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Original Research

Bioactive Natural Compounds of *Zingiber officinale* Using Gas Chromatography-Mass Spectrometry and Evaluation of Its Antibacterial Activity

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Abstract:

Chemical molecules that are found in plants and are sometimes referred to as secondary metabolites are called phytochemicals. The methanolic extract of Zingiber officinale was analyzed, and it was found to contain bioactive phytochemical components. The peak area, molecular weight, molecular formula and retention time, are the three criteria that are used to determine the identity of phytochemical substances. Analysis of Zingiber officinale using gas chromatography-mass spectrometry (GC-MS) uncovered the presence of the Longfolene, 2-(4-Nitrobutyryl)-cyclooctanone, 1, 1-Diphenyl- 4-phenyl - thiobut-3-en-1-ol, Cholestan-3 -ol, 2-methylene-,(3ß,5), ß-Bisabolene, and l-(+)-Ascorbic acid are some of the compounds that were found in the mixture. 2,6-dihexadecanoate, 9,12- Octadecadienoic acid (Z,Z)-, methyl ester, 1-Heptatriacotanol, 10,13-Eicosadienoic acid, methyl ester, E,E,Z-1, 3,12-Nonadecatriene-5,14-diol, 9-Octadecenamide,(Z), and decyl oct- 3- yl ester are the components that make up this compound. The evaluation of antibacterial activity indicated that metabolites of Zingiber officinale were extremely potent against Proteus mirabilis (19.13±0.07).

Key words: *Zingiber officinale*, Phytochemicals, GC-MS, Antibacterial Activity.

Introduction:

There are approximately 53 genera within the Zingiberaceae family, which together contain over 1200 different species. The Zingiberaceae family is found in both southern and eastern parts of Asia. Zingiber officinale , Zingiber zerumbet, and Curcuma long are examples that these characterized plants belonging to this family can be used in the preparation of herbal medicinal remedies [1,2]. Researchers are currently showing a great deal of care and interest in personalized

members of the Zingiberaceae family due to the high use status of Zingiberaceae species as spices and as ingredients and compositions for herbal medicinal remedies in traditional medicine. Here we must mention that the most famous and common member of the Zingiberaceae family is the common and well-known ginger. Although the history of this spice goes back thousands of years to the Sanskrit word "Srngaveram", which literally translates to "root of the horn". [3]

Ginger rhizome, which can be defined as the well-known horizontal stem from which the roots grow, is a well-known medicinal herb that has actually been used very extensively in Chinese herbal medicine for approximately more than 3,000 years throughout Asia, including Indonesia. Japan, India and others such as Arab countries. Because of its beneficial properties, such as its well-known, very pungent flavour, its distinct high aromatic quality, and its distinctive nutritional value.

Here are two different types of substances: volatile compounds, which make up natural essential oil, and non-volatile compounds, which include oleoresin and other natural plant chemicals with biological activities beneficial to human health and life, such as phenols and flavonoids. 4-6]. For this reason, ginger has been transformed into a wide variety of goods, such as ginger tea, ginger powder, sweets, and ginger juice. That's why these elements have become essential thanks to the advancements in science and technology nowadays in the food industry.

The bioactive chemicals found in ginger's essential oils, such as phenolic compounds, flavonoids, terpene, and certain volatile compounds, are mostly responsible for the beneficial effects that ginger has on human health. Other molecules, such as terpene, may also play a role. It has been stated that ginger can treat or prevent a broad variety of conditions, such as nausea and vomiting caused by chemotherapy, lowering blood pressure (anti-hypertension) [7, 8], acting as an anti-diabetic agent by lowering blood glucose levels, acting as an anti-hyperlipidemic agent, and performing other actions. Ginger's capacity to serve as an antioxidant and reduce the production of reactive oxygen species is another reason it's so popular for illness prevention. This property allows ginger to help ward off a variety of persistent conditions [9, 10]. The purpose of this study is to evaluate the effectiveness of *Zingiber officinale* as an antibacterial agent as well as analyze the bioactive chemical components that it contains utilizing GCMS.

Materials and Methods:

Preparation of extract:

In the midst of Iraq, in the city of Hilla, some *Zingiber officinale* was obtained from the local market. After undergoing extensive washing and the elimination of any foreign substances, the seeds were placed in an airtight container to protect them from the damaging effects of humidity. Afterward, they were kept at room temperature until they were required again. After being rinsed with water, the seeds of N. sativa are dried and then pulverized into powder. The powdered seeds of *Zingiber officinale* were macerated in methanol for one to twenty-four hours [11]. For the purpose of separating the plant extract, Whatman No. 1 filter paper was utilized. Additional phytochemical investigation was carried out using the filtrates. In order to remove any remaining traces of moisture, it underwent a second round of filtration using sodium sulfate.

Gas chromatography-mass spectrum (GC-MS) analysis:

In the Agilent 7890, GC-MS analysis procedures for the known plant extract are completed. This device is operated and activated by a computer and is set correctly at 70 volts, as we know in the laboratory. A small syringe containing approximately 1 microliter of methanolic plant extract is used to inject it into the GC-MS device, and here the known scanning process continues for a constant forty-five minutes. During the separation of compounds, work was done to remove them from the column and introduce them to a detector that has the ability to generate an electronic signal at any possible time, in which a chemical substance is detected [11-14]. Hence, we notice that the higher the concentration present in the tested sample, the stronger the signal that is obtained, and

through which it is then analyzed by the computer.

This calculated time period, which we can call the acquisition and retention time (RT), begins when the initial injection is performed (initial time) and this step ends when the rinse is performed. In parallel with the operation of the aforementioned device, the computer carried out the process of building a chromatographic chart by plotting the signal on the known chart. The signal to be produced by a compound when it was eluted from the gas chromatographic column and into the detector was represented in the known chromatogram by each of the peaks in this chromatogram. Here the RT was displayed along the known x-axis, and at the same time the signal intensity was measured on The length of the y-axis. The component present in the measured sample that was first injected was also measured along the y-axis. Meanwhile, after being eluted from the gas chromatographic column, these individual compounds pass to the electron ionization detector (mass spectroscopy). It was then exposed to a barrage of electrons, which caused it to disintegrate into smaller and smaller pieces as it passed completely through the detector.

It is clear from this that the pieces that were extracted are exactly positively charged ions and also have a specific known mass. In the laboratory, the acquired mass/charge ratio (M/Z) was calibrated using what is known as a histogram. At the same time, such a graph was used, called the mass spectrum graph, which is a true fingerprint of the molecule. In order to complete the GC-MS analysis procedure for the above-mentioned extract, the oven temperature, flow rate of the gas used, and the electron gun must be programmed in advance [15]. The device's oven is kept at a constant and specified temperature of 100 degrees Celsius. In this process, helium gas was used as a carrier and suction cup at the same time. The known amount of helium that flows regularly through the system is 1 milliliter per minute. Through this, electrons with an energy of about 70 volts were released by the mass detector's electron gun. The use of the test column known as Elite 1 in this work aims to separate the components from each other. At this time, indices of practical retention of components and fragmentation patterns and shapes of mass spectra were compared with those actually preserved in the archived computer library as well as with published literature on the website in order to identify those components that were actually found in the extracts. The spectra of previously unknown compounds were also compared to those already present in the Wiley and NIST/EPA/NIH collective spectral libraries in order to determine their identities and effectiveness [16].

Determination of antibacterial activity of secondary metabolite compounds of *Zingiber officinale:*

After using a sterile cork-borer to cut wells in the agar with a diameter of five millimeters, 25 microliters of the sample solutions, which were referred to as Metabolites Produced by Zingiber officinale, were injected into each of the wells. On Muller Hinton agar plates, the test pathogens (*Escherichia coli, Proteus mirabilis, and Staphylococcus epidermidis*) were swabbed [17 ,18]. The drilled wells were supplied with a total of 90 l of fungal extracts. The diameter of the wells was cut down to 0.5 millimeters. Following a 24-hour incubation period at 37 degrees Celsius, the plates were examined. The solvent control was performed with methanol.

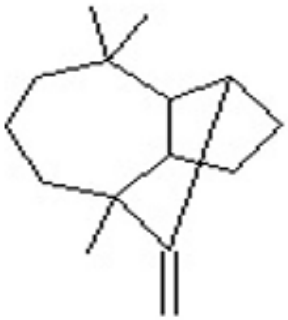
Results and Discussion:

The maceration procedure was used in the process of getting the extract ready to use. The maceration was performed at room temperature using the proper solvent, and it was repeated numerous times while being shaken or stirred. Maceration is a method that can be used for the processing of substances that are not able to tolerate being heated to very high temperatures [19, 20].

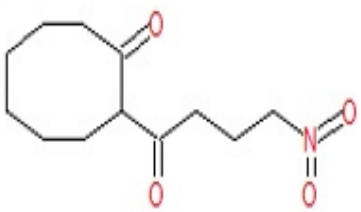
As it is known, this movement actually occurs at the index interlayer, and then spreads to the solvent component itself. The goal of this is precisely to attract known chemical components based on the concept of mass transfer of matter to the actual solvent component. The relative abundance of the molecular mass component parts (m/e) of the

molecular ion (M+) can be used to actually determine the structure whose true presence has been determined in the samples actually tested using the molecular mass spectrometry method. This was done in order to discover the effectiveness of the compounds actually present in the tested samples. When a single molecular fragment is created, its actual stability level determines whether or not it will actually have a true relative abundance of macromolecules, and whether or not it will have a longer lifespan.

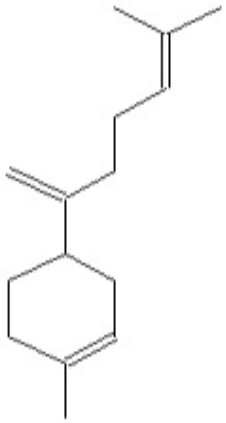
Chromatogram GC-MS examination of the methanol extract of Zingiber officinale revealed the presence of the compounds Longifolene, 2-(4-Nitrobutyryl)cyclooctanone, ß-Bisabolene, 1,1-Diphenyl-4-phenylthiobut-3-en-1-ol, and 1,1-Diphenyl-4-phenylthiobut-3-ol. These compounds were found to l-(+)-Ascorbic acid, Cholestan-3-ol, 2-methylene-,(3ß, 5), and Cholestan-3-ol 2, 6-dihexadecanoate, 9,12-octadecadienoic acid (Z,Z)-methyl ester, 1-heptatriacotanol, 10,13-eicosadienoic acid-methyl ester, E,E,Z-1,3,12-nonadecatriene-5,14- diol, 9-octadecenamide,(Z), and 9-octa-decanamide 2H-Benzo [f] [oxygeneno [2, 3-E] benzofuran-8(9H)-one,9-[2-(di-methylar), phthalic acid, and decyl oct-3-yl ester are the components that make up this compound.



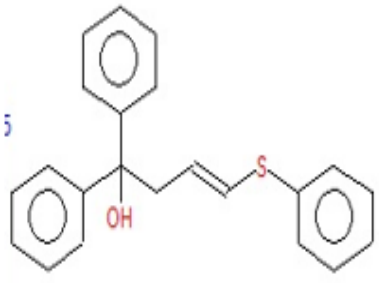
Longifolene
Exact mass: 204.1878
Pharmacological actions : Known anti-nutritional, general anti-inflammatory, antioxidant, anti-tumor



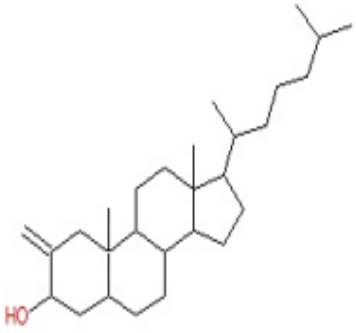
2-(4-Nitrobutyryl)-cyclooctan one
Exact mass: 241.131408
Pharmacological actions : Anti-tumor activity



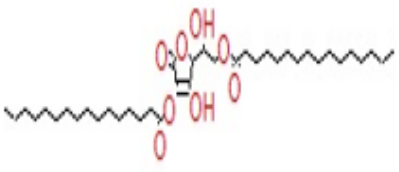
ß-Bisabolene
Exact mass: 204.1878
Pharmacological actions : Anti-ulcer activity



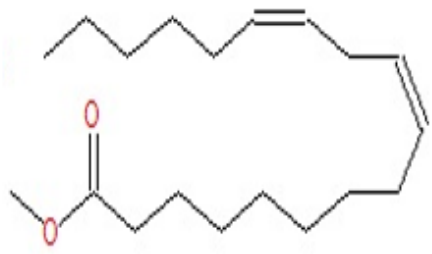
1,1-Diphenyl- 4 – phenyl-thiobut-3-en-1-ol
Exact mass: 332.123486
Pharmacological actions :
Anti-inflammatory properties



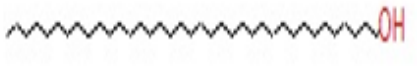
Cholestan-3-ol, 2- methylene-, (3ß,5α)
Exact mass: 400.370516
Pharmacological actions :
Anti-inflammatory properties



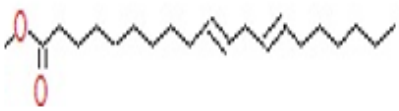
l-(+)-Ascorbic acid 2,6-dihexadecanoate Exact mass: 652.49142
Pharmacological actions: Antioxidant, natural heart protector, cancer preventive, and anti-infertility.



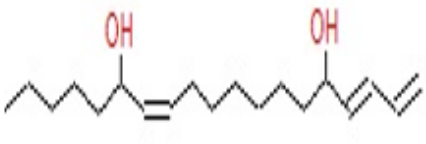
9,12- Octa-decadienoic acid (Z, Z)-, methyl ester
Exact mass: 294.25588
Pharmacological actions: Analgesic, *anti*-inflammatory and ulcerogenic



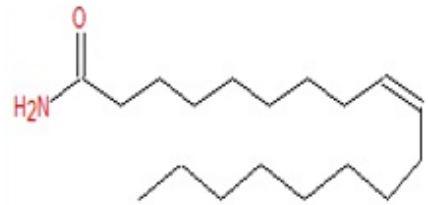
1-Heptatriacotanol
Exact mass: 536.58962
Pharmacological actions: Antioxidant, anticancer, anti-inflammatory and to sex hormone activity



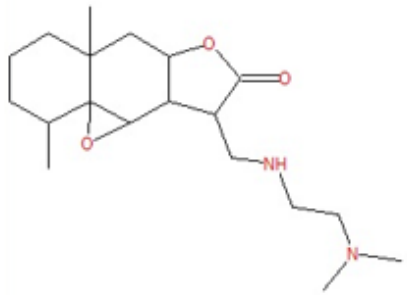
10,13- Eicosadienoic acid, methyl ester
Exact mass: 322.28718
Pharmacological actions :
Anti-bacterial and anti-candidal activities



E,E,Z -1,3,12-Nonadecatriene- 5,14 - diol
Exact mass: 294.25588
Pharmacological actions:
Antimicrobial activity



9-Octadecenamide,(Z)
Exact mass: 281.271864
Pharmacological actions:
Anti-inflammatory activity and antibacterial activity



2H-Benzo[f]oxireno[2,3- E]benzofuran-8(9H)- one,9-[[[2-(dimethylar
Exact mass: 332.123486
Pharmacological actions :
Anti-inflammatory properties

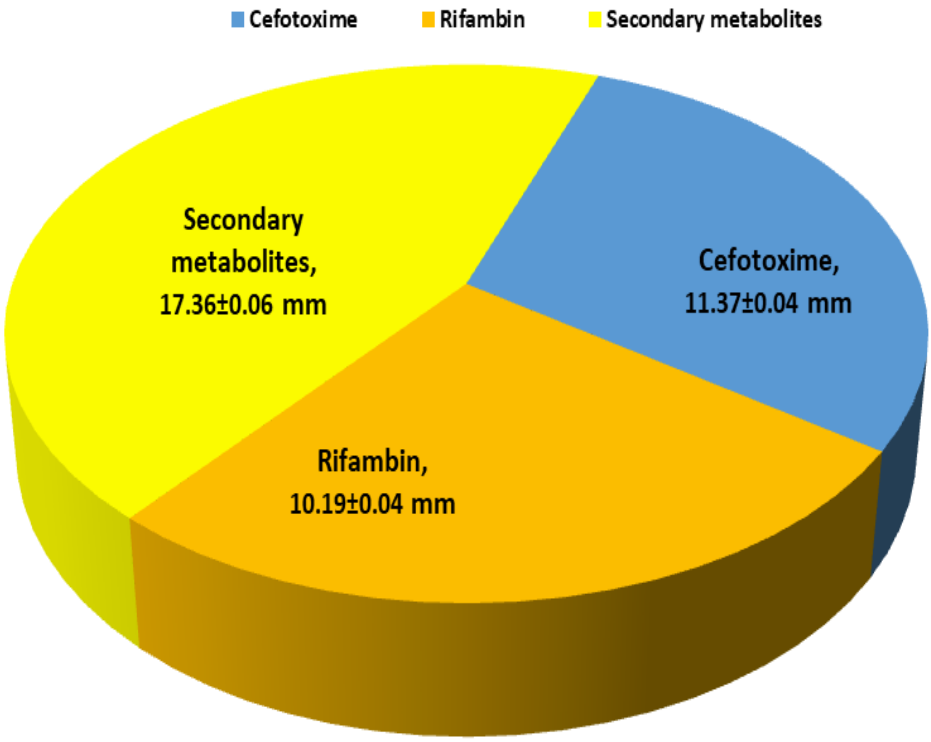


Figure 1. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against Escherichia coli

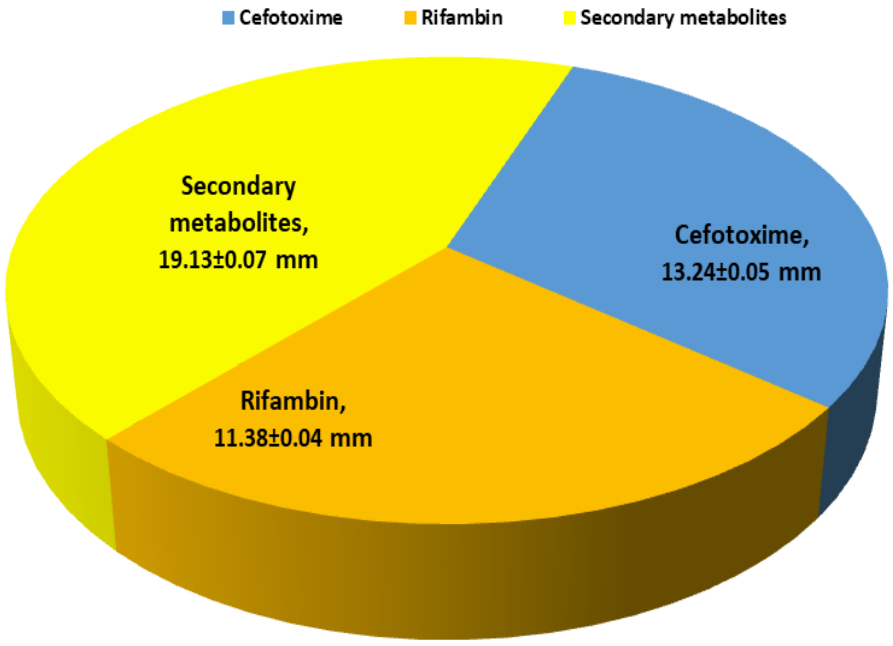


Figure 2. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Proteus mirabilis*

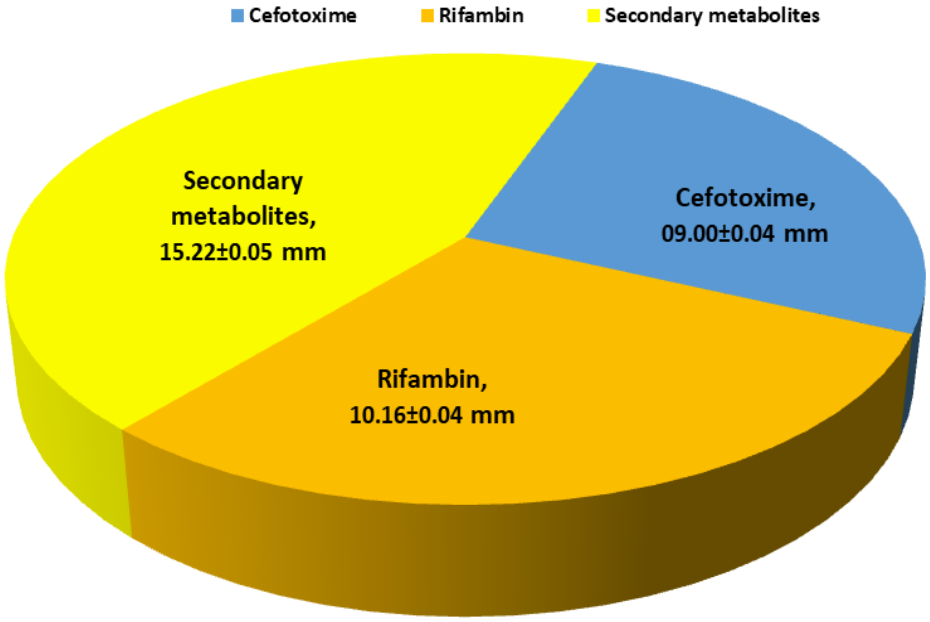


Figure 3. Metabolite products, Rifambin and Cefotoxime as anti- Bacterial activity against *Staphylococcus epidermidis*

There is a well-known belief that plants in general are one of the natural bases for the synthesis and manufacture of effective chemicals that are actually biologically active. In fact, many of these compounds can be used primarily to maintain health and combat known disease disorders, and many of these compounds are marketed and promoted as foods or medicinal herbal medicines. The use of herbal medicine has increased dramatically among the general population for a variety of diseases over the past few years. This is not only due to the ease with which herbal remedies can be obtained without a prescription, the low cost of these remedies, and the availability of appointments with health care specialists [21, 22]; In addition, this is also due to the well-known belief that natural herbal remedies actually have less harmful effects compared to well-known and popular synthetic drugs. There are sterols, triterpenes, tannins, flavanoids, cardiac glycosides,

saponins, volatile oils, and glucosinolates actually present in the ginger plant, according to the results of a qualitative study conducted on this medicinal plant [23-27]. In the current study, Bioactivity of the methanolic extract of *Zingiber officinale* and standard antibiotics Rifambin and Cefotoxime against the five tested pathogens *E. coli* (17.36±0.06, 10.19±0.04 and 11.37±0.04), *Proteus mirabilis* (19.13±0.07, 11.38±0.04 and 13.24±0.05), *Staph. Epidermidis* (15.22±0.05, 10.16±0.04 and 09.00±0.04). Evaluation antibacterial activity found *Zingiber officinale* metabolites was very highly active against *Proteus mirabilis* (19.13±0.07).

Conclusion:

The seed of the Zingiber officinale plant is a promising source for active compounds that have the potential to be used in many clinical contexts as potential therapeutic modalities. However, the severity of the disease should be taken into consideration when evaluating how effective the active components are. It has chemical constituents that may be used in the development of many herbal medicines, including those with anti-inflammatory, analgesic, antipyretic, heart tonic, and antiasthmatic properties.

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