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Inhibitory Effect of Plant Oils of (Rosmarinus officinalis, Zingiber officinale, and Boswellia Serrata) against the Pathogenic *Acinetobacter baumannii* bacteria

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ABSTRACT

Acinetobacter baumannii bacteria are considered one of the most nosocomial types of bacteria that infect the human body. Their treatment has become difficult due to their resistance to known antibiotics nowadays. So, to investigate the possibility of finding and extracting fatty acids from different plants and using them as an alternative to antibiotics. The oils of three types of plants (*Rosmarinus officinalis, Zingiber officinale, and Boswellia Serrata*) were extracted using the Soxhlet device to obtain crude oil. The quantitative and qualitative detection and diagnosis of these fatty acids in these plants were also carried out using the HPLC device. Therefore, the results showed that there is a disparity in the value of inhibition for *Acinetobacter baumannii* bacteria, as it was found that the low concentrations of fatty acids for each of *Rosmarinus officinalis and Zingiber officinale* plants gave the highest inhibition values for bacteria, which were at 12.5 mg. However, giving fatty acids to the *Boswellia Serrata* plant the highest inhibitory value with concentrations (50 and 100 mg. It can be concluded from this paper that there is the possibility of replacing plant extracts and using them as an antibiotic instead of using current anti-life.

Keywords: *Acinetobacter baumannii*, Soxhlet device, Fatty acids, HPLC.

Introduction

Frequently used antibiotics lose their effectiveness against serious bacterial infections. Pathogenic bacteria obtained the most prevalent mechanism of antibiotic resistance from a pool of resistance genes in other microbial genera (1,2). Many medicines, or medicinal compounds, that have been used for millennia to enhance human health are believed to be derived from natural plants. Approximately 65%–80% of the population, especially in developing countries, view herbal medicine as a vital source of healthcare because it is more culturally acceptable, more compatible with the human body, and has fewer side effects (3,4). Plant extracts and essential oils have long been studied as important natural antimicrobials. According to the World Health Organization, traditional medicine uses plant extracts or their active ingredients to treat 80% of the world's population. In clinical settings, over half of the pharmaceuticals are used as natural products (5). Rosemary, or Rosmarinus L., is a member of the Lamiaceae family. This herbal plant grows all over the world and has fragrant, green-picked leaves. It is used in traditional medicine, as a food flavoring, and as a cosmetic. It is an anti-inflammatory, anti-ulcerogenic, anti-diabetic, and choleretic hepatoprotective medication (6,7). Numerous studies have demonstrated the

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antimicrobial effects of rosemary essential oil and its chemical mixture, which also has antioxidant qualities. Throughout the years, researchers have examined the plant's components, including caffeic acid, carnosol, carnosic acid, ursolic acid, and rosmarinic acid. For instance, phenolic acid was discovered in significant amounts (8). Boswellia trees, a common gum resin, are produced by Boswellia trees, mainly B. carteri Birdw. (syn. B. sacra), B. serrata Roxb., B. papyrifera, and B. frereana Birdw. Among other Boswellia species of less commercial importance, B. dalzielii Hutch. (Burseraceae) is an interesting species that has only recently gained attention. Several parts of this tree are used in African traditional medicine because of their anti-diarrhoeal and anti-ulcer qualities, as well as their capacity to treat other gastrointestinal disorders. As a result, some writers looked into the resin's pharmacological characteristics, emphasising its analgesic, anti-inflammatory, and anti-diarrhoea effects. Compared with the more conventional Boswellia species, not much is known to date regarding the chemical makeup of B. dalzielii. The essential oil extracted from its leaves was the subject of the first research. However, it was not until 2019 that the resin essential oil's composition was reported to consist primarily of monoterpenes with trace amounts of the typical diterpenoid found in Boswellia. Likewise, B. dalzielii contains the same boswellic acids found in the resin of most Boswellia species. Generally speaking, these triterpenic components are in charge of certain biological characteristics like anti-inflammatory, antimicrobial, and even anti-cancer effects (9,10). Ginger has been used extensively as a spice, culinary ingredient, and traditional remedy all over the world because it is one of the most important species in the Zingiberaceae family and has important medicinal, nutritional, and indigenous medicinal properties (11,12). Its antibacterial, anti-inflammatory, antipyretic, antioxidant, anti-diabetic, hepatoprotective, renal, and anti-carcinogenic properties are among its medicinal advantages (13,14). The rhizome of ginger usually contains water, fibres, proteins, lipids, carbohydrates, and aromatic oils. In addition to its nutritional and flavourful qualities, ginger has potential applications in traditional medicine for various therapeutic benefits. Despite sporadic increases in the total area for agricultural production due to the immense importance of ginger, its productivity has unfortunately declined over time due to soft rot disease (15,16). Gram-negative, aerobic *A. baumannii* bacteria are a type of coccobacillus that can grow at 44°C. It can be difficult to separate the bacteria from the strain, which is why it is sometimes mislabeled as Gram-positive. Its G+C content ranges from 39% to 44% for deoxyribonucleic acid (DNA), and it is pleomorphic, non-fermentative, non-fastidious, and temperature and pH-tolerant (17). *A. baumannii* Multidrug-resistant (MDR) A. baumannii infections are widespread, and the causes of these infections have been the subject of numerous studies worldwide (18). Fatty acids, which are also essential nutrients and metabolites in living organisms, are abundant in natural fats and dietary oils. For this reason, fatty acid quantification is very interesting for many applications. The fatty acids found in real samples are usually saturated and unsaturated fatty acids with chain lengths ranging from 12 to 22 carbon atoms. Regular measurements of free fatty acids, particularly in plasma, typically measure the total amount rather than the individual fatty acids. One significant drawback of this approach is the widespread presumption that one specific fatty acid is representative of all fatty acids. Chromatographic techniques, which have been employed for many years, can quantitatively analyse individual fatty acids in foods and biological samples because of their inherent separation power (19,20). So, the study aims to investigate the possibility of finding and extracting fatty acids from different plants and using them as an alternative to antibiotics.

Material and Methods

The collection of the plant samples:

The samples of plants used in the study were collected from several areas of Mosul, which included several plants (Rosmarinus officinalis, Zingiber officinale, and Boswellia Serrata) for the purpose of obtaining the oil extract of these plants and conducting laboratory experiments of these extracts on bacteria (*Acinetobacter baumannii)*.

The collection of bacterial samples:

25 samples of bacteria that cause infections, burns, and wounds from patients who were admitted to a hospital in Mosul were taken for various clinical infections after written consent. They were taken to the laboratory for isolation and diagnosis after being placed in sterile, clean plastic boxes. Furthermore, they were purified and isolated by accredited laboratories and underwent many microscopic and normal media examinations (selective media) and biochemical tests.

Cultural identification of the bacteria:

Using the planning method, bacterial colonies were cultivated on the selection medium (MacConkey Agar) and incubated for 24 hours at 37°C. The morphological features of these colonies were examined (21).

Gram stain dye (Gram stain test):

A drop of distilled water was placed on a sterile glass slide, and using a loop, a swab of developing colonies was transferred onto Mrs. Agar medium. The process of mixing the swab and the drop was carried out with the help of a Loop and after exposure to a flame for the purpose of fixing the membrane, and then adding the solutions of the diagnostic kit for the Gramme Stain Kit prepared by ATOM scientific and consisting of the following solutions: (Grystal Violet stain, Lugols Iodin solution, ethyl alcohol ethanol 95%, Safranine stain). After drying, it was examined under a light microscope to see the quality of the response to the dye by the bacterial type, as well as to identify the shape of the cells (22).

Microscopic examination of the bacteria:

The morphological properties of the isolates were tested by making smears of them on slides and dyeing them with Chromatography and light microscopy under 100x power. The developing colonies were then cultured in Mrs. serum medium, and the cells were dyed with Gram stain to identify the shape of the cells and their assemblies (23).

Long-term preservation of Bacteria:

As reported in (24), the sample preservation medium prepared from the materials and methods of action and distributed in Eppendorf tubes with a volume of (1) cm3 was inoculated with pure single colonies of the bacterial isolates under study, and then mixed with a Vortex device, and the tubes were kept by deep freeze Deep cryopreservation at a temperature of -20 ،in this way, bacterial isolates can be preserved for several years (24).

Biochemical Identification of Bacteria:

A number of biochemical tests were performed to diagnose *Acinetobacter baumannii* bacteria according to (25). These included as following: -

- Oxidase test
- Catalase test
- Indole Production test
- Citrate use test
- Urease test

The Extraction of Fatty acids by using the Soxhlet Apparatus:

The Soxhlet apparatus was used in the process of estimating fatty acid compounds for the raw extract of the plant sample. The dry form of the leaves (about 20g) was placed in a thimble (Thumbal), cellulose extraction, and used petroleum ether solvent was used at a temperature of 40-60 degrees Celsius for five hours, with heating, after which then evaporate the solvent was evaporated, and the extracted samples were kept (26).

Detection of the effect of extracts:

1-The diffusion method for investigating the effectiveness of the extracts used under study.

By using Muller-Hinton medium and a 24-h bacterial culture, the Agar well method was used to test the impact of the extracts on the bacteria. Pits with a depth of 6 mm and a width of 8 mm were made in Muller-Hinton's agar. The extracts were injected with 10 microliters of oil from different plants, then the planted petri dishes were incubated for 24 hours at 37 degrees Celsius, and then the inhibition zones formed on the surface were measured using a scientific ruler (27).

2-HPLC Conditions for Analyzing Oil Compounds.

High-performance liquid chromatography (HPLC) using the Sikam model from Germany was used to analyze the samples (the extraction of three plants). The furnace column, the UV (s 2340) detector, the automatic model, and the pump model are all 2100s quadruple gradient pumps. S 4115 is the model number. The detector is UV-280 Nm at a wavelength of 25 cm * 4.6 mm, and the mobile phase was = (methanol: d. Dr.: Formic acid) (70: 25: 5), Column C 18-ozone-depleting substances 1.0 mL/min is the flow rate (28).

Results And Discussion

Cultural identification of bacteria:

Only one isolate out of 25 samples of the bacteria of *Acinetobacter baumannii* was identified and isolated in this investigation. The colonies on the MacConkey medium did not ferment lactose and displayed red colonies encircled by a clear, mucous halo. Moreover, all subsequent experiments were conducted on these bacteria**.**

Microscopic identification of the bacteria:

Acinetobacter baumannii cells appeared under a light microscope at power x100 in the form of Gram-negative, non-fermenting, glucose-negative cells, and are found everywhere in the environment, from soil to water, and they also abound on the surface of the skin of people working in the medical field. There are many types of stagnant bacilli, but the most common types of stagnant bacilli that cause diseases in some categories are the Baumannia bacteria (*Acinetobacter baumannii*), which is called the Iraq bacterium (Iraqibacter), due to its sudden appearance in the Iraq war, especially in military therapeutic barracks.

Biochemical Identification of Bacteria:

Acinetobacter baumannii bacteria are characterized by their positivity and negativity of some biochemical tests for their diagnosis, as shown in **Table 1.**

Table 1 shows the Biochemical tests for the bacterial diagnosis of *Acinetobacter baumannii* bacteria.

Biochemical tests	Results
Catalysis	+Ve
Oxidase	-Ve
Indole	-Ve
Consumption of citrates	+Ve
Urea hydrolysis	-Ve

Acinetobacter baumannii showed a positive catalase test, as shown above in **Table 1**, which indicates their ability to produce this enzyme, which breaks down hydrogen peroxide into oxygen and water, forming gas bubbles during the respiratory process. The ability of the bacteria to produce the catalase enzyme, which converts hydrogen peroxide into oxygen and water and creates gas bubbles during the respiratory process, was verified by a researcher (29) through her investigation of the S. marcescens bacteria (30). As for the oxidase test, *Acinetobacter baumannii* showed a negative result for it, which indicates the inability of bacteria to produce the cytochrome oxidase enzyme. This was also confirmed by a researcher (29) through her study on the bacterium S. *marcescens*. She demonstrated that S. marcescens tested negative for the oxidase test, indicating that the bacteria are unable to produce the cytochrome oxidase enzyme, which promotes the transfer of electrons from the donor part to the oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) to the acceptor part (oxygen). The test is crucial for distinguishing between Enterobacteriaceae species that are positive and those that are negative (31), which stimulates. One of the crucial tests to distinguish between positive and negative genera within the intestinal family is the transfer of electrons from the donor part of the oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) to the receiving part (oxygen) (31). The indole test showed a negative result of the indole test for the absence of a red ring on the surface of the medium after the addition of the reagent. This was also confirmed by a researcher (29) through her study on the marcescens bacteria. This bacterium showed a negative result for the indole test due to the lack of a red ring on the surface of the medium after adding the reagent, which means that it does not possess the tryptophanase enzyme that decomposes the amino acid tryptophan in the medium (32). As well as were *acinetobacter baumannii* can also consume citrate (+ results) as the sole carbon source of energy, as it works to transport citrate permease into the cell effectively by the enzyme Citrate permease, forming pyruvic acid and carbon dioxide, thus turning the medium from Green to blue (33). As for the urea test, the result of the test for bacteria showed a positive result; this was not confirmed by the researcher (29), in her study on urea analysis, as she was given a positive result of the urea decomposition test. She gave all the symptoms as a result of a positive test for possessing the urease enzyme that analyzes urea in the middle, producing ammonia and CO2, causing the acid function of the middle and changing the color of yellow (PH 6.8) to Pink (PH 8.2) (25).

Biological inhibitory efficacy:

After studying the effect of the oil extract of Rosmarinus officinalis, Zingiber officinale and Boswellia Serrata oil on the pathogenic bacteria *Acinetobacter baumannii*, which is causing inflammatory burns and wounds, it turned out that it has a clear inhibitory effect on the growth of pathogenic bacteria as shown in Table (2) as shown by the figure (1) superiority of the plant.

The fatty acids of Rosmarinus officinalis, Zingiber officinale, and Boswellia Serrata plants were used for four concentrations, namely 12.5 mg, 25mg, 50 mg, and 100mg for each plant. The results of **Table 2** showed that all plant oils used in the study on *Acinetobacter baumannii* were close to each other, the highest results were shown for Rosmarinus officinalis and Zingiber officinale plant with a concentration of 12.5 mg had the highest effect on bacteria and with an inhibitory ability of 23.22 mm to 23.22 mm, respectively, as shown in the **Table (2) and Figure (1)** above. It was less inhibited at a concentration of 100 mg for the two plants above, which had an inhibitory capacity of 14 mm. As for the Boswellia Serrata plant, the highest inhibition capacity of colon bacteria was at a concentration of 25 mg and 50 with an inhibition capacity of 23.28 and 23.17, respectively. It was similar to the two plants above, with the lowest inhibition rate at a

concentration of 100 mg, which amounted to 21.14 mm.

The analysis of oil plants by using the HPLC Technique:

High-performance liquid chromatography (HPLC) was used to analyze the sample. Germany's Sikam model. Pump type: automatic 2100 four-way gradient pump. The detector is UV (S2340), the sample model is S5200, and the column oven model is S4115. The column contained CE 18-ozone-depleting substances (25 cm * 4.6 mm), the UV detector was 280 Nm with a flow rate of 1.0 mL /min, and the mobile phase was = (methanol: d. W: formic acid) (70: 25: 5). The analysis of the three plant extract samples to ascertain the concentrations of the plants' extraction oil is displayed in the figures.

Table 3 shows the concentrations of the vegetable oils extracted (Rosmarinus officinalis, Zingiber officinale and Boswellia Serrata) and obtained from different types of plants under study using the HPLC device based on the standards that were injected into the HPLC device at the same concentration of 2%, volume 0.1 ml and operating conditions for the samples under study as well as for the vegetable oil standards, as well as for the oil standards.

It is clear from the standard tables in figures 2,3,4, and 5 that the four fatty acids in the first sample of Rosmarinus *officinalis* are all available and in varying concentrations according to each standard. The peaks of fatty acid standards were matched with the first sample, and after determining the concentration of the standard for each acid that we injected into the HPLC, the concentrations of fatty acids were extracted for the samples under study by determining the area of the graph of the standards present in the sample.

The aforementioned standard figures 2, 3, 4, and 5 make it evident that each of the four fatty acids found in the initial Boswellia serrata sample is present and in a range of concentrations. Following the determination of the standard concentration for each acid injected in the HPLC, the peaks of the fatty acid standards were compared to the first sample. Then, using the area of the graph of the standards in the sample, the fatty acid concentrations for the samples under investigation were extracted.

Table 2 shows the antimicrobial activity of medicinal plant oil using the agar well diffusion method on *Acinetobacter baumannii* bacteria.

Medicinal Plant Oil	Rosmarinus officinalis				Zingiber officinale				Boswellia Serrata			
	The concentrations											
	12.5 mg	25 mg	50 mg	100 mg	12.5 mg	25 mg	50 mg	100 mg	12.5 mg	25 mg	50 mg	100 mg
	Inhibition zone											
Antimicrobial Activity on *Acinetobacter baumannii* bacteria	23.22 mm	21.05 mm	20.74 mm	14.88 mm	23.21 mm	19.76 mm	18.07 mm	14 Mm	22.90 mm	23.28 mm	23.17 mm	21.43 mm

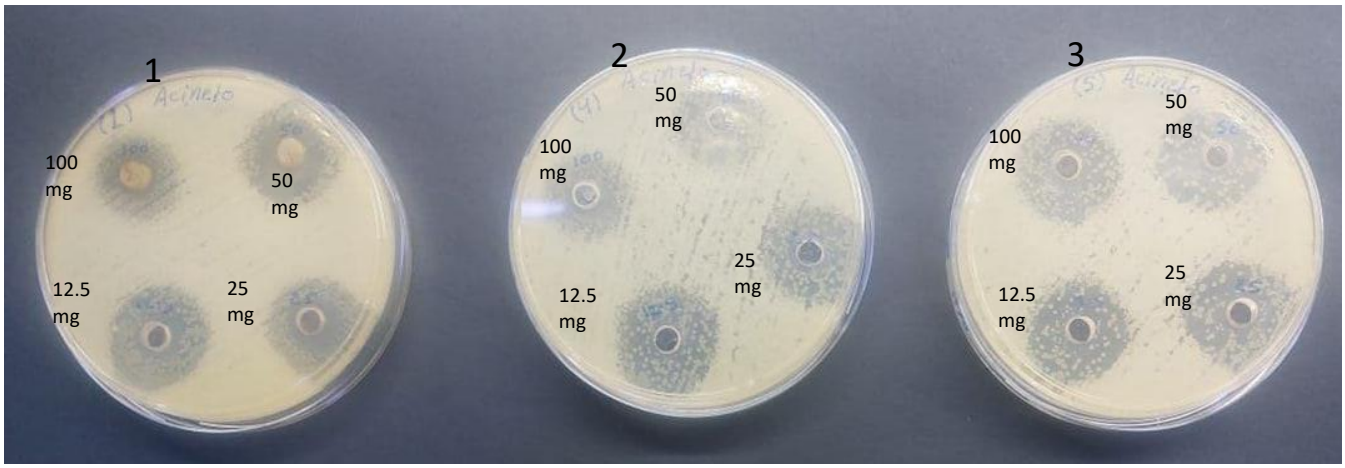


Figure (1) shows the biological activity of fatty acids of a number of plants represented by the Rosmarinus officinalis plant = 1, and Zingiber officinale= 2, and the Boswellia Serrata = 3, and several concentrations, which are 12.5, 25, 50, and 100 mg against *Acinetobacter baumannii* bacteria.

Table 3 shows the concentrations of fatty acids present in the plants under study.

Name %	Rosemary	Zingiber	Boswellia
Palmitic	2.6	6.5	3.9
Oleic	35.9	17.9	14.1
Linoleic	18.9	22.6	15.8
Stearic	1.9	1.3	2.6
Linolenic	0.88	0.81	1.8

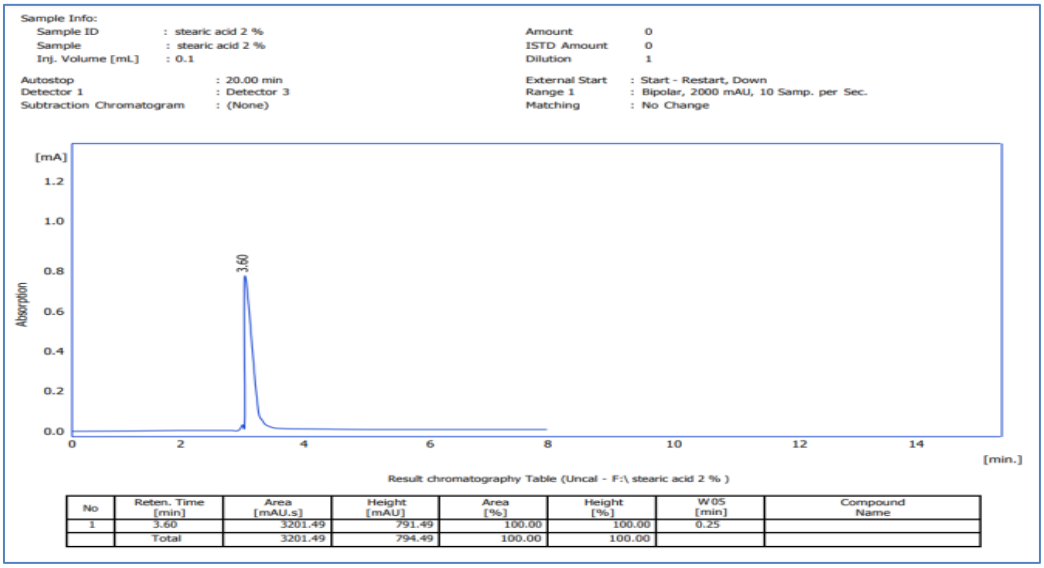
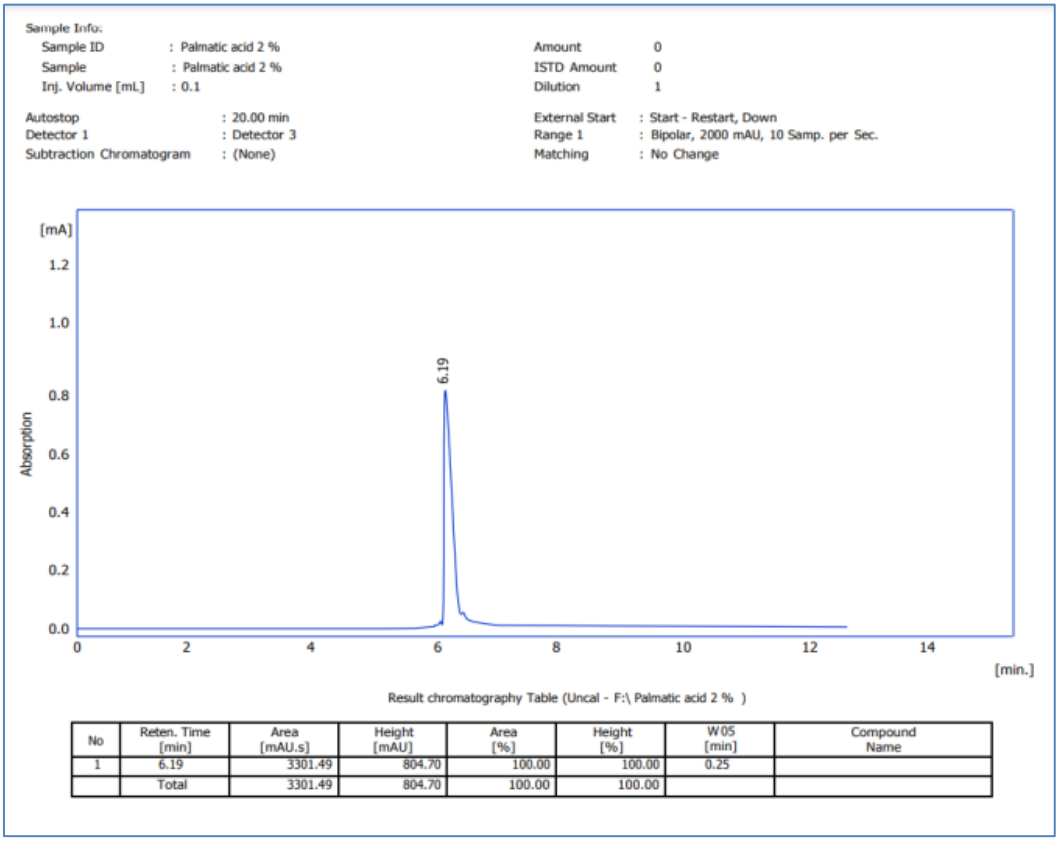
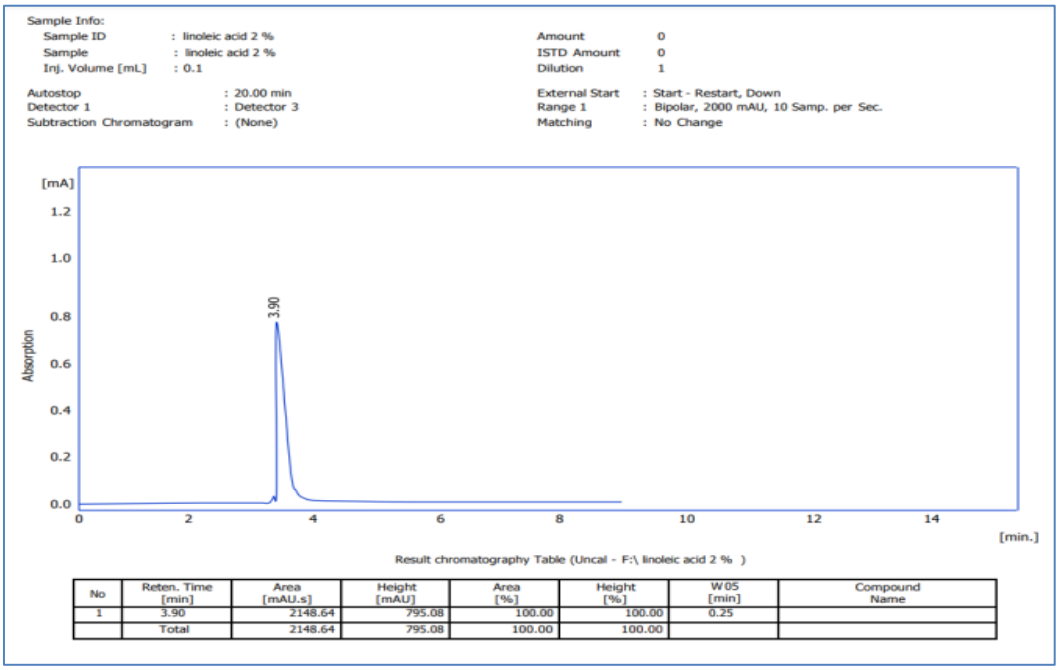


Figure 2 shows one of the most important main standards of fatty acids (Stearic acid) found in plants at a concentration of 2 %.



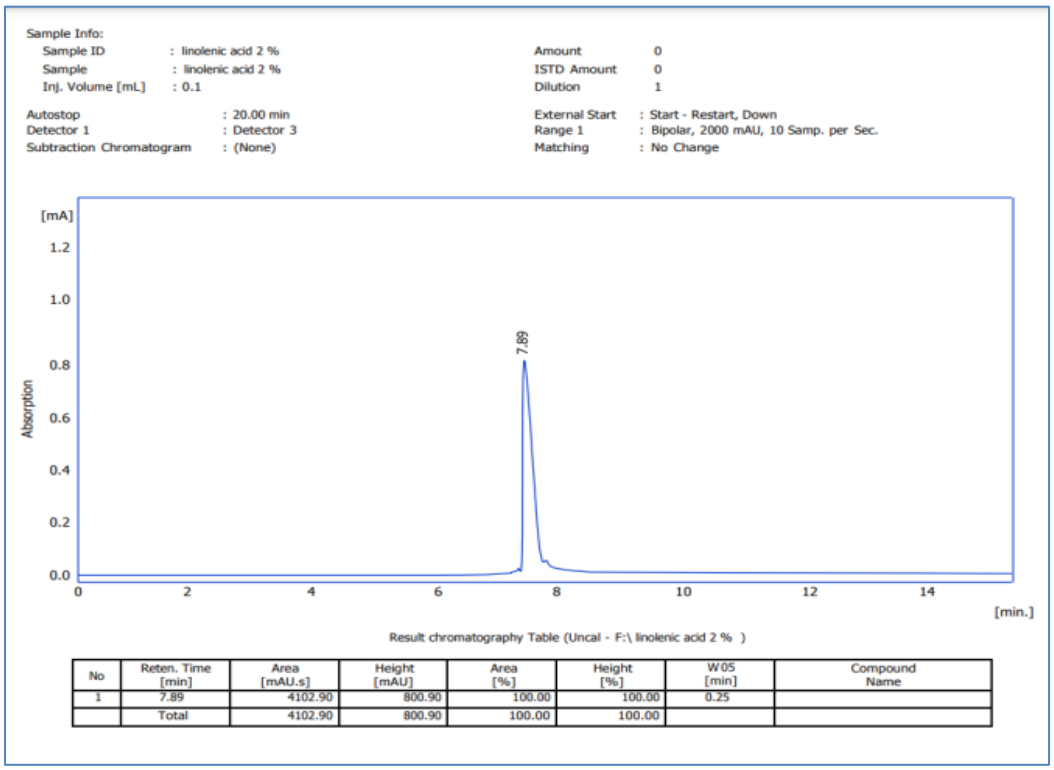
No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	6.19	3301.49	804.70	100.00	100.00	0.25	
	Total	3301.49	804.70	100.00	100.00		

Figure 3 shows one of the most important main standards of fatty acids (Palmitic acid) found in plants at a concentration of 2 %.



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	3.90	2148.64	795.08	100.00	100.00	0.25	
	Total	2148.64	795.08	100.00	100.00		

Figure 4 shows one of the most important main standards of fatty acids (Linolenic acid) found in plants at a concentration of 2 %.



No	Reten. Time [min]	Area [mAU.s]	Height [mAU]	Area [%]	Height [%]	W05 [min]	Compound Name
1	7.89	4102.90	800.90	100.00	100.00	0.25	
	Total	4102.90	800.90	100.00	100.00		

Figure 5 shows one of the most important main standards of fatty acids (Linoleic acid) found in plants at a concentration of 2 %.

The four fatty acids in the first Zingiber officinale sample are all present and in different concentrations in accordance with each standard, as can be seen from the standard Tables 3. The concentrations of fatty acids were extracted for the samples under investigation by using the area of the graph of the standards present in the sample, following the matching of the peaks of the fatty acid standards with the first sample and the determination of the standard's concentration for each acid that we injected into the HPLC.

Through the results of the biological effectiveness of Rosemary and Zingiber plant, it was found that the highest concentration against *Acinetobacter baumannii* bacteria was at a concentration of 12.5 mg as shown in the form of No. 1 and Table No. 2 above, and this may be confirmed by Table No. 3 above in finding many fatty acids in a plant The Rosemary, which was identified quantitatively and qualitatively, with the AGLC device, including the olic acid, with a concentration of 35.9%, and Lennic with a concentration of 18.9%. As for the Zingiber plant, it was the highest rate for fatty acids, which was 22.6%, and the oleic acid was 17.9%, which was the highest concentration in the Rosemary and Zingiber plant. However, it was found that the highest concentration of the sebaceous acids of the Boswellia plant was a concentration of 25 and 50 mg against *A. baumannii* bacteria, as in the form of No. 1 and Table No. 2 above. This may be confirmed by Table No. 3 through the percentage of the fatty acids located in the Boswellia plant, which was for the Eololic acid and was 15.8, and the oleic acid with a 14.1%.

In 2015, research teams from the United States and Germany reported 2015 that they had identified and isolated a novel class of antibiotic that killed specific strains of Gram-positive bacteria (*Mycobacterium tuberculosis* and *Staphylococcus*

aureus) without exhibiting any signs of resistance. This study was different from their use of antibiotics. Teixobactin, a novel antibiotic, was discovered in a screen of uncultured soil bacteria (34). A peptide (secondary metabolism) produced by certain soil-dwelling bacterial species appears to harm bacteria by binding to lipid groups in the cell wall, preventing the synthesis of the cell wall, and ultimately killing the bacteria (35). Additionally, in 2018, a group of researchers from Rockefeller University discovered a novel antibiotic 2018 called malacidins that could eradicate a wide range of Gram-positive bacteria. They are members of a group of substances made by bacteria that live in the soil, and it appears that their activity requires calcium ions. It is thought that the calcium-dependent antibiotic family's conserved Asp-X-Asp-Gly motif facilitates calcium binding. Malacidins appear to be active after binding to calcium; the calcium-bound molecule then binds to the lipid, and the bacterial cell wall generates molecules that lead to the breakdown of the cell wall and the death of the bacteria (35,36).

Conclusion

It can be concluded from the results of the above research that it is possible to strongly recommend the use of plant extracts as one of the best therapeutic and preventive methods to get rid of and treat bacteria instead of antibiotics, which constitute a global problem that cannot be predicted and a global disaster on the world level regarding antibiotics. It has been proven that many generations of antibiotics are at risk from bacteria due to mutations, and thus, it is not possible to get rid of or treat different types of bacteria, while some types of plant extracts, especially fatty acids, have proven the quality of these treatments for bacteria through many published studies in this regard. Accordingly, we recommend the use of these pure fatty acids from these plants in their use as a treatment for bacteria.

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