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Chemical Components GC-MS Analysis of Ginger Essential Oil and Antimicrobial Activity against *Escherichia coli*

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Abstract: The main objective of this investigation were to analyses the chemical component evaluate the antibacterial activities of Ginger Essential Oil (GEO) against bla_{TEM} gene positive Extended Spectrum *Beta* Lactamase (ESBL) producing *Escherichia coli* strains isolated from urine samples of patients with Urinary Tract Infections (UTI). Secondary metabolites of GEO were analyzed using Gas Chromatography-Mass Spectroscopy (GC-MS) techniques, then *in vitro* antibacterial activity was studied. Fresh ginger rhizomes contain (0.168) of Essential Oil (EOs) by weight. The main GEO constituents are Zingiberene (18.61%), α-Curcumene (9.91%), β-Sesquiphellandrene (9.25%), Naphthalene (9.09%), 2-Oxabicyclo‹octane,1,3,3-trimethyl (7.17%). The ESBL producing *E. coli* strains can lead to various infections particularly UTI. The inhibition zone of (100%, 50%, 25% and 12.5%) GEO concentration was (14.51, 9.72, 9.18 and 7.95 mm respectively).

Keywords: *Escherichia coli*, ginger essential oil, GC-MS.

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Introduction

Ginger (*Zingiber officinalis* Roscoe) has been and still is one of the world's most cultivated aromatic plants, being recognized by Asians since the first century as a medicinal herb with rapid effects (1). Ginger is a medicinal plant that has been used traditionally in Ayurvedic, Chinese and Tibb- Unani herbal drugs since ancient times. Now-a-days ginger oil is commercially produced from dry or fresh ginger rhizome (2). EOs and their constituents were utilized to treat a large number of human diseases since ancient times. Today, EOs act as an alternative source with its oral, topical and aromatherapy treatment. Extract of ginger was confirmed effective against four test organisms-two drugs resistant and two non-drug resistant bacteria. Thus, it is remarkable to recognize the potential use of the ginger extract in treating infections caused by *Staphylococcus aureus*, *E. coli*, Methicillin Resistant *S. aureus*, and ESBL *E. coli* (3). EOs represent a source of antimicrobial, antioxidants and anticancer components, they are currently attracting increasing interest in the scientific community and there is much research being performed on their

pharmacological activities, particularly their antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (4-11). Analysis of EOs using GC-MS: The EOs was analysed for its chemical composition using GC-MS systems, GC-MS were used for the identification and quantification of chemical constituents in EOs. This study presents a new perspective on the benefits of GEO as a solution with antibacterial action. Using GEO as an agent in the prevention of the exposure to pathogens in public spaces may prove helpful in public health policies (1). The main objective of this investigation were to analyses the chemical components and estimate the antibacterial activities of GEO against bla_{TEM} gene positive ESBL producing *E. coli* strains isolated from urine samples of patients with UTI.

Materials and methods

Isolation and identification of *Escherichia coli*

Bacteria samples were collected in sterilized containers from inpatients that admitted in hospitals in Baghdad in Al-Karkh. The isolates that were obtained from the 100 samples were identified according to the following characters, observed. Growth on EMB agar was used to differentiate between lactose fermenter and non-fermenter microbes for the isolation and detection of Enterobacteriacae from mixed specimens, lactose fermenters metabolize the lactose in the media and give acid byproducts resulting in a color change in the colonies. Weaker fermenter of lactose produced pinkish-purple color while non-lactose fermenters produced colorless colonies (12). Were prepared according to the manufacturing company instruction, the constituents were dissolved in distilled water (D.W), pH was adjusted to 7.2± 0.2 then boiled in water to dissolve all constituents completely. The sterilization of media was done by autoclaving at 121ºC for 15min at 15 pound/inch^2, thereafter distributed into sterile petri dishes, otherwise, the media were incubated at 37 ºC for 24 hours to ensure sterility. Conventional PCR was used for the detection of the *16SrRNA* specific gene for the identification of the *E. coli* shows in Table (1).

Table (1): Listed the sequences of the primers used for conventional PCR to detect *Escherichia coli 16SrRNA* genes.

Name of primer	Sequence '5---3'	Product Size(bp)	Primers Design
***16SrRNA*_F**	CTTAATCGACCATACGCTTTG	**482**	Designed in current study
***16SrRNA*_R**	ATGAATAATCGAGTCCACCAG		

Zingiber officinale (Ginger)

Zingiber officinale (Ginger) fresh rhizomes was bought from market of Baghdad city and classified in the College of Science, Department of Life Biology, University of Baghdad as *Zingiber officinale* Roscoe. The EOs was extracted from the Ginger plant rhizomes using specialized Clevenger device (13). As the result the yield of oil that obtained for every run was calculated by using (1).

$$\text{Essential oil (\%)} = \frac{\text{Amount of essential oil (g) obtained}}{\text{Fresh ginger rhizomes (g) used}} \times 100\ \% \quad (1)$$

An analysis was performed using an Agilent 15977 A Network (GC-MS) System was equipped with an Agilent 7890B Series auto-injector, coupled to an Agilent 5977A Mass Selective Detector. The carrier gas used was He with the flow rate of 40 ml/min with DB 1 as the columns (pressure 8.8085 psi) and Electron Impact (EI) as the ionizer. The sample was heated from 70°C up to 250°C with a heating rate of 10°C/min. The detector and injector temperature was 250°C with the initial time of 1 minute (14).

Antibacterial activity

In this study was determined by agar well diffusion method loop full (1µl) growths from bacterial isolate that were inoculated into nutrient agar then incubated at 37°C for 24 hours. The bacterial suspensions were diluted with normal saline. Adjust the turbidity and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU/ml. Cotton swab was dipped and streak into adjustment suspension the entire Mueller-Hinton agar (for all tested bacteria) surface of plates. Media were cut into five wells (6mm diameter) by corn borer; the oil was diluted with 10 % DMSO. 100µL of GEO were added into wells (The plates were performed in triplicates) at a concentration of 100%, 50%, 25%, and 12.5%. All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 hours of incubation, each plate was noted for zone of inhibition for all isolates. Diameters of inhibitions zone were measured by measuring scale in millimeter (mm) (13, 15).

Results and discussion

Isolation and identification of *Escherichia coli*

The isolates after streaking a loop of lactose fermented colonies on (EMB) agar plates, they were incubated at 37°C for 24 hours. The aniline dyes (Eosin and Methylene Blue) in this medium combined to form a green metallic sheen precipitate at acidic pH, which served as an indicator of lactose acid production (16, 17). Figure 1 shows the metallic appearance of *E. coli* colonies on EMB agar.

Figure (1): Growth of *Escherichia coli* on eosin methylene blue agar at 37°C for 24 hrs.

Molecular Identification of *E. coli* of Detection *16SrRNA* Gene. The results of 71 samples Polymerase Chain Reaction (PCR) were performed for all 71 samples and the PCR result showed 46 samples (64.78%) with a positive

result by using *16SrRNA* detection. Figure 2 show PCR results of *16SrRNA* gene detection (480pb), and this was done to confirm the accuracy of our tests and methods used for identifying of this gene.

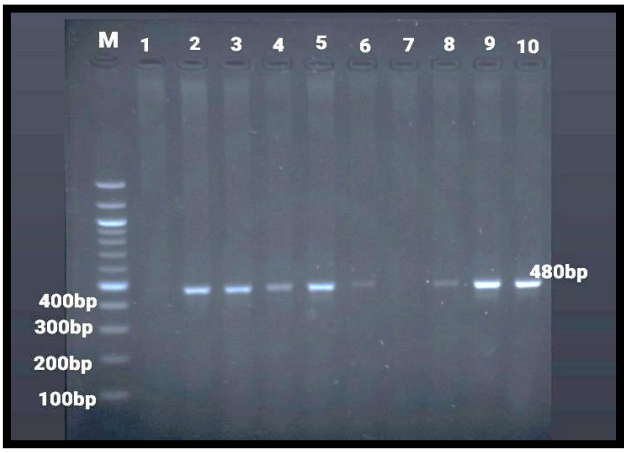

Figure (2): Agarose gel electrophoresis of PCR products of *16SrRNA* gene using 2% agarose at 7V for 1 hr. Lane (M): l00bp ladder, Lane (1- 10): PCR products of *16SrRNA* gene of *Escherichia coli*, Lane 7: Negative result.

Touch-down PCR is modified PCR techniques to increase the specificity of PCR product by using initial primer annealing temperature higher than the optimal temperature. Later, the temperature is reduced gradually until reach the specific one (18). In this study, the optimal annealing temperature of reaction is 57°C. However, the annealing temperature of reaction was started at 67°C and decreased in about 1°C every second cycle until reach the optimum annealing temperature of primer, followed by 15 additional cycles at 57°C. The minimum number of cycles needed during the earlier part of the TD program to eliminate nonspecific priming would be dependent on the efficiency of amplification during high temperature cycling (18).

GC-MS analysis

Some of the compounds present in the GC-MS chromatogram Figure 3. Fresh ginger rhizomes contain (0.168) of EOs by weight. Were identified using the data library of the device, with a match > 72. The 20 identified compounds with retention times and areas under curves are shown in Table 2. Also, the percentage of each identified compound in the GEO composition is reported. When considering the areas under the total curve, the highest percentage belongs to Zingiberene (18.61%), followed by *α*-Curcumene (9.91%), *β*-Sesquiphellandrene (9.25%) and Naphthalene (9.09%).

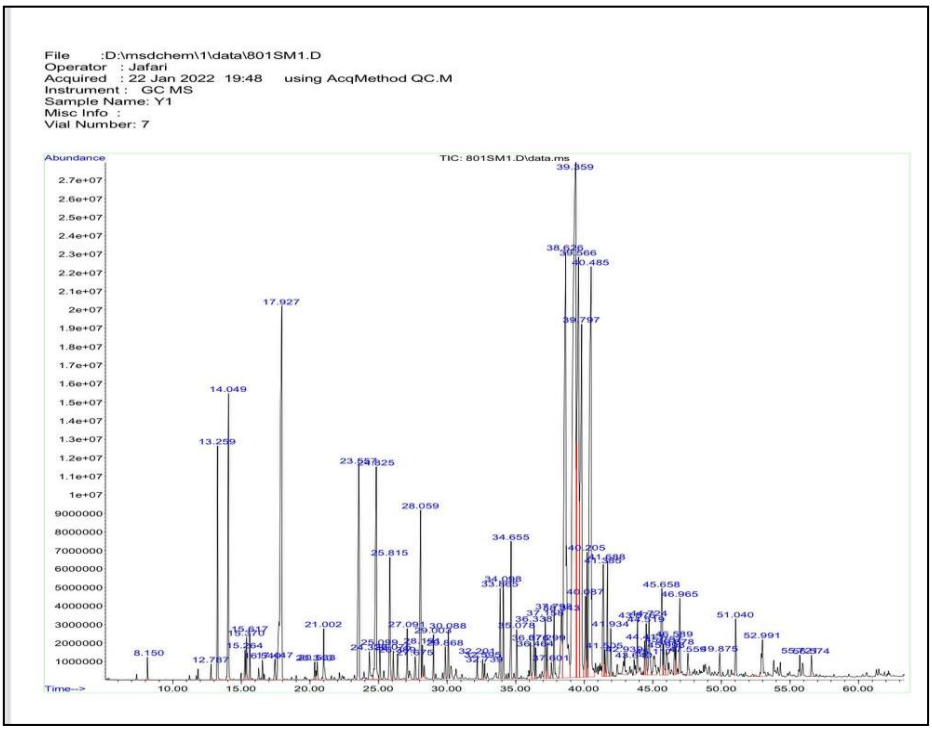

Figure (3): Chromatogram GC-MS of Ginger Essential Oils.

Table (2): Chemical compound of *Ginger* essential oils.

No.	Compound	R. Time	Area%
1	- (-)Zingiberene	39.356	18.61
2	*α*-Curcumene	38.625	9.91
3	*β*-Sesquiphellandrene	40.488	9.25
4	Naphthalene	39.568	9.09
5	2-Oxabicyclo‹ octane, 1,3,3-trimethyl	17.930	7.17
6	Cyclohexene	39.796	6.21
7	Baros camphor	24.822	2.99
8	- (-)Alcanfor	23.559	2.64
9	Camphene	14.049	2.60
10	2-Methyl-3-phenyl-propanal	28.057	1.86
11	Acintene A	13.260	1.83
12	*α*-Terpinyl propionate	25.817	1.32
13	*β*-Eudesmol	45.660	1.29
14	Cyclohexane	34.653	1.28
15	Elemol	41.385	1.26
16	1,7-di-epi-α-Cedrene	38.345	1.14
17	Nerolidol	41.688	0.99
18	*delta*. -Cadinene	40.208	0.95
19	(+)-epi-Bicyclosesquiphellandrene	40.088	0.94
20	1H-Cycloprop[e]azulene, decahydro-	44.723	0.79
21	Other compounds	----------	17.79

The literature highlights the influence of many factors on the quantity and quality of GEO, pedoclimatic conditions, fresh or dry use (19) and extraction methods (20, 21). Mentions the presence of Camphene, Phellandrene, Curcumene, Terpineol, Borneol and Citral in GEO (22), which are compounds that were also found in the sample analysed in the present study. GEO substances act individually or in synergy with one another include but not limited to sesquiterpene compounds like Bisapolene, Zingiberene, Zingiberol, Sesquiphellandrene, Curcurmene, phenolic compounds like Shogaols and Gingerols, and other compounds like 6-dihydrogingerdione, Galanolactone, Gingesulfonic acid, Zingerone, Geraniol, Neral, Monoacyldigalactosylglycerols and Gingerglycolipis (23, 24, 25). May reduce postoperative nausea and vomiting (26).

Antibacterial activity

The results of antibacterial activity of GEO are shown in Figure 4. The antibacterial property its inhibitory activity against ESBL *E. coli*. The utilized the wells method, was utilized after the 24 hours incubation period, for a total of 40 samples it was used based on the presence of beta lactamase genes (contain genes bla_{TEM}) (14,27), qualified as the final study sample (each sample was repeated three times). As expected, higher GEO concentration produced wider zones of inhibition. Based on the result, the 12.5 % GEO has no inhibitory activity against ESBL *E. coli* (7.95 mm), but 25% and 50% GEO has weak to moderate inhibitory activity against ESBL *E.coli* (9.18 and 9.72 mm respectively), the 100 % GEO concentration was against ESBL *E.coli* (14.51 mm). The inhibitory activity of the ginger may be due to the presence of potential active chemical constituents in the rhizome.

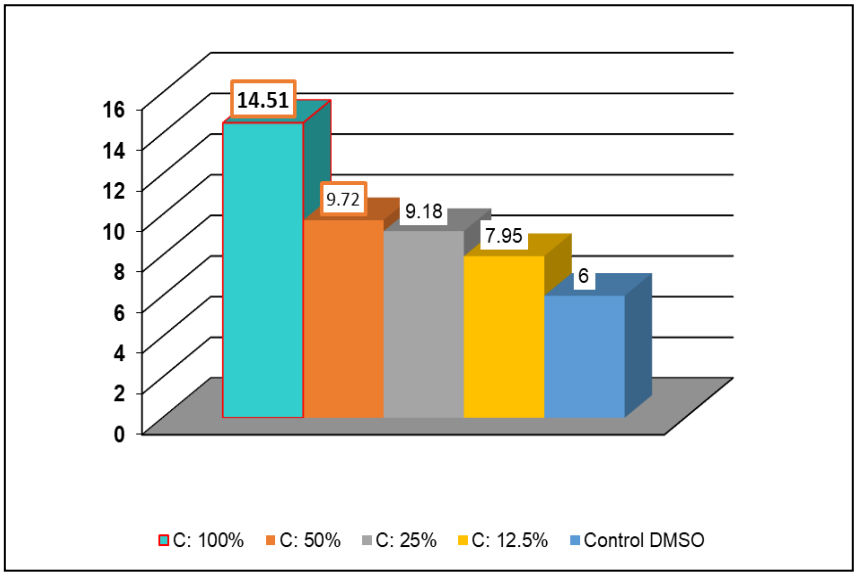

Figure (4): The Antibacterial activity of Ginger Essential Oil against Extended Spectrum *Beta* Lactamases *Escherichia coli*.100 %: (14.51 ± 0.10 a mm), **50%:** (9.72 ± 0.06 b), **25%:** (9.18 ± 0.11 bc), **12.5%:** (7.95 ± 0.07 c), **Control DMSO:** (6.00 ±0.00 d). Means having with the different letters in same column differed significantly. ** (P≤0.01)

Conclusions

GEO showed inhibitory effects against ESBL producing *E. coli* strains which were previously isolated from UTI patients. The results of our investigations may propose a good treatment option against resistant infectious bacteria.

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