asthma, fighting cancerous diseases and improving bowel function (Sultan, *et al*., 2020b and Sultan, 2018).

The effect of the chemical compounds separated from frankincense resin on some pathogenic bacteria and by using an sensitivity test (disc diffusion).

Use the sensitivity test (disc diffusion) to find out the effect of the effective chemical compounds against some positive and negative germs, as the extract contained free phenols and different concentrations with somewhat higher effect than the fatty acid extract against the pathogen bacteria used in the study and compared with the antibiotic ciprofloxacin as shown in table 3 and Pictures (1 + 2).

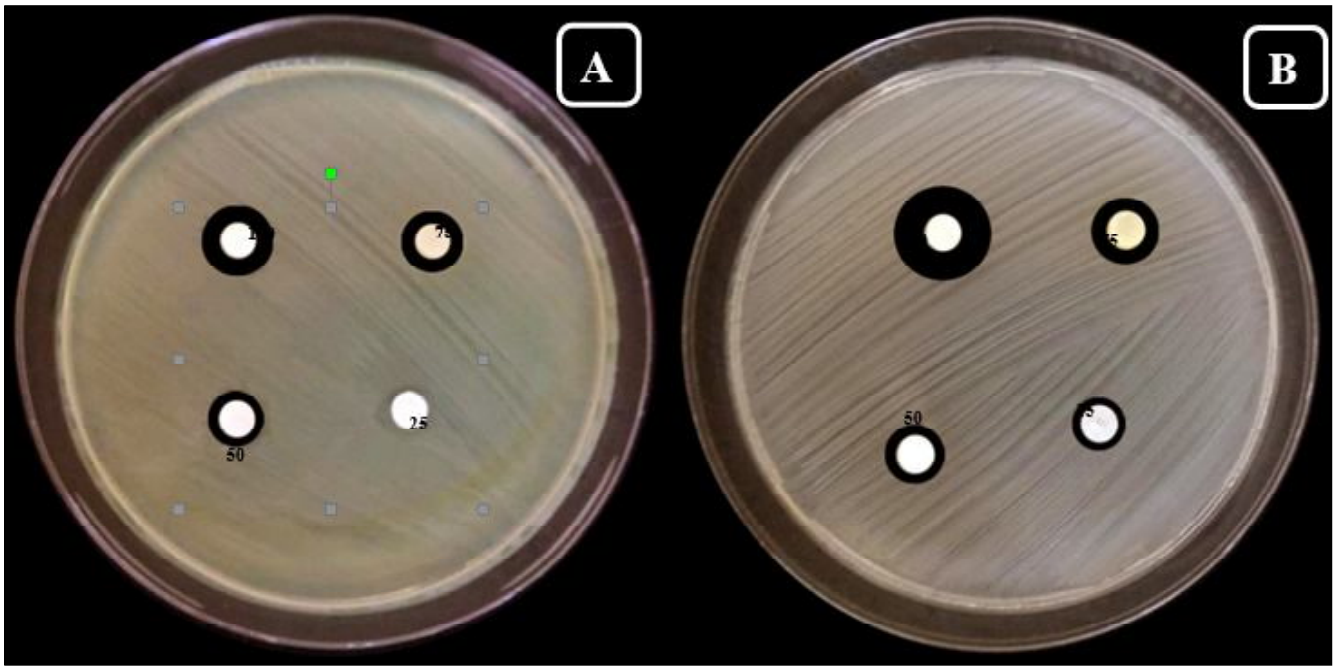
It was the highest effect of fatty acids against a bacterium *Bacillus subtilis* with a diameter of 18 mm inhibition, the effect was higher than the standard antibiotic, the highest effect of free phenols was 22 mm against *Streptococcus pneumoniae*.

It is also the highest effect of the antibiotic under study, the inhibitory effect is due to the separated chemical compounds as many researchers the role of fatty acids in getting rid of microorganisms and the resulting infection

**Table 3:** Antimicrobial efficacy of chemical composition of *Boswellia serrata* extract (mm).

| Microorganism | Chemical Composition | Concentration (mg/c³) / Zone inhibition in (mm) | | | | |
|---|---|---|---|---|---|---|
| | | 100 | 75 | 50 | 25 | Ciprofloxacin (5µg/ml) |
| *Bacillus* | F | 18 | 15 | 10 | 8 | 15 |
| *subtilis* | ph | 20 | 16 | 13 | 10 | |
| *Streptococcus* | F | 22 | 12 | 8 | - | 18 |
| *pneumoniae* | ph | 14 | 11 | 9 | - | |
| *Proteus* | F | 12 | 8 | - | - | 10 |
| *vulgaris* | ph | 14 | 12 | 9 | - | |

F = Fatty acid, PH = Phenolic Compound.




**Photos (1+2):** Antibacterial effect of A. fatty acids agents *Bacillus subtilis*.
B. Phenolic compounds agents *Proteus vulgaris*.

from (Ali, *et al*., 2008 and Ahmed, *et al*., 2015).

Fatty acids also affect the energy complex by changing the oxygen pathway and thus cell destruction The bacterium, in addition to the inhibition of a large number of vital components of the bacterial cell and this was confirmed by researchers (Yuan, *et al*., 2006 and Abd Emaged, *et al*., 2009).

The results of the study are consistent with the findings of the researchers, Ahmed, *et al*., 2015 in the ability of phenolic compounds to treat bacterial infections and the resulting diseases By inhibiting metabolic reactions by affecting the enzymes of these reactions, they interfere with proteins and lead to denaturation protein that stops the biological activities of the bacterium and its death (Williams and Lewis, 2011).

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