

Antimicrobial Activity of Volatile Oils Derived from Ethanolic Garlic Extract

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The aims of this study wereto test the antibacterial activity and chemical composition of various crude extracts of garlic bulbs (*Allium sativum*).The extract was obtained usingorganic solvent ethanol to extractgarlic bulbs.The extract was prepared and evaluated for antimicrobial activity against sixe bacterial strains by determining minimum inhibitory concentration (MIC). The results revealed that theethanolic extract is potent in inhibiting bacterial growth of both gram-positive and gram negativebacteria. The chemical composition of *Allium sativum*was analyzed by gas chromatography/mass spectroscopy (GC/MS).Results of the present study sign the interesting assurance of designing a potentially active antibacterial agent from*Allium sativum*.

Key words: Antimicrobial, *Allium sativum*, Garlic, GC-MS.

Traditional medicinal plants and natural foods has many antimicrobial activities. Even though, the medicinal plants can not considered safer than synthetic antibiotics, herbal antibiotics are considered as a valuable medicinal source by the health care professionals^{1,2}.

Garlic (*Allium sativum*) is one of the most extensively investigated medicinal plants in use since ancient times due to its antibacterial, antifungal, and antiviral properties³. Garlic antimicrobial activities have been recognized for centuries being many of its therapeutic properties first mentioned in 1500 BC in an Egyptian recipe named *Papirus ebers*. Currently, it is used in folk

medicine for the treatment of many diseases⁴, and for the preservation of food products due to its antiseptic and disinfectant properties⁵. Allicin is produced by the enzymatic activity of alliinase (a cysteine sulfoxide lyase) after crushing a garlic clove. There is extensive support that allicin and other thiosulfinates are responsible for the range of remedial effects described for garlic⁶. Garlic extract has been shown to have a wide spectrum inhibitory effect on the growth of various gram-positive and gram-negative bacteria such as: *Micrococcus*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Lactobacilli*, *Pseudomonas*, *Salmonella*, *Shigella*, *Proteus*, *Helicobacter pylori*, *Staphylococcus*, and *Streptococcus* species^{2,6,7}. Moreover, it is also active against multi-drug resistant (MDR) organisms such as *Pseudomonas aeruginosa*, *Klebsiella pneumonia*

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and *Mycobacterium tuberculosis*². Garlic extract mouth wash is effective at reducing total salivary bacterial counts^{7, 8}. Pure garlic extract had more efficient antimicrobial properties than tetracycline against human-caecum bacteria⁹. Garlic also showed to be a strong fungicide agent against *Candida albicans*, which is a fungi usually present in the oral surfaces¹⁰.

This study aimed to evaluate the antimicrobial activity of garlic bulbs (*Allium sativum*) ethanolic extracts and identify the active volatile compounds of *Allium sativum* bulbs extract. To our knowledge this is first report on the study of anti-microbial activity of volatile components derived from garlic bulbs against the human clinical Pathogens. The assessment might provide a basis for searching the potent active compounds for the antimicrobial related search and improve the medicinal application of garlic bulbs.

MATERIALS AND METHODS

Fresh garlic rootless bulbs (*Allium sativum*) were obtained from the local market, Riyadh, Saudi Arabia. The garlic bulbs were cleaned, next dried by oven at 70°C for overnight, then ground to a fine powder using an electric grinder to pass a 0.4 mm screen. All chemical reagents used in this study were of analytical grade.

Preparation of extracts

About 25 g of garlic bulbs powder were soaked in 100 ml ethanol with agitation at 40°C overnight. The extract was filtered and dried over anhydrous sodium sulfate and finally evaporated under steam of nitrogen using sample concentrator model Techne DB.3 (Techne, UK). Ethanol was used in these study as it is less toxic than methanol, and so it could be an interesting solvent for the extraction of polar volatile compounds from garlic bulbs. The yield of the ethanol extract was 3.7 g, the extract was stored at -20°C until further use. Aliquot of the extract was resolved in dimethyl sulfoxide (DMSO) to get a final concentration of 1.0 mg/mL.

Test Microorganisms

Six different bacterial strains were used in this study, including *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and

Salmonella typhi. These Six bacterial strains were obtained from ATCC (American Type and Collection Center). The bacteria rejuvenated in Mueller-Hinton broth MHB (Difco, USA) at 37°C for 18 h and then stocked at 4°C in Mueller-Hinton Agar MHA (Sigma, USA).

Analysis and identification of compounds

The identification of volatile compounds which obtained from the ethanolic of garlic bulbs (*Allium sativum*) extract was carried out by gas chromatography mass spectrometry (GC/MS) series Agilent 7890 with Ion-trap series 240 (Agilent Technologies, USA) (Agilent Technologies, J&W Scientific Products, Palo Alto, CA, USA), equipped with an Agilent Technologies capillary DB-5MS column (30 m length; 0.25 mm i.d.; 0.25 µm film thickness), GC inlet was split/splitless injector used in the splitless mode, inlet temperature was 250°C. The carrier gas was He and was used at 1 mL min⁻¹ flow rate. The oven temperature program was as follows: 2 min at 100°C ramped from 100 to 150°C at 5°C min⁻¹ and 1 min at 200°C. then ramped from 200 to 260°C at 5°C min⁻¹, total run time 40 min. Filament delay, 4 min; ionization voltage 70 eV; emission current 10A; scan rate 1 scan/s; mass range 70–500 m/z; ion source temperature 200°C all Agilent, Santa Clara, CA). Identification of components was assigned by matching their mass spectra with Wiley and NIST library data, standards of the main components and comparing their Kovats Retention Indices (KRI) with reference libraries^{11, 12}. The component concentration was obtained by semi-quantification by peak area integration from GC peaks and by applying the correction factors.

Antibacterial assay

The method reported by Baqir 1985¹³, has been adopted. The tests were run in triplicate. Petri plates (23x23 mm) were prepared with Trypticase soy agar and an adequate amount of inoculum was flooded onto each plate, excess inoculum was removed and the plates were dried for 30 min at 37°C. Holes (6 mm diameter) were made in the inoculated agar and filled with samples of plant extracts, plates were incubated for 24 hrs at 37°C. Inhibition zones when present were measured in millimeter.

Antimicrobial activity assay

The Antimicrobial activities were determined by Kirby Bauer Disc diffusion method described by¹⁴. The extracts were prepared and the sterile blotting paper disc (5 mm) was soaked

in the diluted extract in two different final concentrations (50 μ l and 100 μ l/disc). The prepared disc were dried in controlled temperature (at 37 °C overnight) to remove excess of solvent and used for study.

Determination of Minimum Inhibitory Concentrations (MIC)

The antimicrobial activity of the ethanolic of garlic bulbs (*Allium sativum*) extract, were determined using micro dilution broth method as described by Brantner and Grein, 1994¹⁵. Different antibiotics [Ampicillin, amikacin, gentamicin, kanamycin, and tetracycline (10–32 μ g/ml)] were used as reference standards (CLSI, 2011). The *Ziziphus jujuba* extract solution was prepared to obtain final concentrations of 0.1-10 mg/ml for antibacterial testing. One microliter of an overnight culture of each bacterial strain, containing approximately 10⁴ CFU, was applied onto a 96-well microtiter plate in the presence of MHB. The microtiter plates were incubated at 35°C for 18 h. Observations were performed at least in replicate and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth, MIC.

RESULTS AND DISCUSSION

With the increase in the incidence of resistance to antibiotics, alternative natural products of plants could be of interest. Some plant extracts and phytochemicals are known to have antimicrobial properties, which could be of great importance in the therapeutic treatments. In the last years, various studies have been conducted in different countries, demonstrating the efficacy of this type of treatment¹⁶. The mean of minimum

inhibition concentrations (MIC) of these volatile compounds which derived from ethanolic of garlic bulbs (*Allium sativum*) extract against certain of pathogenic bacteria strains at 24 and 48 hours were presented in Table 1.

The MIC of ginger extract against the test isolates ranged from 0.17-4.5 mg/mL, and from 0.17–6.0 for 24 and 48 hours, respectively. The MIC results of garlic bulbs extract was gotten from the extrapolation diameter zone of inhibition of the concentration.

Each MIC determination was performed in triplicate per bacterial isolate

A side from concerns with food quality degradation, these microorganisms may be causal agents of intestinal infections in humans. According to the values of microbial growth rate in the presence of different extract concentrations garlic bulbs (*Allium sativum*) extract was presented antimicrobial capacity following the order: *Bacillus subtilis* > *Listeria monocytogenes* > *Klebsiella peunomoniam* > *Proteus vulgaris* > *Escherechia coli* > *Pseudomonas aeruginosa* were the most sensitive microorganisms even at lower concentration. *Pseudomonas aeruginosa* was the most resistant microorganism even at higher concentration.

The MIC as low as μ g mL⁻¹ of a semi-purified fraction against gram negative and positive bacteria is suggestive of good antibacterial potential of the volatile compounds extracted from garlic bulbs. Hence *Allium sativum* may yield potential molecules in the treatment of infections caused by pathogenic bacteria which have developed resistance against the known antibiotics, Singleton, 1999¹⁷.

Table 1. Mean 24-h MICs of ethanolic garlic bulbs (*Allium sativum*)

Enteric isolate	24 h MIC (mg/ml)	84 h MIC (mg/ml)
<i>Bacillus subtilis</i>	0.17	0.17–0.68
<i>Proteus vulgaris</i>	1.37	2.74
<i>Escherechia coli</i>	2.75	5.5
<i>Pseudomonas aeruginosa</i>	4.5	6.0
<i>Klebsiella peunomoniam</i>	0.5	1.35
<i>Listeria monocytogenes</i>	0.2	0.8

Extract against six pathogenic bacteria.

Table 2. Identified compounds of ethanolic extract of garlic bulbs (*Allium sativum*)

#	Retention Time (min.)	Name	Chemical structure	MW	% of Total
1	10.467	Vanillin lactoside	$C_{20}H_{28}O_{13}$	476	0.836
2	12.392	α -Curcumene	$C_{15}H_{22}$	202	4.334
3	12.736	α -Bergamotene	$C_{15}H_{24}$	204	2.416
4	13.514	β -Sesquiphellandrene	$C_{15}H_{24}$	204	1.924
5	16.322	β -Guaiene	$C_{15}H_{24}$	204	1.081
6	16.525	Gingerone	$C_{11}H_{14}O_3$	194	17.087
7	16.907	β -Eudesmol	$C_{15}H_{26}O$	222	1.576
8	17.799	Methyl 2,5-octadecadiynoate	$C_{19}H_{30}O_2$	290	3.063
9	18.974	1,2-Methyl-5-(1,2,2-trimethylcyclopentyl)phenol	$C_{15}H_{22}O$	218	1.59
10	20.363	cis-Z- α -Bisabolene epoxide	$C_{15}H_{24}O$	220	3.863
11	21.574	Longipinocarveol	$C_{15}H_{24}O$	220	1.742
12	22.122	Spiro[4.5]decan-7-one, 1,8-dimethyl-8,9-epoxy-4-isopropyl-	$C_{15}H_{24}O_2$	236	1.009
13	26.765	1,3,3,6-Trimethyl-4,5-heptadien-2-one	$C_{10}H_{16}O$	152	3.163
14	27.48	geranyl- α -terpinene	$C_{20}H_{32}$	272	0.973
15	29.547	Gingerol	$C_{17}H_{26}O_4$	294	42.391
16	28.682	[6]-Paradol	$C_{17}H_{26}O_3$	278	3.091
17	29.938	Benzenepropanamide, 3,4-dimethoxy-N-(5-methyl-3-isoxazolyl)-	$C_{15}H_{18}N_2O_4$	290	0.737
18	33.098	6-(3,5-Dimethyl-furan-2-yl)-6-methyl-hept-3-en-2-one	$C_{14}H_{20}O_2$	220	3.797
19	33.695	Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390	5.328

Chemical composition of *Ziziphus jujuba* seeds extract

Fig. 1 presented the typical GC/MS chromatogram of a total of 20 compounds were recorded in solvent extracts as indicated in Table 4. Most of these identified compounds are playing a role in the biological activity of natural extracts. Some of these compounds are reported for the first

time in *Ziziphus jujuba* seeds. The typical GC-MS chromatogram of volatile compound derived from ethanolic of garlic bulbs extract is given in Fig. 1.

The highest compounds were identified in garlic bulbs (*Allium sativum*) extract given in Table 2 and Fig. 2. 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- (17.08%), is phenolic compound and one of the major compounds. Phenolic

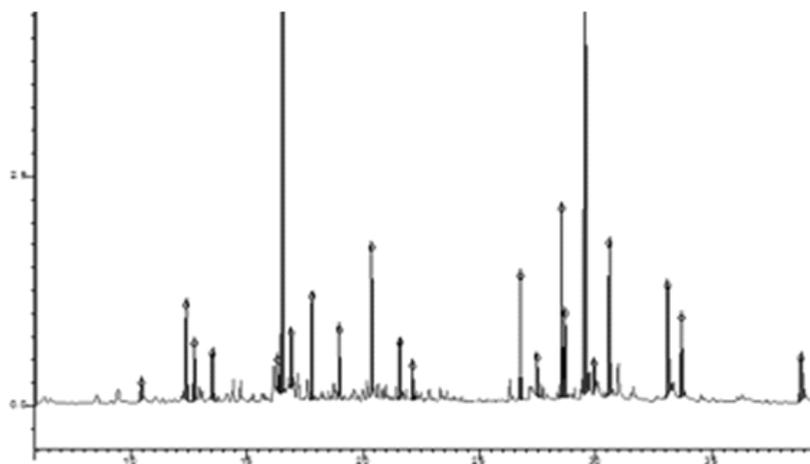


Fig. 1. Typical GC-MS chromatogram of volatile compound derived from ethanolic of garlic bulbs extract

compounds were found to inhibit the cell growth and fermentation and used as antioxidant^{12,17}. Furthermore, its derivatives have also been used for therapeutic purposes. For instance, Hydroxymethylfurfural is a potential candidate for treating sickle cell anemia¹¹. Gingerol, has been found to possess many interesting pharmacological and physiological activities, such as anti-inflammatory, analgesic, and cardiotoxic effects. Gingerone results from the thermal degradation of gingerols during extraction¹⁸. Gingerol (42.39%), 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl) (17.08%), also play a role in the activity of garlic bulbs (*Allium sativum*) extracts. While for the first

time we identified diisooctyl phthalate (5.32%), and Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (4.33%) (4.81%) in garlic bulbs (*Allium sativum*) extracts. Garlic bulbs extract were found to contain small amounts of other compounds, this in line with other investigators¹⁹.

The highest abundant compounds were identified in the ethanolic extract of garlic bulbs (*Allium sativum*), were Gingerol (42.39%), 2-Butanone, 4-(4-hydroxy-3-methoxyphenyl)- (17.08%), Diisooctyl phthalate (5.32%), and Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl- (4.33%) (Fig. 2).

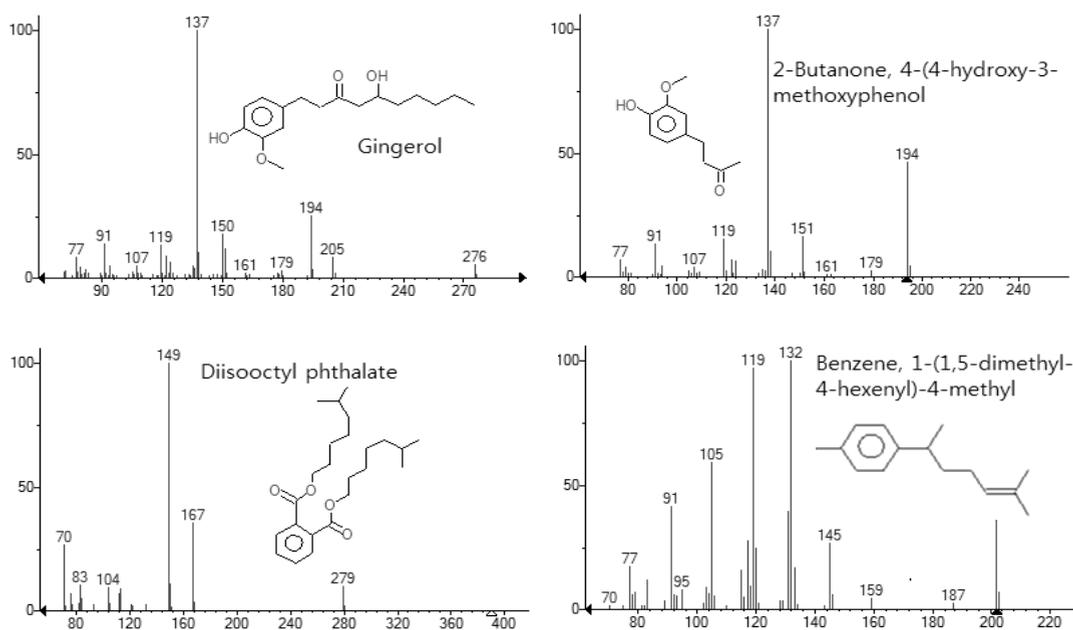


Fig. 2. Chemical structure of the highest abundant compounds were identified in the ethanolic extract of garlic bulbs (*Allium sativum*)

CONCLUSION

In conclusion, our study was one of very few studies have confirmed that the antimicrobial activity of garlic bulbs (*Allium sativum*) extract against certain microorganisms. Results of this study showed that the have found for the first time that garlic bulbs extracts are effective in inhibiting the growth of *Listeria monocytogenes*, *Escherechia coli*, *Pseudomonas aeruginosa*,

Klebsiella pneumoniae and *Listeria monocytogenes*. Our data provide a strong rational base for the use in Traditional Chinese Medicine of garlic bulbs extracts in the treatment of many diseases. Moreover, our results highlight that *Allium sativum* are valuable bulbs rich in bioactive compounds with potential human health benefits. More experiments are in progress to understand the molecular targets and pathways affected by *Allium sativum*.

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