ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 11, Issue 4, 2018

Online - 2455-3891
Print - 0974-2441

Research Article

ANIBACTERIAL EFFICACY AND GAS CHROMATOGRAPHY-MASS SPECTROMETERY ANALYSIS OF BIOACTIVE COMPOUNDS PRESENT IN DIFFERENT EXTRACTS OF *ALLIUM SATIVUM*

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Received: 01 December 2017, Revised and Accepted: 10 January 2018

ABSTRACT

Objective: Medicinal plants are rich libraries containing wide variety of compounds of therapeutic values. *Allium sativum* commonly known as garlic is a very well-known medicinal plants being used with food products. In the present study, the antibacterial activity of different extracts of *A. sativum* was investigated along with their phytochemical analysis by gas chromatography-mass spectrometery (GC-MS) to explore antimicrobial compounds present in extracts.

Methods: The antibacterial activity of *A. sativum* was evaluated against 9 reference bacterial strains and 3 MDR bacterial strains including *Escherichia coli* MDREC1, *Klebsiella pneumoniae* MDRKP2, and *Pseudomonas aeruginosa* MDRPA3 by microbroth dilution and agar well diffusion method.

Results: The results obtained from agar well diffusion assay showed the zone of inhibition from 12 to 26 mm for different extracts. The methanol and acetone extracts were found most potent against reference and MDR bacterial strains. MIC values were in the range of 1.87–7.5 mg/ml. Further, GCMS analysis confirmed the presence of 35 compounds including dodecanoic acid, hexadecanoic acid, and methyl ester in common.

Conclusion: The varied antimicrobial activity of extracts was due to the presence of different concentrations of the identified compounds which can be isolated and used for the treatment of various infectious diseases caused by MDR strains of *E. coli*, *P. aeruginosa,* and *K. pneumoniae*.

Keywords: Antibacterial activity, *Allium sativum*, Gas chromatography-mass spectrometery, Bioactive compound, MDR Bacterial strains.

 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i4.24053

INTRODUCTION

Microbial infections are the main cause of morbidity and mortality in developed as well as developing countries, even though a large number of antimicrobial compounds are available for the treatment and management of these diseases. Overexploitation and indiscriminate use of the antibiotics can lead to the development of drug resistance and it is one of the major health problems [1]. Thus, there is an urgent need of the natural products which can be used for the treatment of various infectious diseases.

Plants serve as a source of various effective and powerful drugs right from the time of human civilization. The herbal medicine has a long history in the treatment of several diseases and has been widely spread all around the world [2]. The plant products have played a significant role in the history of drug discovery [3]. It is belief that natural substances have fewer side effects than synthetic pharmaceuticals and are easily available from the surroundings [4]. Herbal medicines are in great demand both in developed and developing countries for primary health care. Today also, many rural areas of developing countries are depend on traditional medicine for their primary health care [5]. India is one of the oldest, richest, and most diverse culturals traditionally associated with the use of medicinal plants [6].

Alliaceae is an important medicinal plant family and the genus *Allium* has over 700 members with special tastes, forms, and colors [7]. Species in the Alliaceae family are perennial herbs. The leaves grow from a bulb at the base of the plant. The leaves vary from flat to circular in cross-section and may be hollow, with parallel veins running lengthwise along the blade. The family was formerly included in a broadly defined Liliaceae. Plants of this family are widely distributed in temperate, tropical, and semiarid regions. Common examples of this family are onion, garlic, and chives.

Allium sativum is a species of Allium commonly known as garlic, which is being used as a spicy flavoring agent in food as well as folk medicine across the globe since centuries [8]. *A. sativum* is a hardy, bulbous, perennial plant, 1.2 m in height native to Mediterranean regions of Africa and Europe. The most commonly used part of *A. sativum* is its bulb. This plant contains carbohydrates, reducing sugars, lipids, flavonoids, ketones, alkaloids, steroids, and triterpenes [9]. It is a remarkable plant, reported to have various pharmacological activities such as antimicrobial, antithrombotic, hypolipidemic, antiarthritic, hypoglycemic, and antitumor activity [10,11]. The characteristic pungent smell of garlic is due to the presence of allicin which is also responsible for the antibacterial activity. Many of the health benefits associated with garlic consumption have been attributed to the thiosulfinates, the most abundant class of organosulfur compounds, found in freshly chopped or crushed garlic. Oil-macerated heated garlic products contained mainly vinyldithiins, ajoene, and small amount of sulfides [12,13].

Keeping these aspects in view, the efforts were made for better understanding of qualitative, quantitative chemical composition by gas chromatography-mass spectroscopy (GC-MS) analysis and to explore the antibacterial activity of organic solvent extracts of *A. sativum.*

METHODS

Preparation of plant extracts

Garlic (*A. sativum*) bulbs were purchased from the local market of Rohtak (28.8909°N and 76.5796°E), Haryana, India. The bulbs were peeled off, followed by washing and subjected to shade dry. The dried material was grinded in electrical grinder to obtain powder form. Six organic solvents, i.e. methanol, acetone, chloroform, ethyl acetate, petroleum ether, and benzene were used for extraction in 1:10 ratio by cold percolation for 48–72 h (50 g dried powder). The obtained extracts were filtered using Whatman No. 1 filter paper and then concentrated

using rotatory evaporator at 40°C. The total yield of the extracts was measured and % age of extracted value was calculated by the following formula: Quantity of extract obtained× 100/weight of initial quantity of dried extract.

Antibacterial activity

The antibacterial assay was carried out using agar well diffusion against 9 reference strains, namely, (1) *Shigella flexneri* ATCC 12022, (2) *Enterococcus faecalis* ATCC 29212, (3) *Staphylococcus aureus* ATCC 259323, (4) *Proteus mirabilis* ATCC 43071, (5) *Salmonella typhi* ATCC 13311, (6) *Serratia marcescens* ATCC 27137, (7) *Klebsiella pneumoniae* ATCC 700603, (8) *Escherichia coli* ATCC 25922, and (9) *Pseudomonas aeruginosa* ATCC 27853 and three MDR bacterial strains, namely, (1) *E. coli* MDREC1, (2) *K. pneumoniae* MDRKP2, and (3) *P. aeruginosa* MDRPA3 [14]. A sterile wire-loop was used to pick up the isolated colonies of tested bacteria and to emulsify into 10ml of peptone water solution. Turbidity of the suspension of test organism was compared with McFarland turbidity standard. Using a sterile swab stick, the test organisms were spreaded on autoclaved solidified nutrient agar media. The inoculated plates were then allowed to solidify. The wells were made using borer of 6 mm diameter. In each well, from a stock solution of 100 mg/ml, the 20, 40, 60, and 80 µl of plant extracts were poured. The Petri plates were wrapped with parafilm to avoid the environmental contamination and incubated at 37°C for 24 h. Streptomycin discs of 10 µg (HiMedia Laboratories Pvt. Ltd. India) were used as a positive control and diluted dimethyl sulfoxide (DMSO) was used as a negative control. After incubation, the plates were examined for zones of inhibitions. The zones of inhibition were measured in mm using a plastic ruler HiAntibiotic ZoneScaleTM-C (HiMedia Laboratories Pvt. Ltd., India). All the experiments were performed in triplicates, and their mean and standard deviation was calculated for further statistical analysis using MS Excel program.

Minimum inhibitory concentration (MIC)

MIC is the lowest concentration of an antimicrobial compound able to inhibit the observable growth of a microorganism after overnight incubation. MIC for different extracts against different tested bacterial strains was determined in 96 multi-well microtiter plates using the method of Sarker *et al*. with slight modifications, and the plates were prepared in triplicate and incubated at 37°C for 18–24 h [15]. The resazurin dye was used as indicator and color changes from purple to pink or to colorless indicated growth of microbes. The lowest concentration at which no color change occurred was recorded as the MIC value of that extract. The streptomycin was taken as a positive control and diluted DMSO was taken as a negative control for antimicrobial susceptibility test of extracts.

GCMS analysis

Phytochemical analysis of all six extracts of *A. sativum* was carried out using GCMS analyzer (BRUKER SCION 436-GC SQ). Extracts were dissolved in methanol (high-performance liquid chromatography grade) and filtered through Whatman™ FILTER DEVICE (0.2 µm). Helium (99.99%) was used as carrier gas, at a flow rate of 1 ml/min in split mode. RESTEK Rtx®-5 (Crossbond® 5% diphenyl/95% dimethyl polysiloxane) with 30 m length, 0.25 µm df, and 0.25 mm ID column was used for separation of phytochemicals. 2 µL of sample was injected to column. The injector temperature was 280°C. The temperature of oven starts at 70°C and hold for 2 min, and then, it was raised at a rate of 7°C per minute up to 320°C, hold for 1 min. Temperature of ion sources was maintained at 250°C. The mass spectrum was obtained by electron ionization at 70eV and detector operates in scan mode 30–500 Da atomic units. Total running time was 38.71 min including 3 min solvent delay.

RESULTS

Yield of extracts

The total yield of extracts is shown in Table 1. Maximum yield 13.70% was obtained in methanol extract.

Antibacterial activity

Antibacterial activity of extracts of *A. sativum* was investigated against nine reference bacterial strains and 3 MDR bacterial strains at different concentrations by agar well diffusion assay. Results obtained in agar well diffusion assay are shown in Table 2. All extracts except benzene showed considerable antibacterial activity with the diameter of inhibition zones ranging from 10 to 26 mm against different bacteria used in the study. Methanol and acetone extracts of *A. sativum* exhibited antibacterial activity against wide range of bacterial strains based on the study included MDR strains of *E. coli*, *P. aeruginosa,* and *K. pneumoniae*. However, methanol extract showed higher antibacterial activity as compared to other extracts. Methanol extract of *A. sativum* showed concentration-dependent activity. The highest dose of 8 mg/ml was found to be most effective. Highest zones of inhibition were reported of 26 and 23 mm against *S. flexneri* and *S. marcescens,* respectively. Similarly, acetone extract showed 24 and 22 mm zone of inhibition against *S. flexneri* and *S. marcescens,* respectively. In case of MDR strains, the acetone extract was reported to have the highest activity against *E. coli* MDREC1 and *K. pneumonia* MDRKP2 with 24 and 20 mm zone of inhibition, respectively, while methanol extract was reported to have the highest activity against *P. aeruginosa* MDRPA3 with a zone of inhibition of 17 mm.

MIC

MIC values of different extract for tested bacteria have been shown in Table 3. Outcomes of the study showed that MIC values of *A. sativm* extracts were found to be in a range of 1.87 mg/ml to 7.50 mg/ml. A good correlation of antibacterial activity in agar well diffusion assay and microbroth dilution has been observed. It was found that methanol extract inhibited the total growth total growth of *S. flexneri* at a concentration of 1.87 mg/ml that is well correlated to agar well diffusion assay where is formed a largest zone of inhibition.

In case of MDR strains, acetone extract was found to have better activity as compared to other extract. MIC values of acetone extracts were to be in the range of 1.87–3.75 mg/ml that is well correlated with the results of agar well diffusion assay.

GCMS analysis

A total number of 35 organic compounds were identified by comparing the GCMS spectra result with the NIST MS library. The list of compounds identified from different extracts, i.e. methanol, acetone, ethyl acetate, chloroform, petroleum ether, and benzene is given in Table 4. The major compounds identified by matching the spectra with NIST library were dodecanoic acid, hexadecanoic acid, methyl ester, di (2-ethylhexyl) adipate, 9-dodecanoic acid-methyl ester, and methyl stearate. The spectra of the GCMS are given in Figs. 1-6.

DISCUSSION

The rising population is the reason to worry about various health problems due to which the production of natural antimicrobial drugs is being used to control the various infectious diseases. Medicinal plants and spices are generally used natural antimicrobial agents in foods and have been used conventionally for thousands of years by many cultures for controlling common health problems. The secondary metabolites isolated from the plants have gained very high importance due to their effectiveness against multidrug-resistant microbes.

Table 1: Yield of extracts and % age of extracted values in different solvents

Solvents	Yield of extracts (g)	%age of extracted value
Methanol	6.85	13.70
Acetone	5.0	10
Ethyl acetate	3.30	6.6
Chloroform	1.28	2.56
Benzene	0.925	1.85
Petroleum ether	1.20	2.4

A. sativum is traditionally being used as dietary and anti-infective agent [16]. Several *in vitro* studies support its antibacterial, antifungal, and antiviral properties [17,18]. The antimicrobial activity of garlic has been reported due to the presence of phenolic and organosulfur compounds.

It is believed that the secondary metabolite of plants, i.e., tannins, saponins, phenolic compounds, essential oils, and flavonoids is responsible for their bioactive potential [19]. The crude extracts from several plants have been reported for their antibacterial activity against MDR bacterial strains where modern antibiotic therapies have many side effects. In this study, *in vitro* efficacy of *A. sativum* extracts on reference strains and MDR strains of bacteria organisms have been analyzed. The agar well diffusion assay is a practical approach to study the efficacy of compounds against bacteria, and by measuring the size of inhibition zone, it is not an adequate method because zone of inhibition might be affected by the solubility, volatization, and rate of diffusion in agar medium or its volatilization, and thus, the results could be affected. Hence, other methods to evaluate antibacterial activity of extracts should be used parallel as the microbroth dilution method confirms this in the present study with a good correlation.

In the previous study, the antibacterial activity was evaluated of ethanol extract of *A. sativum* against MDR clinical isolates of *E. coli*, *Enterobacter* sp., *P. aeruginosa*, *Proteus* sp., *Klebsiella* sp., *S. aureus,* and *Bacillus* sp. and the inhibition zone reported was 18.50,13.50, 19.45, 13.65, 11.50, 14.55, and 16.55 mm, respectively [20]. In the present study, the antibacterial activity of methanol extract of *A. sativum* was maximum against reference bacterial strains *S. flexneri*, *E. faecalis,* and *S. marcescens* with the inhibition zone of 26, 22, and 23 mm, respectively, while in case of MDR strains, the acetone extract was found most active against MDR *E. coli* MDREC1 and MDR *K. pneumonia* MDRKP2 with the inhibition zone of 24 and 20 mm, respectively. This higher zone of inhibition may be due to the climatic and geographical conditions from where the plant has been collected and the used concentration in that study was 200 µg/ml. The methanolic extract of *A. sativum* also shows good activity against the MDR strain of *P. aeruginosa* MDRPA3 with inhibition zone of 17 mm.

It has been reported that ethanol and aqueous extracts of *A. sativum* showed antibacterial activity against methicillin-resistant *S. aureus* and penicillin-susceptible *S. aureus* strains with the MIC value of 2 mg/ml, while in case of *in vivo* study, the extracts of garlic do not inhibit or kill the bacteria significantly [21]. In our study also, the methanol, acetone, chloroform, and petroleum ether extracts of *A. sativum* show the inhibition of *S. aureus* with the inhibition zone of 17, 13, 12, and 12 mm, respectively, but MIC value of these extracts was 7.5 mg/ml which is higher than the above study which may be due to the use of pepton water medium in spite of Muller-Hinton broth.

Rath and Padhy tested a total of 26 Indian spices against nine MDR bacterial strains which infects urinary tract and *A. sativum* was found active against 6 bacterial strains, namely, *S. aureus*, *Acinetobacter baumannii*, *Citrobacter freundii*, *Enterobacter aerogenes*, *K. pneumoniae*, and *P. mirabilis*, with the inhibition zone of 17, 17, 18, 22, 17, and 21, respectively, and inactive against remaining three bacterial strain, namely, *E. faecalis*, *E. coli,* and *P. aeruginosa* which is contradictory to this study as the methanol and acetone extracts of *A. sativum* showed good activity against *E. coli* and *P. aeruginosa* which may be due to the difference in solvent used for extraction and the methods used for the analysis of antibacterial activity [22].

Many phytochemical analysis studies have been carried out on *A. sativum* and the different classes of compounds reported in these studies were

Table 2: Zone of inhibition (mm) of different extracts of *A. sativum* against reference and MDR bacterial strains

Bacterial strain	Methanol	Acetone	Ethyl acetate	Chloroform	Petroleum ether	Benzene	Streptomycin (10 µg disc)
S. flexneri	26±0.76*	24±1.00	15±0.57	-	16±0.57	-	24±0.57
E. feacalis	22±0.57	20±1.00	12±0.76	-	-	-	24±0.57
S. aureus	17±0.76	13±0.76	-	12±0.57	12±1.00	-	23±0.76
P. mirabilis	16±0.57	10±1.00	19±1.00	10±0.76	-	-	23±1.00
S. typhi	14±1.00	15±0.57	-	-	15±1.00	-	20±1.00
S. marcescens	23±0.76	22±0.76	20±1.00	-	18±0.76	-	20±0.57
K. pneumoniae	19±0.76	20±0.57	20±1.00	15±1.00	-	-	19±0.57
E. coli	18±0.76	17±0.57	-	-	17±1.00	-	18±0.57
P. aeruginosa	20±0.57	17±1.00	15±0.57	-	12±1.00	-	25±0.76
E. coli MDREC1	15±0.76	24±1.00	15±0.57	-	16±0.57	-	20±0.57
K. pneumoniae MDRKP2	16±0.57	20±1.00	12±0.76	-	-	-	19±0.57
P. aeruginosa MDRPA3	17±0.76	13±0.57	-	12±1.00	12±1.00	-	20±0.76

*The zone of inhibition showed as mean±standard deviation (n=3), S. *flexneri: Shigella flexneri, E. faecalis: Enterococcus faecalis, S. aureus: Staphylococcus aureus, P. mirabilis: Proteus mirabilis, S. typhi: Salmonella typhi, S. marcescens: Serratia marcescens, P. aeruginosa: Pseudomonas aeruginosa, K. pneumonia: Klebsiella pneumonia, E. coli: Escherichia coli*

Table 3: MIC values (mg) of different extracts of *A. sativum* against different reference bacterial strains and MDR strains

Bacterial strain	Methanol	Acetone	Ethyl acetate	Chloroform	Petroleum ether	Benzene
S. flexneri	1.87	1.87	7.5	-	3.75	-
E. feacalis	1.87	3.75	7.5	-	-	-
S. aureus	7.5	7.5	-	7.5	7.5	-
P. mirabilis	7.5	7.5	3.75	7.5	-	-
S. typhi	7.5	7.5		-	7.5	-
S. marcescens	3.75	3.75	1.87	-	3.75	-
K. pneumoniae	3.75	3.75	1.87	3.75	-	-
E. coli	3.75	3.75	-	-	3.75	-
P. aeruginosa	3.75	7.5	3.75	-	7.5	-
E. coli MDREC1	7.5	1.87	3.75	-	3.75	-
K. pneumoniae MDRKP2	3.75	1.87	7.5	-	-	-
P. aeruginosa MDRPA3	3.75	3.75		7.5	7.5	-

The MIC values are showed as mean (n=3), *S. flexneri: Shigella flexneri, E. faecalis: Enterococcus faecalis, S. aureus: Staphylococcus aureus, P. mirabilis: Proteus mirabilis, S. typhi: Salmonella typhi, S. marcescens: Serratia marcescens, P. aeruginosa: Pseudomonas aeruginosa, K. pneumoniae: Klebsiella pneumoniae, E. coli: Escherichia coli*

Table 4: Bioactive compounds of six extracts of *A. sativum* screened by GCMS analysis with area and % of total

Name of compound	Area						% of total						Formula
	ASM	ASA	ASEA	ASC	ASPE	ASB	ASM	ASA	ASEA	ASC	ASPE	ASB	
Dodecanoic acid	1.275e+11	1.501e+9	5.526e+7	5.054e+7	5.414 e+7	-	45.306	32.715	5.788	4.240	11.665	-	$C_{12}H_{24}O_2$
Tetradecanoic acid	4.170e+10	4.324e+8	-	-	9.456 e+6	-	14.822	9.425	-	-	2.037	-	$C_{14}H_{28}O_2$
Pentadecanoic acid	3.586e+8	-	-	-	-	-	0.127	-	-	-	-	-	$C_{15}H_{30}O_2$
Hexadecanoic acid, methyl ester	5.592e+8	1.896e+8	2.492e+8	2.118e+8	9.335 e+7	2.949e+8	0.199	4.132	26.103	17.768	20.110	10.848	$C_{17}H_{34}O_2$
n-Hexadecanoic acid	3.647e+10	3.144e+8	-	-	-	-	12.963	6.853	-	-	-	-	$C_{16}H_{32}O_2$
9-Octadecenoic acid (Z)-, methyl ester	5.694e+8	-	-	-	-	-	0.202	-	-	-	-	-	$C_{19}H_{36}O_2$
cis-Vaccenic acid	1.649e+10	-	-	-	-	-	5.862	-	-	-	-	-	$C_{18}H_{34}O_2$
Octadecanoic acid	1.625e+9	-	-	-	-	-	0.578	-	-	-	-	-	$C_{18}H_{36}O_2$
Oleanitrile	4.375e+8	1.153e+8	-	-	-	7.437e+8	0.155	2.512	-	-	-	27.359	$C_{18}H_{33}N$
Bis (2-ethylhexyl) phthalate	2.738e+8	1.286e+8	2.242e+7	2.192e+7	2.366 e+7	-	0.097	2.803	2.541	1.839	5.097	-	$C_{24}H_{38}O_4$
13-Docosenamide	5.485e+10	-	-	-	-	-	19.498	-	-	-	-	-	$C_{22}H_{43}NO$
Lauric anhydride	5.349e+8	-	-	-	-	-	0.190	-	-	-	-	-	$C_{24}H_{46}O_3$
Dodecanoic acid, methyl ester	-	1.550e+8	8.806e+7	9.024e+7	7.480e+7	-	-	1.378	8.469	7.571	16.115	-	$C_{13}H_{26}O_2$
Methyl tetradecanoate	-	8.740e+7	-	-	-	-	-	1.905	-	-	-	-	$C_{15}H_{30}O_2$
9,12-Octadecadienoyl chloride, (Z, Z)-, methyl ester	-	2.600e+8	-	-	-	-	-	5.667	-	-	-	-	$C_{18}H_{31}ClO$
Methyl stearate	-	2.883e+7	1.988e+7	1.794e+7	1.202 e+7	-	-	0.628	2.082	1.505	2.590	-	$C_{19}H_{38}O_2$
Squalene	-	1.426e+8	-	-	-	-	-	3.107	-	-	-	-	$C_{30}HSO$
Dodecanoic acid. 1,2,3-propanetriyl ester	-	5.543e+8	-	-	-	-	-	12.081	-	-	-	-	$C_{39}H_{74}O_6$
Oleic Acid	-	2.805e+7	-	-	-	-	-	0.611	-		-	-	$C_{18}H_{34}O_2$
Tridecanoic acid, 12-methyl-, methyl ester	-	-	3.996e+7	3.932e+7	3.318 e+7	-	-	-	4.186	3.299	7.148	-	$C_{15}H_{30}O_2$
Methyl 12,13-tetradecadienoate	-	-	4.732e+8	-	-	-	-	-	49.565		-	-	$C_{15}H_{26}O_2$
Eicosanoic acid, methyl ester	-	-	1.210e+7	-	-	-	-	-	1.267		-	-	$C_{21}H_{42}O_2$
1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester	-	-	-	9.511e+6	6.526 e+6	-	-	-	-	0.798	1.406	-	$C_{16}H_{22}O_4$
9,12-Hexadecadienoic acid, methyl ester	-	-	-	5.995e+7	1.056 e+8	-	-	-	-	50.296	22.756	-	$C_{17}H_{34}O_2$
Tritetracontane	-	-	-	2.305e+7	7.776 e+6	-	-	-	-	1.934	1.675	-	$C_{43}H_{88}$
Hentriacontane	-	-	-	3.405e+7	8.274 e+6	-	-	-	-	2.856	1.782	-	$C_{31}H_{64}$
13-Tetradecen-1-ol acetate	-	-	-	1.475e+7	-	-	-	-	-	1.237	-	-	$C_{16}H_{30}O_2$
Octacosane	-	-	-	1.835e+7	-	-	-	-	-	1.540	-	-	$C_{28}H_{58}$
9-Octadecenamide, (Z)-	-	-	-	1.632e+7	1.173 e+7	8.005e+8	-	-	-	1.369	2.526	29.448	$C_{18}H_{35}NO$
1-Heptacosanol	-	-	-	1.952e+7	-	-	-	-	-	1.638	-	-	$C_{27}H_{56}O$
Methyl 8-methyl-nonanoate	-	-	-	-	6.515 e+6	-	-	-	-	0.733	-	-	$C_{11}H_{22}O_2$
Methyl 11-hexadecenoate	-	-	-	-	6.515 e+6	-	-	-	-	1.403	-	-	$C_{17}H_{32}O_2$
trans-13-Octadecenoic acid	-	-	-	-	1.373 e+7	-	-	-	-	-	2.957	-	$C_{18}H_{34}O_2$
11,14-Octadecadienoic acid, methyl ester	-	-	-	-	-	5.541e+8	-	-	-	-	-	20.385	$C_{19}H_{34}O_2$
Hexanedioic acid. bis (2-ethylhexyl) ester	-	-	-	-	-	3.251e+8	-	-	-	-	-	11.960	$C_{22}H_{42}O_4$

ASM: *Allium sativum* methanol extract, ASA: *Allium sativum* acetone extract, ASEA: *Allium sativum* ethyl acetate extract, ASC: *Allium sativum* chloroform extract, ASPE: *Allium sativum* petroleum ether extract, ASB: *Allium sativum* benzene extract

Table 5: Identified compounds of *A. sativum* and their biological activity reported in literature

Name of the compounds	Biological activity	References
Dodecanoic acid	Anti-*Mycobacterium tuberculosis,* antibacterial, antiviral and antifungal	[31,32]
Tetradecanoic acid	Larvicidal and repellent activity	[33]
Pentadecanoic acid	Antioxidant	[34]
hexadecanoic acid, methyl ester 9	Antibacterial and antifungal	[35]
n-Hexadecanoic acid	Anti-inflammatory, antioxidant, hypocholesterolemic nematicide, pesticide, antiandrogenic flavor, hemolytic, 5-alpha reductase inhibitor, potent mosquito larvicide	[36,37]
9-Octadecenoic acid (Z) methyl ester	Antioxidant, anticancer	[38]
Octadecanoic acid	Antimicrobial activity	[37]
Dodecanoic acid, methyl ester	Antibacterial, antiviral, antifungal	[39]
Squelene	Antibacterial, antioxidant, antitumor, cancer preventive, chemopreventive, immunostimulant, lipoxygenase inhibitor	[34]
Oleic acid	5-Alpha-reductase-inhibitor, allergenic, alpha-reductase-inhibitor, anemiagenic, antialopecic, antiandrogenic, anti-inflammatory, antileukotriene, cancer preventive, choleretic, dermatitigenic, flavor, hypocholesterolemic, insectifuge, irritant, percutaneostimulant	[34]

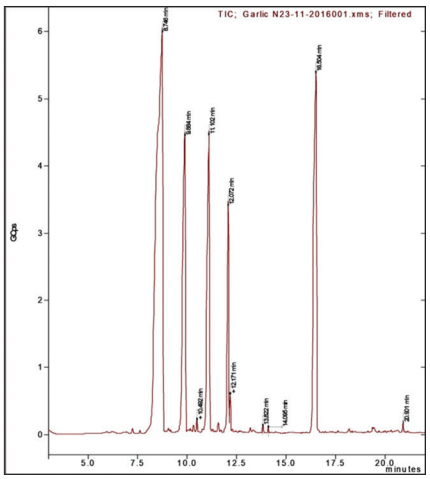

Fig. 1: Gas chromatography-mass spectrometry spectra of methanol extract

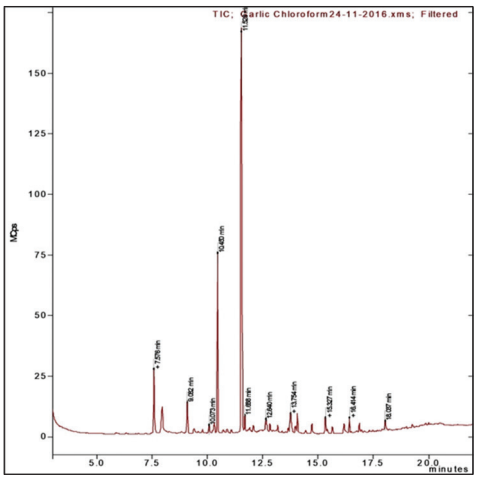

Fig. 3: Gas chromatography-mass spectrometry spectra of chloroform extract

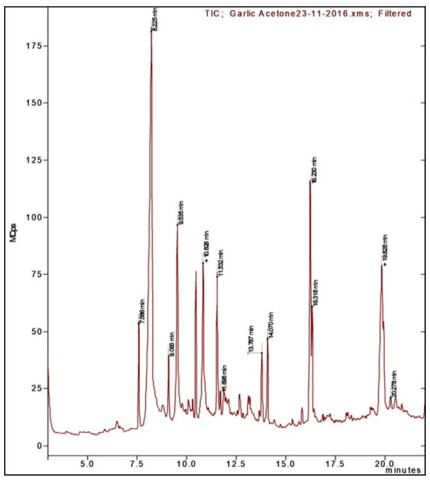

Fig. 2: Gas chromatography-mass spectrometry spectra of acetone extract

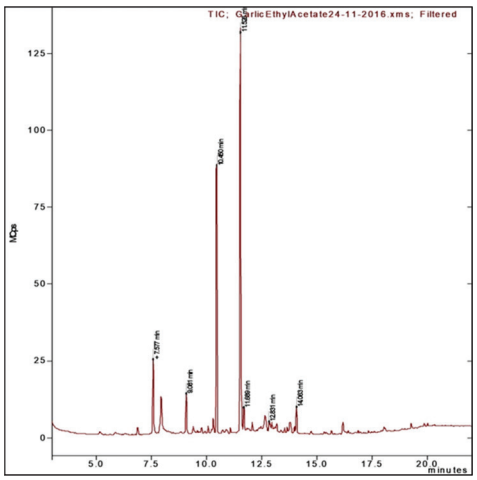

Fig. 4: Gas chromatography-mass spectrometry spectra of ethyl acetate extract

carbohydrates, alkaloids, cardiac glycosides, saponins, flavonoids, terpenes, and steroids [23-25]. Allicin, ajoene, thiosulfinates, and a wide range of other organosulfurate compounds are the major constituents which are responsible for the antimicrobial activity of *A. sativum* [26].

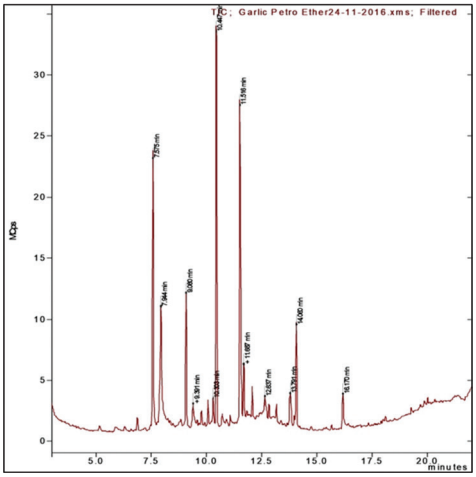

Fig. 5: Gas chromatography-mass spectrometry spectra of petroleum ether extract

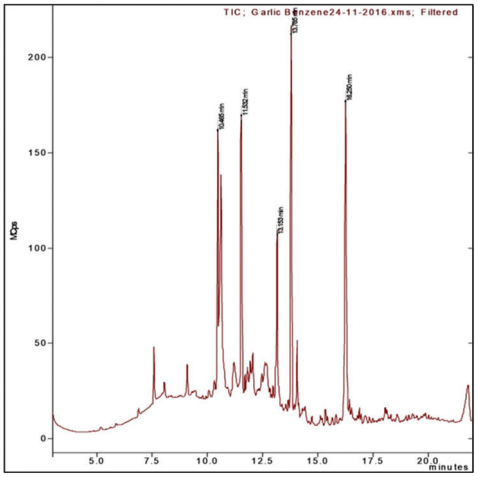

Fig. 6: Gas chromatography-mass spectrometry spectra of benzene extract

The bioactive compounds of six different garlic extracts were compared through GC-MS analysis as summarized in Table 4, and a total number of 35 compounds from six different extracts were identified. In methanol extract, the % of total of dodecanoic acid, tetradecanoic acid, n-hexadecanoic acid, and 13-docosenamide was higher than other extracts, i.e., 45.306, 14.822, 12.963, and 19.948, respectively. The dodecanoic acid and n-hexadecanoic acid have been reported previously to have antibacterial activities that may be the reason of highest antibacterial activity of methanol extract against reference strains [27].

The results showed that hexadecanoic acid and methyl ester were detected in all six extracts while two compounds, dodecanoic acid and Bis(2-ethylhexyl) phthalate were present five extracts except benzene extract. These substances are produced by the plants as secondary metabolites and may have beneficial or adverse effects [28-30]. As the benzene extract was found inactive against all the bacterial strain, we can conclude that the antibacterial activity against reference strains was due to the presence of dodecanoic acid and in case of MDR due to the presence of derivative of dodecanoic acid, i.e., dodecanoic methyl ester in acetone extract as shown in Table 4.

Some of the major identified compounds have been found to have remarkable biological activities against certain illnesses and/or microbial pathogens as shown in Table 5 with references from literature. These compounds might be responsible for the antibacterial efficacy of the plants extract.

The acetone extract was found most active against MDR strains. The main phytoconstituents identified from acetone extract were dodecanoic acid, tetradecanoic acid, 9-octadecenamide (Z), and dodecanoic acid 1, 2, 3-propanetriyl ester. The presence of the derivative of dodecanoic acid, namely, dodecanoic acid, methyl ester, and dodecanoic acid, 1, 2, 3-propanetriyl ester in acetone extract may be the reason of its potential against MDR strains. Some other additional compounds identified in acetone extracts were methyl tetradecanoate, 9, 12-octadecadienoyl chloride, (Z,Z), methyl stearate, squalene, and oleic acid also may be the reason for the antibacterial potential against MDR strains.

CONCLUSION

There are many scientific reports which stated that *A. sativum* has great potential for the treatment of various infectious diseases. In the present study, garlic was found to have good antibacterial activity against reference as well as MDR bacterial strains. The GCMS analysis shows the presence of bioactive compounds mainly dodecanoic acid and dodecanoic acid, methyl ester which may be responsible for the antibacterial activity of *A. sativum*. Thus, these compounds should be isolated and their pharmacokinetic properties and toxicity should be analyzed.

ACKNOWLEDGMENT

This research work was supported by grant from UGC, New Delhi, in form of UGC BSR [F.4-1/2006 (BSR/7-371/2012] & UGC-SAP [F.20/2012(SAP-II)].

AUTHORS CONTRIBTION

Dushyant Sharma has performed experimentation work and data collection and drafted the manuscript. Reena Rani made significant involvement in the interpretation of data and revising the manuscript. Monika Chaturvedi participated in the design of the study and performed the statistical analysis. Jaya Parkash Yadav helped in designed the study and finalization of the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest between authors.

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