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Original article

LC-MS-based metabolite profiling of aqueous garlic (*Allium sativum* L.) bulb extract

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Abstract

Garlic (*Allium sativum*) is a well-known plant as a natural product source as a spice and medicinal plant. However, garlic also become attention in agriculture due to its potential for the allelopathy effect which beneficial in cropping by its allelochemicals. This study aims to identify potential compounds with allelopathic effects by literature study on previous allelopathic or biological activity research in macerated garlic bulb extract using a distilled water solution. Analysis was conducted using LC-MS instrumentation. The results revealed 79 compounds detected and classified into 12 phytochemical groups. Organosulfur compounds were the main group. Ten of 29 organosulfur compounds have demonstrated the potential to function as allelochemicals. These compounds were ajoene, allicin, alliin, allyl methyl sulfide, diallyl disulfide, diallyl sulfide, diallyl trisulfide, dimethyl disulfide, dimethyl sulfide, and dipropyl disulfide. Alliin and allicin were the two most abundant organosulfur compounds, with percentage compositions of 9.286% and 6.986%, respectively. Future studies may explore the potency of other phytoconstituents detected in garlic extract for the allelopathic effect.

Keywords: Allelochemicals, *Allium sativum*, bulb, LC-MS analysis, phytochemicals
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Introduction

Natural products are defined as chemical contents produced by bacteria, plants, and animals (Andila et al., 2019). There are two main categories of plant natural products, i.e., primary metabolites and secondary metabolites. Primary metabolites are organic substances required for plant normal growth, development, cellular functions, and reproduction, while secondary metabolites are organic substances crucial for plant resistance and defense against infection. Several examples of primary metabolites are carbohydrates, fats, and protein, while secondary metabolites are phenolics, terpenoids, and nitrogen-containing compounds (Elshafie et al., 2022). Interestingly, secondary metabolites have various biological activities, thus encouraging research about their utilization in agriculture and pharmacological activities (Yan et al., 2018; Andila et al., 2019). One of the plants utilized as a natural product in human civic history is garlic.

Garlic (*Allium sativum* L.) is a layered bulbous perennial herb that belongs to the Alliaceae family which has a pungent flavor as the unique feature causing it to be recognized worldwide (Macpherson et al., 2005; Bayan et al., 2014; Baswarsiati et al., 2023). This plant is used for spice, food, and medical purposes (Chanda et al., 2011). Garlic is native to Asia (Kovarovič et al., 2019). According to Statista (2025), Indonesia ranked 11th as a garlic producer in the Asia-Pacific region in 2023. The provinces of Central Java (40.49%), West Nusa Tenggara (39.45%), East Java (6.56%), West Java (6.34%), West Sumatra (2.48%), and East Nusa Tenggara (1.08%) are the primary producers provinces of garlic in Indonesia (Hadiawati et al., 2022). In 2021, East Java's garlic production reached 4,220 tons, as Statistics Indonesia (2023) reported. The cultivation of garlic is concentrated in specific districts or cities, including Malang Regency, Batu City, Lumajang Regency, and Probolinggo Regency. Specifically, Malang Regency produced 1,774.6 tons in 2021 (Statistics East Java 2023). In Malang, various ethnobotanical reports prove the beneficial usage by the local community, i.e., Dampit and Pamotan villagers in Dampit District consume garlic for health purposes (Hadiarti et al., 2025); Tengger community applies garlic mash to stab wounds (Indriyani et al., 2012) as well as for treating colds and flatulence (Batoro et al., 2013); seller, customer, and distributor in Pasar Besar Malang mentioned garlic usage as spices on various foods such as tofu, *tempe*, and stir-fried vegetables and treating gum disease (Cahyono, 2019). Moreover, Malang has seven varieties of garlic, i.e., Geol, Kusuma, Lumbu Hijau, Lumbu Kuning, Lumbu Putih, Sangga Sembalun, and Tawang Mangu Baru. These varieties are different in morphological (leaf color, leaf orientation, and clove color) and yields (bulb weight, clove length, clove diameter, and number of cloves/tubers) (Baswarsiati et al., 2024).

Nowadays, garlic is used not only for food and medicinal purposes but also in cropping systems for its allelopathic effects. Allelopathy is defined as the natural phenomenon of allelochemicals released by microorganisms, fungi, or plants to the environment that affects other organisms (Kostina-Bednarz et al., 2023;

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Kumar et al., 2024). Allelochemicals can be primary or secondary metabolites (Chaïb et al., 2021). Initially, Theophrasus, a Greek botanist, observed the allelopathy phenomenon around 3000 BCE on destroyed weeds and depleted soil medium by chickpea plants. However, the term ‘allelopathy’ was initiated by Hans Molisch in 1937 and derives from the Greek ‘allelos’, defined as mutual, and ‘pathos’, defined as suffering (Chaïb et al., 2021). Allelopathy affects either beneficial or detrimental on target organisms because it stimulates or inhibits growth and development (Cheng & Cheng, 2015; Kumar et al., 2024). Allelochemical mechanisms in influencing growth and development are multiple pathways, including photosynthesis, respiration, cell wall polysaccharide synthesis, phytohormone synthesis, protein expression, and nucleic acid metabolism (Li et al., 2022). Allelopathy has several applications, including maintaining soil health, managing weeds and pests, protecting crops, and supporting crop re-establishment (Cheng & Cheng, 2015; Kiely et al., 2023; Kumar et al., 2024). Allelochemicals are an appropriate substitute for synthetic compounds to prevent the toxic effects of synthetic compounds (Chinchilla et al., 2014). Garlic promises to be an allelopathic agent for various purposes and is applied in many crops. Previous studies revealed garlic allelopathy effect as (1) crops growth and yield stimulator on cucumber (*Cucumis sativus*) (Xiao et al., 2012; Xiao et al., 2019), lettuce (*Lactuca sativa*) (Han et al., 2013), female ginseng (*Angelica sinensis*) (Zhang et al., 2015), pepper (Ding et al., 2016), quinoa (*Chenopodium quinoa*) (El-Rokiek et al., 2019), and eggplant (*Solanum melongena*) (Ali et al., 2019a; Ali et al., 2019b); (2) herbicide for weeds management on various weeds, i.e. *Cyperus rotundus* (El-Rokiek et al., 2018), *Digitaria ciliaris*, *Echinochloa oryzoides*, *Calystegia sepium*, *Quamoclit coccinea* (Jang et al., 2019), *Amaranthus retroflexus*, *Cichorium intybus*, and *Echinochloa crusgalli* (Mansour et al., 2014); (3) pesticide for managing various pest insects, i.e. *Podisus maculiventris* (Mamduh et al., 2017), *Rhynchophorus ferrugineus* (Al-Shuraym et al., 2020), *Parlatoria blanchardi* (Al-Hussainawy et al., 2021), and *Cerataphis orchidearum* (Al-Shuraym, 2022); (4) anti-fungal activity against phytopathogenic fungi of crops such as *Phytium* species (Yuan et al., 2012), *Botrytis cinerea*, *Fusarium oxysporum*, *Phytophthora capsici*, *Verticillium dahliae* (Hayat et al., 2016), etc.

Liquid Chromatography – Mass Spectrometry (LC-MS) is a sophisticated tool for quantitative phytochemical screening. It compromises the separation, identification, and quantification of various compounds in a sample (Mukherjee, 2019). LC-MS is suitable as a compound analyzer for non-volatile and polar compounds (Nair, 2017). LC-MS working principle is that the sample is injected into a chromatographic column and then separated over time depending on the interaction of the content of the sample with the column (stationary phase) and solvent (mobile phase). Separation compounds are based on the compounds' polarity. After that, the sample is ionized and the mass spectrum is used to detect sample analysis as a function of mass ratio/load (m / z) (French, 2017; Gerona & French, 2022). The advantages of LC-MS instruments are applicability for small-sample analysis, increased sensitivity, large mass range, and soft ionization (Want et al., 2005; Wilson et al., 2020). Various plants have been investigated for their metabolite profile contents by LC-MS, i.e., calabash (*Cresentia cujete*) seed ethanolic extract (Gaspersz et al., 2024), *Eurya acuminata* leaf ethanolic extract (Alamsjah et al., 2024), *Archidendron bubalinum* seed shells methanolic extract (Hanafi et al., 2018), and many more.

Indonesia is a mega biodiversity country with a wide variety of plant species, many of which have the potential to contain allelochemicals. Garlic is an example of Indonesian flora that needs to be investigated as an allelopathy source plant. Therefore, screening of the phytochemical contents of garlic bulbs was acquired. Several studies reported LC-MS analysis of garlic bulb from Neipu, Taiwan (Dewi et al., 2017), Hisar, India (Monika et al., 2024), and Aomori Prefecture, Japan (Zhu et al., 2016). Non-aseptically macerated garlic is an extraction procedure that easy to do by traditional garlic farmers for garlic allelopathy application. Therefore, this research was conducted to explore various active compounds in the aqueous garlic bulb extract by non-aseptically maceration so that their potency as allelochemicals can be recognized by literature study on various research that reported the allelopathy effect or biological activity of these compounds.

Methods

Extraction Procedure

Extraction was done by non-aseptically maceration. Garlic cultivated from Jabung, Malang Regency was obtained from the local Sawojajar, Malang, East Java, Indonesia market. Cloves were peeled from the outer skin. Cloves were ground using a grinder. 2,250 g cloves were mixed with 4,500 mL distilled water. Extraction was done within 48 hours, soaking at room temperature. Filtrate and residue were separated using a sieve 18 mesh (Atun et al., 2021).

LC-MS Analysis

The extract dilution began with 0.1 g of the extract sample dissolved in methanol to a final volume of 100 mL. Homogenization was done with a vortex for 10 minutes. The sample was then filtered by filter vacuum. Solids were separated by centrifugation at 8000 rpm for 10 minutes. Supernatant used for protein precipitation and purification via Solid Phase Extraction (SPE). After purification, the solution was filtered over a 0.45 µm cellulose acetate membrane and prepared for LC-MS analysis (Shimadzu LC-MS – 8040 LC-MS). One µL of the sample was injected into the liquid chromatography apparatus. The column used was Shimadzu Shim Pack FC-ODS (2 mm x 150 mm, three µm) isocratic mobile phase mode at a flow rate of 0.5 mL/min and temperature at 35°C. The mobile phase was 95% ethanol as the solvent—MS analysis with electrospray ionization (ESI) with positive ion mode. Mass spectra were acquired with a scan range from 0,6 sec/scan (mz: 10-1500) (Mastuti et al., 2023). Database used for chemical compound identification was the National Institute of Standards and Technology (NIST) Mass Spectral Library 17 (Ljoncheva et al., 2022).

Data Analysis

Detected compounds were classified based on the chemical compound groups. Compounds group of every metabolite were searched by literature review from articles journals, books, and Pubchem website.

Results and Discussion

Figure 1 and Table 1 showed 79 phytoconstituents identified in the aqueous garlic bulb extract. This study identified 12 chemical groups, i.e., organosulfur, flavonoid, phenolic, terpenoid, steroid, sterol, aromatic compound, ester, furan, selenium, aldehyde, and tripeptide. Organosulfur was the main group of compounds detected by LC-MS analysis, including 29 compounds, followed by flavonoids with 22 compounds. Organosulfur is a compound containing sulfur atoms (Puri et al., 2023). This finding is consistent with Hwang & Kim (2022) reported Gas Chromatography – Mass Spectrometry (GC-MS) analysis showed organosulfur compounds dominantly presence in commercial garlic powders. There were 20 organosulfur compounds from 35 compounds in total. Moreover, by GC-MS analysis, Plata-Rueda et al. (2017) found 14 organosulfur compounds in garlic essential oil. Alliin and allicin were major organosulfurs with 9.286 and 6.986 % composition, respectively. In addition, kaempferol (4.035%) and quercetin (4.033%) were major flavonoids with high composition percentages, among others. Emir et al. (2019) reported kaempferol also became a major flavonoid but ranked second in the LC-MS analysis of *Allium nigrum* aerial parts. However, kaempferol in bulb was a minor flavonoid same as quercetin.

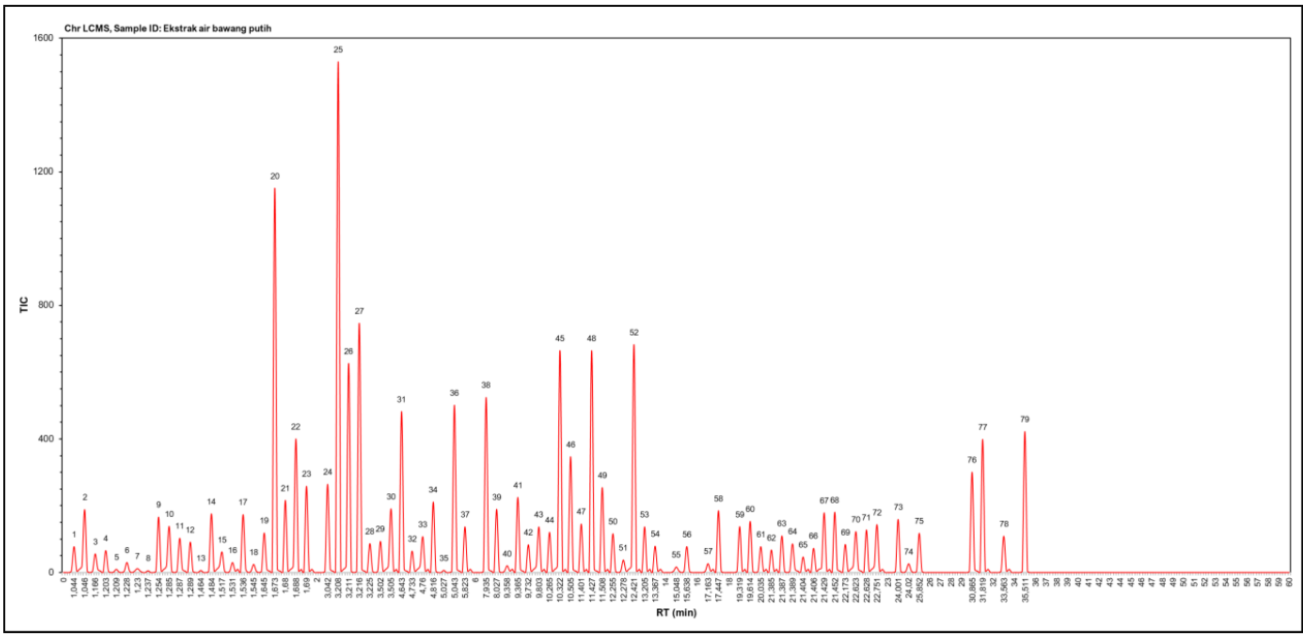

Figure 1. LC-MS chromatogram of aqueous garlic bulb extract

Table 1. Chemical composition of aqueous garlic bulb extract using LC-MS analysis

Peak Number	Compounds Name	Chemical Formula	RT (min)	Molecular Weight	Composition (%)	References*
Organosulfur						
1	dimethyl sulfide	C_2H_6S	1.044	62.1	0.472	Marcinkowska & Jeleń (2022)
2	allyl mercaptan	C_3H_6S	1.046	74.1	1.145	Marcinkowska & Jeleń (2022)
3	allyl methyl sulfide	C_4H_8S	1.166	88	0.34	Marcinkowska & Jeleń (2022)
4	dimethyl disulfide	$C_2H_6S_2$	1.203	94	0.401	Marcinkowska & Jeleń (2022)
9	diallyl sulfide	$C_6H_{10}S$	1.254	114.2	1.009	Devi (2021)
10	methyl allyl disulfide	$C_4H_8S_2$	1.285	120.2	0.84	Radulović et al. (2015)
11	methyl propyl disulfide	$C_4H_{10}S_2$	1.287	122.2	0.626	Liu et al. (2022a)
14	dimethyl trisulfide	$C_2H_6S_3$	1.484	126.3	1.069	Marcinkowska & Jeleń (2022)
15	2-vinyl-4-H-1,3-dithiin	$C_6H_8S_2$	1.517	144.3	0.375	Ammelia et al. (2020)
17	diallyl disulfide	$C_6H_{10}S_2$	1.536	146	1.054	Devi (2021)
18	S-methyl 2-propene-1-thiosulfinate	$C_4H_8OS_2$	1.545	136.2	0.149	Guillamón et al. (2023)
19	dipropyl disulfide	$C_6H_{14}S_2$	1.645	150.3	0.7211	Marcinkowska & Jeleń (2022)
20	allicin	$C_6H_{10}OS_2$	1.673	162	6.986	Devi (2021)
21	methiin	$C_6H_{10}OS_2$	1.68	151.2	1.313	Marcinkowska & Jeleń (2022)
22	(-) S-allyl-L-cysteine	$C_6H_{11}NO_2S$	1.688	161.1	2.43	Devi (2021)
23	trans-S-(1-propenyl)-L-cysteine	$C_6H_{11}NO_2S$	1.69	161.2	1.57	PubChem (2025)
25	alliin	$C_6H_{11}NO_3S$	3.208	177.2	9.286	Devi (2021)

26	cycloalliin	$C_6H_{11}NO_3S$	3.211	177.2	3.801	Ammelia et al. (2020)
27	isoalliin	$C_6H_{11}NO_3S$	3.216	177	4.531	Devi (2021)
28	di-(2-propenyl)trisulfide	$C_6H_{10}S_3$	3.225	178.3	0.5279	Hossain et al., (2023)
29	diallyl trisulfide	$C_6H_{10}S_3$	3.502	178.3	0.569	Devi (2021)
30	allyl trisulfide	$C_6H_{10}S_3$	3.505	178.3	1.16	Radulović et al. (2015)
32	3,5-diethyl-1,2,4-trithiolane	$C_6H_{12}S_3$	4.733	180.3	0.39	Park et al. (2001)
37	diallyl tetrasulfide	$C_6H_{10}S_4$	5.823	210.4	0.8305	Iciek et al. (2009)
38	ajoene	$C_9H_{14}OS_3$	7.935	234.4	3.183	Devi (2021)
39	allyl pentasulfide	$C_6H_{10}S_5$	8.027	242.4	1.15	Block (2010)
43	diallyl hexasulfide	$C_6H_{10}S_6$	9.803	274.5	0.83	Lu et al. (2011)
46	γ-glutamyl-S-trans-1-propenyl-cysteine	$C_{11}H_{18}N_2O_5S$	10.51	290.3	2.108	Petropoulos et al. (2017)
49	diallyl heptasulfide	$C_6H_{10}S_7$	11.51	306.6	1.546	Iciek et al. (2009)
Flavonoid						
40	5-deoxykaempferol	$C_{15}H_{10}O_5$	9.358	270	0.125	Telezko & Wojdylo (2014)
41	apigenin	$C_{15}H_{10}O_5$	9.365	270	1.367	Telezko & Wojdylo (2014)
42	naringenin	$C_{15}H_{12}O_5$	9.732	272.1	0.506	Telezko & Wojdylo (2014)
44	luteolin	$C_{15}H_{10}O_6$	10.27	286.2	0.733	Telezko & Wojdylo (2014)
45	kaempferol	$C_{15}H_{10}O_6$	10.32	286.2	4.035	Telezko & Wojdylo (2014)
48	quercetin	$C_{15}H_{10}O_7$	11.43	302.2	4.033	Telezko & Wojdylo (2014)
62	isovitexin	$C_{21}H_{20}O_{10}$	21.39	432.4	0.411	Tsifsoglou et al. (2019)
63	vitexin	$C_{21}H_{20}O_{10}$	21.39	432.4	0.667	Liu et al. (2022b)
64	apigetrin	$C_{21}H_{20}O_{10}$	21.39	432.1	0.523	Liu et al. (2022b)
67	kaempferol-3-rhamnoside	$C_{21}H_{20}O_{11}$	22.62	448.4	0.745	Telezko & Wojdylo (2014)
68	reynoutrin	$C_{20}H_{18}O_{11}$	21.45	434.1	1.098	Zheng et al. (2024)
69	astragalin	$C_{21}H_{20}O_{11}$	22.17	448.4	0.512	Zheng et al. (2024)
70	kaempferol-3-O-D-glucoside	$C_{21}H_{20}O_{10}$	21.43	432.4	1.088	Telezko & Wojdylo (2014)
71	luteolin-7-glucoside	$C_{21}H_{20}O_{11}$	22.63	448.4	0.774	Telezko & Wojdylo (2014)
72	agigenin	$C_{27}H_{44}O_5$	22.75	448.6	0.872	Devi (2021)
73	quercetin-3-O-glucoside	$C_{21}H_{19}O_{12}$	24	463.4	0.967	Telezko & Wojdylo (2014)
74	hyperoside	$C_{21}H_{20}O_{12}$	24.02	464.4	0.16	Tsifsoglou et al. (2019)
75	isorhamnetin-3-O-β-D-galactopyranoside	$C_{22}H_{22}O_{12}$	25.85	478.4	0.715	Tsifsoglou et al. (2019)
76	kaempferol-3-(6"- malonylglucoside)	$C_{24}H_{22}O_{14}$	30.87	534.4	1.826	Telezko & Wojdylo (2014)
77	quercetin-3-O-malonylglucoside	$C_{24}H_{22}O_{15}$	31.82	550.4	2.421	Telezko & Wojdylo (2014)
78	naringin	$C_{27}H_{32}O_{14}$	33.56	580.5	0.662	Motallebi et al. (2022)
79	quercetin-3-glucoside-7-rhamnoside	$C_{27}H_{30}O_{16}$	35.51	610.5	2.563	Telezko & Wojdylo (2014)
Phenolic acid						
7	benzaldehyde	C_7H_6O	1.23	106.1	0.0736	Jackson (2017)
12	benzoic acid	$C_7H_6O_2$	1.289	122	0.556	Kato et al. (2023)
16	3-phenyl-2-propenol	$C_9H_{10}O$	1.531	134.1	0.183	Zeb (2021)
24	gallic acid	$C_7H_6O_5$	3.042	170	1.606	Kato et al. (2023)
31	caffeic acid	$C_9H_8O_4$	4.643	180.2	2.928	Kato et al. (2023)
36	ferulic acid	$C_{10}H_{10}O_4$	5.043	194.2	3.042	Kato et al. (2023)
47	ellagic acid	$C_{14}H_6O_8$	11.4	302.2	0.883	Wang et al. (2022)
50	5-O-caffeoylshikimic acid	$C_{16}H_{16}O_8$	12.26	336.3	0.707	Kato et al. (2023)
51	4-p-coumaroylquinic acid	$C_{16}H_{18}O_8$	12.28	338.1	0.227	Kato et al. (2023)
52	chlorogenic acid	$C_{16}H_{18}O_9$	12.42	354.3	4.144	Kato et al. (2023)
Terpenoid						
54	squalene	$C_{29}H_{48}$	13.37	396.4	0.48	Musa et al. (2024)
59	β-amyrin	$C_{30}H_{50}O$	19.32	426.7	0.835	Musa et al. (2024)
60	lupeol	$C_{30}H_{50}O$	19.61	426.7	0.93	Musa et al. (2024)
Steroid						
58	tigogenin	$C_{27}H_{44}O_3$	17.45	416.6	1.125	Sobolewska et al. (2016)
61	laxogenin	$C_{27}H_{42}O_4$	20.04	431	0.472	Sobolewska et al. (2016)
65	gitogenin	$C_{27}H_{44}O_4$	21.4	432.6	0.283	Sobolewska et al. (2016)
66	β-chlorogenin	$C_{27}H_{44}O_4$	21.41	432.6	0.441	Sobolewska et al. (2016)
Sterol						
55	campesterol	$C_{28}H_{48}O$	15.05	400.7	0.102	Khan et al. (2022)
56	stigmasterol	$C_{29}H_{48}O$	15.64	412.7	0.473	Khan et al. (2022)
57	β-sitosterol	$C_{29}H_{50}O$	17.16	414.7	0.162	Khan et al. (2022)
Ester						
13	isopropyl butyrate	$C_7H_{14}O_2$	1.464	130.2	0.04	Bampidis et al. (2022)
35	isopentyl benzoate	$C_{12}H_{16}O_2$	5.027	192.1	0.042	Bampidis et al. (2022)
Furan						

5	furfural	$C_5H_4O_2$	1.209	96	0.06	Li et al. (2020)
6	2.4-dimethylfuran	C_6H_8O	1.228	96.1	0.184	Li et al. (2020)
Selenium						
33	methylselenocysteine	$C_4H_9NO_2Se$	4.76	182.1	0.6547	Rayman (2020)
34	dimethyl diselenide	$C_2H_6Se_2$	4.816	188	1.283	Rayman (2020)
Aldehyde						
8	4-heptenal	$C_7H_{12}O$	1.237	112.2	0.035	Zhao et al. (2024)
Tripeptide						
53	S(2-carboxypropyl)glutathione	$C_{14}H_{23}N_3O_8S$	13.21	393.4	0.831	Yamaguchi & Kumagai (2019)

*Reference information for chemical compound group

Out of 79 compounds, we focus on the profile of 10 organosulfur compounds with potential allelopathic properties. Organosulfur compounds are the main chemical group in garlic, and previous research has reported their allelopathic effects or potential as allelochemicals based on their biological activity, either from isolated compounds or commercial products.

Dimethyl sulfide (DMS)

This compound had the fastest retention time, detected at 1.044 min with a composition of 0.472% (Figure 1, Table 1). Scherb et al. (2009) reported DMS also appeared in other crops like tomato, asparagus, cabbage, celery, beetroot, leek, orange, and strawberry using LC-MS screening. The role of DMS is producing cabbage-like odour in vegetables and flavour for fruits (Scherb et al., 2009; Parker, 2015). Generally, DMS is responsible to odorant character of food and beverages (Deed, 2019). In addition, DMS produced by green algae as well (Alcolombri et al., 2015). Van Alstyne et al. (2023) reported green algae *Ulva fenestrata* was affected by airborne dimethyl sulfide exposure. It induced increasing dimethylsulfoniopropionate (DMSP) production. Moreover, DMS is one of DMSP breakdown product. DMS and DMSP are important for marine algae as antioxidant (Sunda et al., 2002).

Allyl methyl sulfide (AMS)

The retention time of this compound was 1.166 min with 0.34% composition (Figure 1, Table 1). AMS is a volatile organosulfur compound which responsible for garlic odour. In addition, persistent garlic odour in human breath due to long digesting process of AMS. AMS is a product from allicin biosynthesis. Allicin converts directly to allyl mercaptan or intermediated by diallyl disulfide (DADS) as allicin derivates then forms allyl mercaptan, then allyl mercaptan transforms to AMS (Sato et al., 2020). Viner-Mozzini & Sukenik (2012) reported AMS influenced photosynthetic activity and growth of various cyanobacteria and algae cultures of *Nitzschia* sp., *Chlorella* sp., *Microscystis* sp., *Aphanizomenon ovalisporum*, and *Peridinium gatunense*. AMS inhibit maximum photochemical quantum yield of photosystem II (PSII) on *Aphanizomenon ovalisporum*. *Peridinium gatunense* motility cells declined as higher AMS concentration with 1% induce full cell motility inhibition.

Dimethyl disulfide (DMDS)

The retention time of this compound was 1.203 min with 0.401% composition (Figure 1, Table 1). In aged garlic extract, DMDS being a key aroma-active compound (Abe et al., 2020). Odour description of DMDS is sulfury, fried garlic, and towngas (Hwang & Kim, 2022). Tea (*Camellia sinensis*) also contained DMDS which contributes an odour of cooked corn-like (Zhai et al., 2022). Synthesis of DMDS is derived from methionine as initial precursor (Denawaka et al., 2016). DMDS is a predominant content with > 80% on canola (*Brassica napus*) and camelina (*Camelina sativa*) root and shoot extracts. These extracts suppress germination, root fresh weight, and shoot fresh weight of flax (*Linum usitatissimum*), radish (*Raphanus sativus*), and wild oat (*Avena fatua*). However, radish shoot fresh weight was increased (Walsh et al., 2014).

Diallyl sulfide (DAS)

The retention time of this compound was 1.254 min with 1.009% composition (Figure 1, Table 1). DAS is classified as a member of the group of monosulfides which responsible for fresh garlic and pungent odour (Hwang & Kim, 2022). DAS is one of second-generation organosulfur compound from allicin degradation and being the main derivative compounds of allicin in aqueous extract (Hitchcock et al., 2020; Plata-Rueda et al., 2017). In addition, heat-treated garlic contains higher quantities of these chemicals than raw garlic (Hwang & Kim 2022). Studies of aged garlic extract and black garlic reveal DAS was identified as one of key-aroma active compounds (Abe et al., 2020; Yang et al., 2019). Bai et al. (2023) applied DAS solution commercial product as herbicide on *Echinochloa crusgalli* and the result was the grass' growth was inhibited. Viner-Mozzini & Sukenik (2012) also applied DAS in addition to AMS on cyanobacteria and algae *Aphanizomenon ovalisporum*, *Chlorella* sp., *Microscystis* sp., *Nitzschia* sp., and *Peridinium gatunense* and induce higher suppression on PSII photochemical quantum yield than AMS. In addition, DAS induce similar result on motility inhibition on *Peridinium gatunense* as AMS treatment.

Diallyl disulfide (DADS)

The retention time of this compound was 1.536 min with 1.054% composition (Figure 1, Table 1). DADS is derivative product from allicin decomposition process (Hwang & Kim, 2022). DADS formed at 100 ºC by steam (Block, 1985). Odour produced by DADS possesses fresh garlic and pungent. DADS is a key aroma-active compound found in aged garlic and black extract (Hwang & Kim, 2022). Other *Allium* species which contain DADS are garlic chives (*Allium tuberosum*) and onion (*Allium cepa*) (Wiczkowski, 2011; Bastaki et al., 2021). Cheng et al. (2016) reported DADS solution increase antioxidant enzymes activities and soluble protein content. Ali et al. (2021) applied DADS solution on healthy and infected eggplant by *Verticilium dahliae* and both increase in growth parameters, antioxidant enzymes activities, phytochemical content, hormone signalling, and physiological responses. In addition, *Verticilium dahliae* is inhibited. DADS solution also conducted as herbicide on *Echinochloa crusgalli* (Bai et al., 2023).

Dipropyl disulfide (DPDS)

The retention time of this compound was 1.645 min with 0.7211% composition (Figure 1, Table 1). DPDS is a derivate compound from allicin (Wiczkowski 2011). It found in all *Allium* species (Bastaki et al., 2021). DPDS applied by Viner-Mozzini & Sukenik (2012) as another volatile organosulfide compounds treatment. DPDS showed better result than AMS and DAS on motility inhibition of *Peridinium gatunense* in low concentration. 0.01 and 0.05 % concentration induce higher non-motile cells than AMS and DAS in the same concentration. Nevertheless, DPDS showed uneffective result compared with AMS and DAS on reduction of maximum quantum yield on cyanobacteria and algae *Aphanizomenon ovalisporum*, *Chlorella* sp., *Microscystis* sp., *Nitzschia* sp., and *Peridinium gatunense*.

Allicin

The retention time of this compound was 1.673 min with 6.986% composition (Figure 1, Table 1). Allicin is unstable in high temperature (Plata-Rueda et al., 2017). It decomposes to other organosulfur such as DAS, DADS, and diallyl trisulfide (DATS), DMDS, and allyl methyl trisulfide (Hwang & Kim, 2022; Malla et al., 2021). Allicin degradation process is non-enzymatic degradation (Hwang & Kim, 2022). According to heat sensitivity, allicin is the main compound in raw garlic (Malla et al., 2021). Hayat et al. (2016) applied allicin aqueous standard (AAS) commercial product to against various crops pathogenic fungi, *Fusarium oxysporum*, *Botrytis cinerea*, *Verticillium dahliae*, and *Phytophthora capsici*. All fungi are effective inhibited by AAS. Additionally, leaf disk bioassay on infected pepper by *Phytophthora capsici* and infected eggplant by *Verticillium dahliae* showed AAS decrease significantly on severity index percentage and disease severity percentage.

Alliin

The retention time of this compound was 1.203 min with 0.401% composition (Figure 1, Table 1). Alliin is a precursor of allicin derived from L-cysteine with odorless characteristic. When fresh garlic cloves are crushed, alliin converts to allicin by alliinase enzyme (Hwang & Kim, 2022). On the other hand, Block (1985) report allicin convertion in water and ethyl alcohol occurred at 25^{o}C. The research about allelopathy effect of alliin is absence. However, previous study reported the biological properties of alliin thus future study may conducted for its allelopathy potency. Alliin act as inhibitor for lipoxygenase and cyclo-oxygenase enzymes (Bayer et al., 1988) and become anti-thromobotic agent (Wagner et al., 1990).

Diallyl trisulfide (DATS)

The retention time of this compound was 3.502 min with 0.569% composition (Figure 1, Table 1). DATS is another organosulfur second-generation from allicin (Hitchcock et al., 2020). It contributes on characteristic aroma on black garlic and roasted garlic (Cadwallader et al., 2011; Yang et al., 2019). DATS commercial product able to inhibit toxic cyanobacterium *Microcystis aeruginosa* performance by Wang et al. (2016) experiment. DATS decrease significantly cell density, chlorophyll a content, chlorophyll fluorescence, and relative electron transport rates compared with untreated group. Moreover, Zhou et al. (2018) treated algae *Alexandrium tamarense* by DATS and induce inhibition on the algae. Longer time observation showed higher inhibitory rate.

Ajoene

The retention time of this compound was 1.203 min with 0.401% composition (Figure 1, Table 1). Ajoene is one of abundant compound of second-generation organosulfur compounds. Therefore, garlic aged preparations contain higher ajoene than fresh preparations (Hitchcock et al., 2020). Allicin decomposes to ajoene when organic oils being the solvent (Malla et al., 2021). Ajoene biosynthesis started with S-thioallylation allows two allicin molecules to join, resulting in a sulfonium ion. After beta-elimination, a thiocarbocation can be formed through gamma-addition with 2-propenesulfenic acid, resulting in ajoene (Hitchcock et al., 2020). There is no research about ajoene allelopathy effect in agriculture. However, this compound might potential as allelochemical due to it has been reported for the antifungal property. Ajoene reported inhibit growth of fungus *Aspergillus niger* and *Candida albicans* by induce depression of hypha surface (Yoshida et al., 1987). In addition, another research by Maluf et al. (2008) also conducting ajoene antifungal activity on *Paracoccidioides brasiliensis*. The results showed ajoene was capable to suppress viable cell of fungus and reduced antibodies levels. Therefore, ajoene shows considerable promise as an antifungal agent for crops.

Conclusion

LC-MS analysis identified 79 phytochemicals in the water extract of garlic bulbs, with organosulfur compounds as the main constituents. Out of 29 organosulfur compounds, ten were identifies as potential allelochemicals, i.e. dimethyl sulfide, allyl methyl sulfide, dimethyl disulfide, diallyl sulfide diallyl disulfide, dipropyl disulfide, allicin, alliin, diallyl trisulfide, and ajoene. The most abundance constituents were alliin with 9.286% composition and allicin with 6.986% composition. Further investigation is needed for compounds suspected of having allelopathic effects in agriculture such as ajoene and alliin as well as other phytochemicals that have not yet been studied but may have potential as allelochemicals.

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