EXTRACTION AND CHARACTERIZATION OF ESSENTIAL OIL EXTRACTED FROM THE BULBS OF *ALLIUM SATIVUM* LINN. (GARLIC)

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Abstract

Garlic, a member of the Alliaceae family has been used for thousands of years as a food additive, spice, and medicine. Garlic extract has been shown to reduce serum cholesterol levels and increase blood coagulation time. Alkaloids, α- amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, tannin, hydrolysable tannin, flavonoids, steroids are present in garlic bulbs, but starch is not. From the study of the elemental analysis by EDXRF (Energy Dispersive X-Ray Fluorescence), the essential metal potassium (1.321 %), sulphur (1.176%), calcium (0.081%), and iron (0.006%) were found in the garlic. The toxic heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) were not detected in the bulb sample. The essential oil was isolated from *Allium sativum* L. (garlic) by steam distillation. The mixture of essential oil with water was separated with *n*-hexane. The antioxidant activity of extracted essential oil, ethanol and watery extracts was screened by the DPPH free radical scavenging assay method. The IC_{50} value of extracted essential oil was found to be 514.79 µg/mL, while the 95 % ethanol and watery extracts were 55.70 µg/mL and 147.55 µg/mL. So, 95% ethanol has the most potent antioxidant activity. The organic constituents in the extracted essential oil of garlic were investigated at the Department of Research and Innovation National Laboratory (DRI), Yangon. Seven compounds (methyl-1-propenyl disulfide, diallyl disulphide, 2-vinyl-1, 3-dithiane, 3-vinyl-1, 2-dithicyclohex-4-ene, 3-vinyl-1, 2-dithicyclohex-5-ene, di-2-propenyl trisulphide and di-2-propenyl tetrasulfide) were detected in the essential oil of garlic. So, from all of these experimental data, it can be inferred that *Allium sativum* L. (garlic) can be useful for medicinal purposes.

Keywords: *Allium sativum* Linn., DPPH, steam distillation method, GC-MS, EDXRF

Introduction

Garlic is one of those plants that has been used for centuries to fight infectious diseases. Botanically, garlic is known as *Allium sativum* and is a member of Alliaceae family. Garlic is closely related to the onion, shallot, leek, chive, and rakkyo (Ali and Mohsen, 2014).

Garlic is native to Central Asia, from where its cultivation has spread to Southwest Asia and the Mediterranean region. Today, garlic is cultivated in regions with a moderate or subtropical climate all over the world (Al-Snafi, 2013).

Garlic extract has been shown to reduce serum cholesterol levels and increase blood coagulation time (Singh *et al*., 2001). So, garlic is a natural health promoter and a wonder drug available from Mother Nature. Moreover, garlic possesses anticancer, antiviral, antioxidant, and anti-inflammatory properties. The parts of the plant used medicinally include fresh bulbs, dried bulbs and oil extracted from the garlic. So, garlic is the super food to maintain health, and it has been used as a medicinal plant since ancient times, and it is still used in folk medicine all over the world (Sethi *et al.*, 2014). Garlic essential oil is obtained through steam distillation of garlic.

Antioxidant compounds in food are found to have a health-protecting factor. Antioxidants are whole grains, fruits, and vegetables. In this study, the DPPH assay was a rapid, easy and economical method to measure antioxidant activity of the sample, which could be viewed by the naked eye (Rahman *et al*., 2012).

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Gas chromatography-mass chromatography (GC-MS) is a method that combines features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample (Silverstein, 1998). GC-MS has long been the method of choice for identifying volatile compounds in complex mixtures (Karasek, 1988). For the identification of organic constituents in the extracted essential oil of garlic, a GC-MS spectrometer was used to analyse the molecular weight and molecular structure of organic compounds. Mineral elements play an important role in the health and disease of humans and animals. The functions of minerals in humans and animals are interrelated. Deficiencies in trace elements have been implicated in various health problems.

Material and Methods

Sampling

The fresh bulbs of *Allium sativum* L. (garlic) were collected from Pakokku Township, Magway Region (Figure 1).

The sample's fresh bulbs were washed, sliced with a knife, and dried in the shade for 7 days. The dried samples were crushed into fine powder using a blender and stored in an airtight container to prevent moisture changes and other contaminations.

Botanical Aspects

Botanical name	: *Allium sativum* L.
Genus	: *Allium*
Species	: *Sativum*
Family	: Amaryllidaceae
English name	: Garlic
Myanmar name	: Kyat-Thon- Phyu

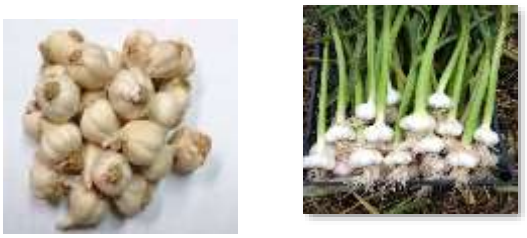


Figure 1. Photographs of *Allium sativum* Linn. (garlic)

Phytochemical Investigation of the Bulbs of *Allium sativum* L. (Garlic)

Phytochemical tests for *Allium sativum* L. (garlic) were carried out according to the test tube method to investigate the presence or absence of phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, tannins, hydrolysable tannins, flavonoids, steroids, and starch. The results are shown in Table 1.

Determination of Qualitative Elemental Analysis of *Allium sativum* L. (Garlic) Bulbs by EDXRF Technique

Some elements were present in dried powder sample; qualitative elemental analysis was performed by the EDXRF (Energy Dispersive X-Ray Fluorescence) method at the Physics Department, Maubin University. The results are shown in Figures 2 and 3.

Screening of Antioxidant Activity of Extracted Essential Oil and Crude Extracts of *Allium sativum* L. (Garlic) by DPPH free Radical Scavenging Assay Method

A DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was chosen to assess the antioxidant activities of extracted essential oil, ethanol and watery extracts of sample. This assay has been widely used to evaluate the free radical scavenging effectiveness of various flavonoids and polyphenols in food systems. The results are shown in Table 2 and Figures 4 and 5.

Extraction of Essential Oil from the Bulbs of *Allium sativum* L. (Garlic) by Steam Distillation Method

A round-bottomed flask was filled with freshly prepared garlic bulb paste (100 g) and 500 mL of distilled water. The flask was fitted with a steam distillation set. When the flask was heated on the heating mantel for about 4 h, the condensed mixture of oil and water separated out and was collected in the receiver flask. From this mixture, the essential oil was isolated in the separation funnel by using n-hexane. The resulting solution was dried over anhydrous sodium sulphate and filtered to get the essential oil.

Identification of Organic Compounds Present in Extracted Essential Oil of *Allium sativum* L. (Garlic) Bulbs by GC-MS Spectroscopic Method

In order to determine the organic constituents in the extracted essential oil of garlic, the GC-MS method was performed at the Department of Research and Innovation National Laboratory (DRI), Yangon. The results are shown in Figures 6 – 12.

Results and Discussion

Preliminary Phytochemical Tests of *Allium sativum* L. (Garlic)

Garlic bulbs were found to contain alkaloids, α- amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, tannins, flavonoids, steroids, hydrolysable tannins, and no starch (Table 1).

Qualitative Elemental Analysis of *Allium sativum* L. (Garlic) by Energy Dispersive X-ray Fluorescence (ED XRF) Technique

X-ray spectrometer permits simultaneous analysis of light elements and heavy metals (Griken *et al*., 1986). In this research work, the relative abundance of elements present in the bulbs of *Allium sativum* L. (garlic) was determined by an ED XRF spectrometer. The ED XRF spectrum is shown in Figures 2 and 3. It can be seen that mineral elements such as K (1.321%), S (1.176%), P (0.387%), Ca (0.081%), Fe (0.006%), Zn (0.004%),Cu(0.003 %), Mn (0.001%),Ni(0.001%), and Sr (0.001%) are present in the sample bulbs. It was found that there is no contamination with toxic metals such as Cd, As, Pb, and Hg.

Screening of Antioxidant Activity of Extracted Essential Oil and Crude Extracts by DPPH Free Radical Scavenging Assay Method

The antioxidant activity was studied on essential oil and crude extracts (95% EtOH and H_2O) of the bulbs of *Allium sativum* L. (garlic) by using a DPPH free radical scavenging assay method. DPPH (1,1-diphenyl-2-picryl-hydrazyl) method is based on changing colour to reduce free radical DPPH in essential oil and crude extracts (95% EtOH and H_2O) of various concentrations. The antioxidant activities were expressed as a 50% oxidative inhibitory concentration (IC_{50}).

In the determination of antioxidant activity, the values of essential oil, 95 % EtOH, and H_2O extracts were found to be 514.79, 55.70, and 147.55 µg/mL respectively. The antioxidant activity of 95% ethanol extract is more potent than the H_2O extract (Table 2, Figures 4 and 5).

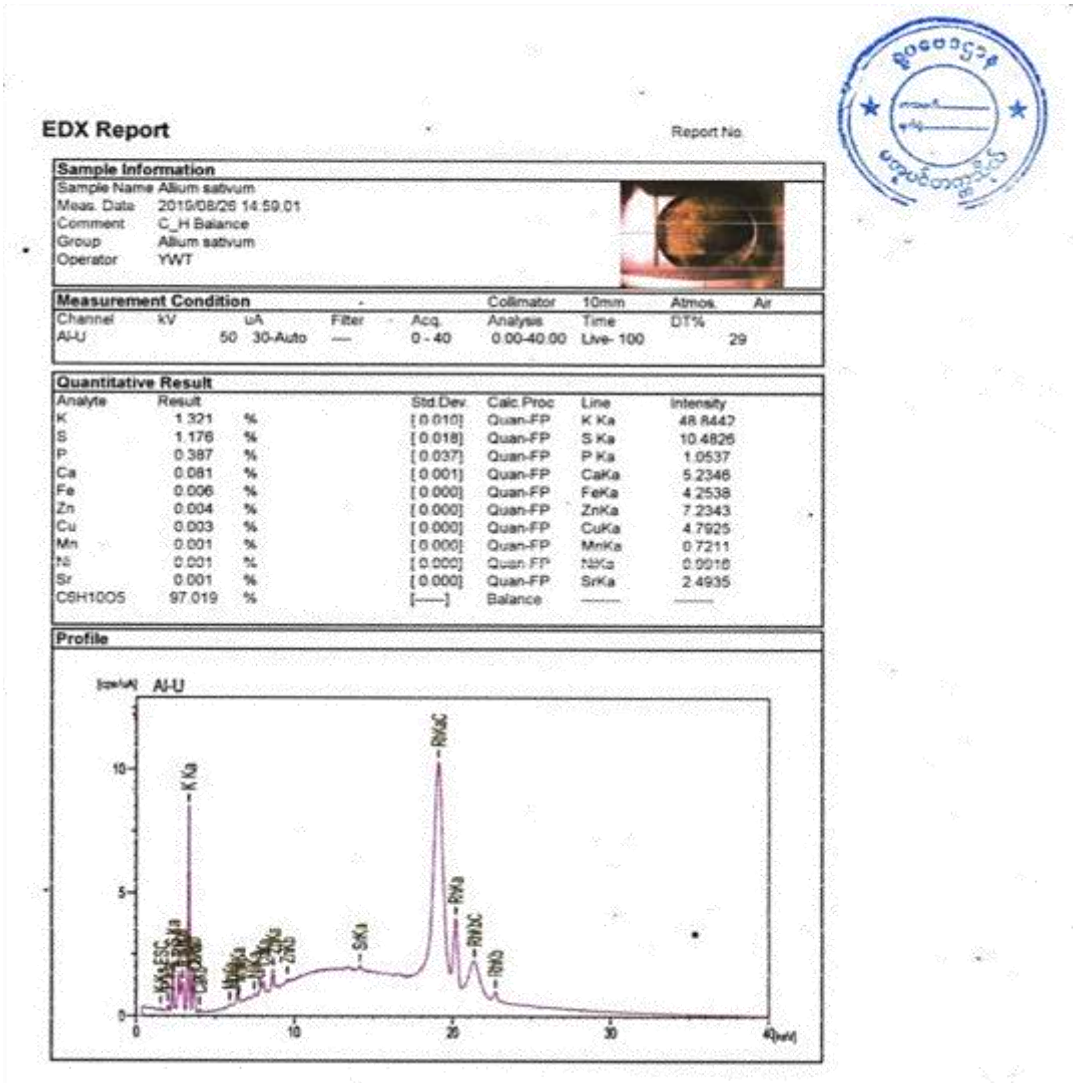
Extraction of Essential Oil from the Bulbs of *Allium sativum* L. (Garlic) by Steam Distillation Method

The essential oil of the bulbs of *Allium sativum* L. (garlic) was extracted by steam distillation. The yield percent of essential oil was 21.3 %.

Table 1. Results of Phytochemical Investigation of the Bulbs of *Allium sativum* L. (Garlic)

No.	Tests	Extract	Test reagents	Observation	Results
			(i) Dragendorff's reagent	orange ppt	+
1.	Alkaloids	1 % HCl	(ii) Mayer's reagent	white ppt	+
			(iii)Wagner's reagent	reddish brown ppt	+
2.	α -amino acids	H_2O	Ninhydrin reagent	purple spot	+
3.	Carbohydrates	H_2O	10 % α-Naphthol and conc:H_2SO_4	violet ring	+
4.	Glycosides	H_2O	10 % lead acetate	white ppt	+
5.	Phenolic compounds	H_2O	10 % ferric chloride	deep blue colour	+
6.	Reducing sugars	H_2O	Benedict's solution	green colour	+
7.	Saponins	H_2O	Distilled water	frothing	+
8.	Starch	H_2O	1 % Iodine solution	no deep blue colour	-
9.	Tannin	H_2O	1 % Gelatin sol^n: and 5 % $FeCl_3$	white ppt	+
10.	Hydrolysable Tannin	H_2O	10 % NaOH	emulsion on shaking	+
11.	Flavonoids	95 % EtOH	Mg ribbon and conc: HCl	pink colouration	+
12.	Steroids	MeOH	$CHCl_3$ and conc:H_2SO_4	red colour	+

(+) present, (-) absent, ppt = precipitate



EDX Report

Report No.

Sample Information

Sample Name	Allium sativum
Meas. Date	2019/08/26 14:59:01
Comment	C_H Balance
Group	Allium sativum
Operator	YWT

Measurement Condition

Channel	kV	uA	Filter	Acq.	Collimator	10mm	Atmos.	Air
					Analysis Time	Live- 100	DT%	
Al-U	50	30-Auto	---	0 - 40	0.00-40.00		29	

Quantitative Result

Analyte	Result		Std.Dev.	Calc.Proc	Line	Intensity
K	1.321	%	[0.010]	Quan-FP	K Ka	48.8442
S	1.176	%	[0.018]	Quan-FP	S Ka	10.4826
P	0.387	%	[0.037]	Quan-FP	P Ka	1.0537
Ca	0.081	%	[0.001]	Quan-FP	CaKa	5.2346
Fe	0.006	%	[0.000]	Quan-FP	FeKa	4.2538
Zn	0.004	%	[0.000]	Quan-FP	ZnKa	7.2343
Cu	0.003	%	[0.000]	Quan-FP	CuKa	4.7925
Mn	0.001	%	[0.000]	Quan-FP	MnKa	0.7211
Ni	0.001	%	[0.000]	Quan-FP	NiKa	0.0016
Sr	0.001	%	[0.000]	Quan-FP	SrKa	2.4935
C9H10O5	97.019	%	[----]	Balance	------	------

Profile

Figure 2. ED XRF spectrum of the bulbs of *Allium sativum* L. (garlic)

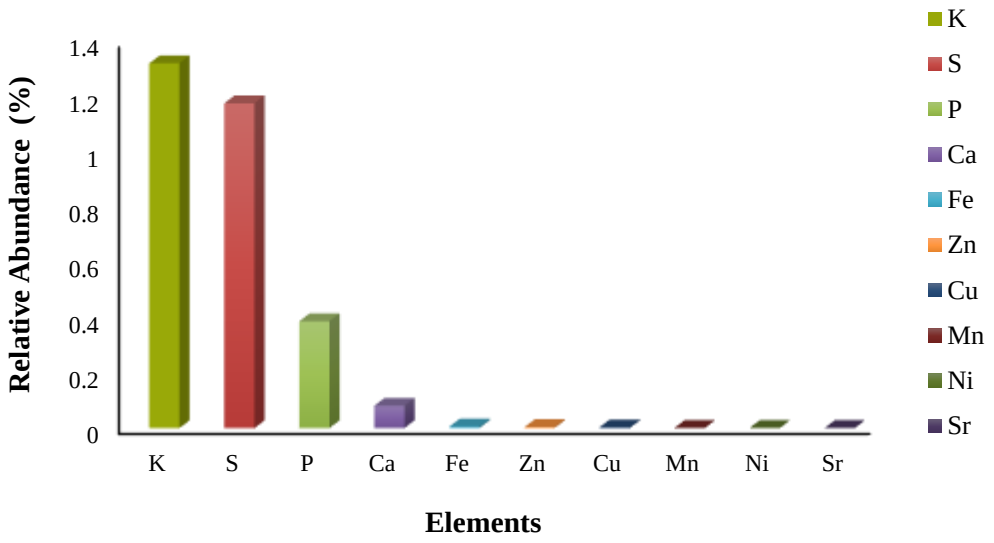


Figure 3. The bar graph of relative abundance of elements in the *Allium sativum* L. (garlic) by ED XRF

Table 2. Percent RSA and IC_{50} Value of Essential Oil and Crude Extracts (95 % EtOH and H_2O) from the Bulbs of *Allium Sativum* L. (Garlic) and Standard DPPH in Various Concentrations

Extracts	Percent RSA ± SD of different concentrations of extracts (%)							IC_{50}
	12.5	25.0	50.0	100.0	200.0	400.0	800.0	(µg/mL)
Watery	37.77 ± 0.009	39.29 ± 0.004	43.68 ± 0.003	47.53 ± 0.004	52.88 ± 0.005	65.66 ± 0.010	78.22 ± 0.015	147.55
95% Ethanol	30.82 ± 0.004	37.95 ± 0.002	47.81 ± 0.001	67.40 ± 0.006	92.74 ± 0.004	94.79 ± 0.004	102.34 ± 0.007	55.70
Essential oil	30.39 ± 0	31.22 ± 0.0007	32.53 ± 0.0014	35.39 ± 0.0042	37.30 ± 0.0007	45.411 ± 0.0148	77.82 ± 0.0153	514.79
DPPH (Std.)	49.23 ± 0.002	59.36 ± 0.006	71.63 ± 0.002	86.20 ± 0.000	97.24 ± 0.001	98.47 ± 0.000	99.06 ± 0.003	13.46

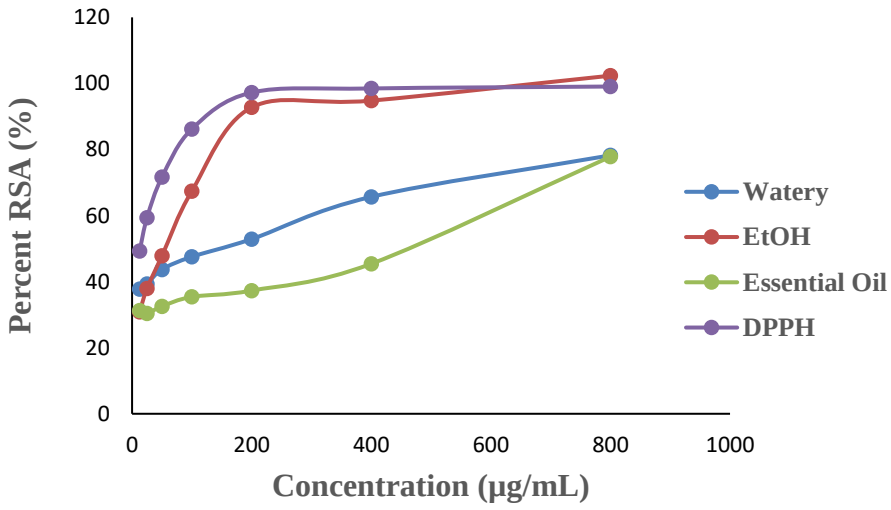


Figure 4. Plot of percent radical scanvenging activity (% RSA) vs. concentration (µg/mL) of extracted essential oil and crude extracts of *Allium sativum* L. (garlic) and standard DPPH

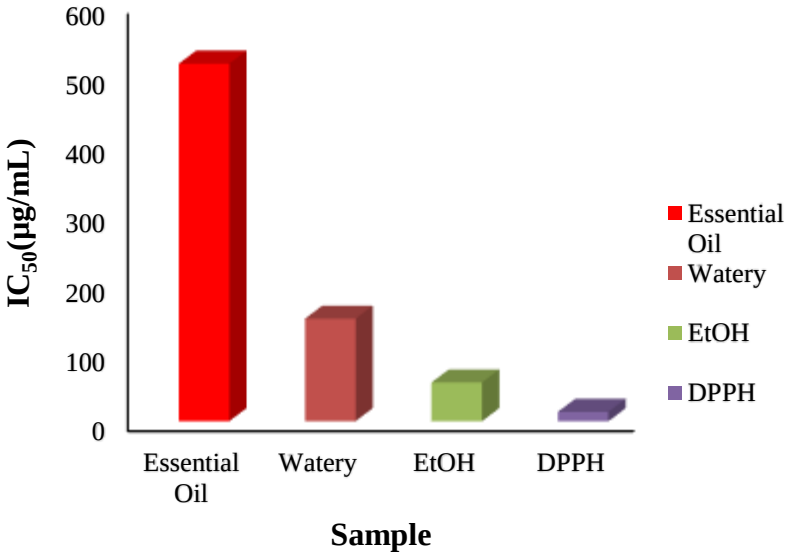


Figure 5. The bar graph of IC_{50} values of extracted essential oil and crude extracts of the bulbs of *Allium sativum* L. (garlic)

Identification of Organic Compounds in Essential Oil of *Allium sativum* L. (Garlic) by GC-MS Spectroscopic Method

Gas chromatography-mass spectrometry (GC-MS) is the most important tool for the identification of unknown organic compounds, both by matching spectra with reference spectra and by a priori spectral interpretation. According to the GC-MS chromatogram, the peak appears at the retention time of 6.60 min with 100 % abundance. At this retention time of 2.925 min, the GC-MS spectra (Figure 6) show the molecular ion peak at *m/z* 120, indicating the molecular weight of the compound is 120 and the molecular formula is $C_4H_8S_2$, and so this compound is methyl-1-propenyl disulphide. At this retention time, 4.728 min, the GC-MS spectra (Figure 7) show the molecular ion peak at *m/z* 146, indicating the molecular weight of the compound is 146 and the molecular formula is $C_6H_{10}S_2$, and so this compound is diallyl disulphide. At this retention time of 4.736 min, the GC-MS spectra (Figure 8) show the molecular ion peak at *m/z* 146, indicating the molecular weight of the compound is 146 and the molecular formula is $C_6H_{10}S_2$, and so it is 2-vinyl-1,3-dithiane. At this retention time of 6.254 min, the GC-MS spectra (Figure 9) show the molecular ion peak at *m/z* 144, indicating the molecular weight of the compound is 144 and the molecular formula is $C_6H_8S_2$, and so it is 3-vinyl-1,2-dithiacyclohex-4-ene. At this retention time of 6.596 min, the GC-MS spectra (Figure 10) show the molecular ion peak at *m/z* 144, indicating the molecular weight of the compound is 144 and the molecular formula is $C_4H_8S_2$, and so it is 3-vinyl-1,2-dithiacyclohex-5-ene. At this retention time of 7.700 min, the GC-MS spectra (Figure 11) show the molecular ion peak at *m/z* 178, indicating the molecular weight of the compound is 178 and the molecular formula is $C_6H_{10}S_3$; and so, it is di-2-propenyl trisulphide. At this retention time of 10.772 min, the GC-MS spectra (Figure 12) show the molecular ion peak at *m/z* 210, indicating the molecular formula is $C_6H_{10}S_4$, and so it is di-2-propenyl tetrasulphide.

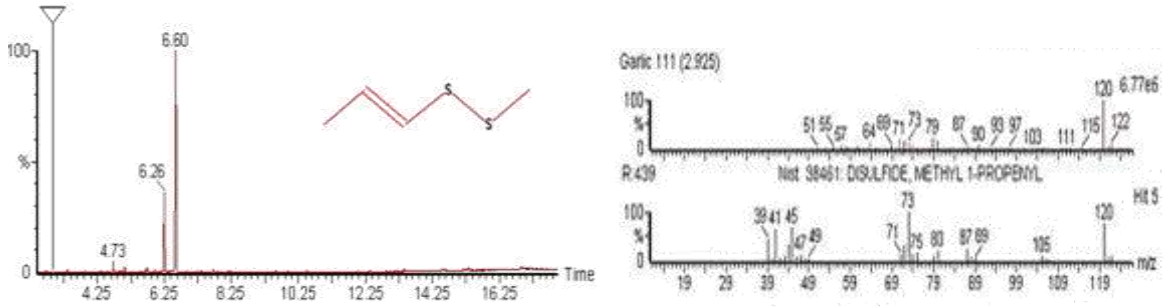


Figure 6. Gas chromatogram and mass spectra of methyl-1-propenyl disulphide

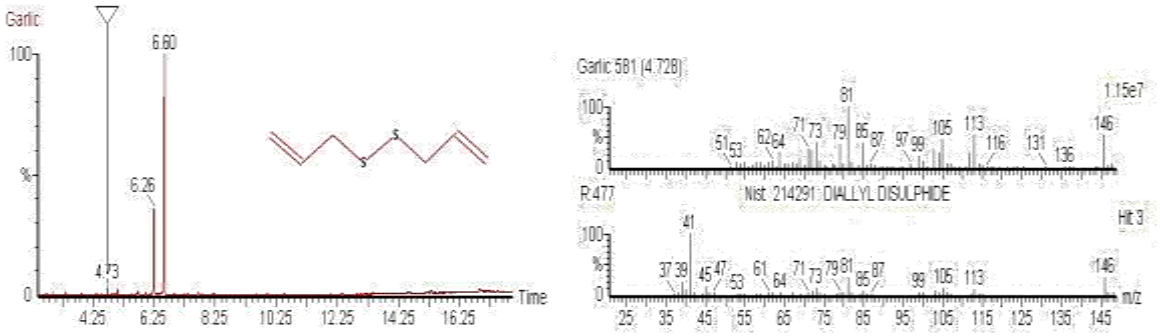


Figure 7. Gas chromatogram and mass spectra of diallyl disulphide

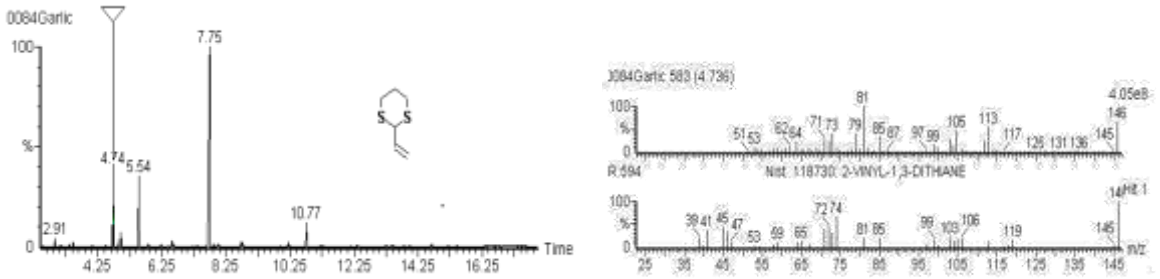


Figure 8. Gas chromatogram and mass spectra of 2-vinyl-1, 3-dithiane

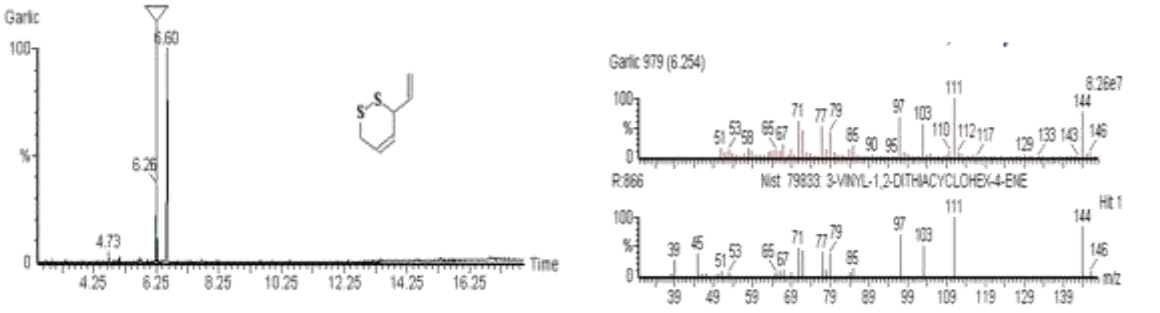


Figure 9. Gas chromatogram and mass spectra of 3-vinyl-1,2-dithiacyclohex-4-ene

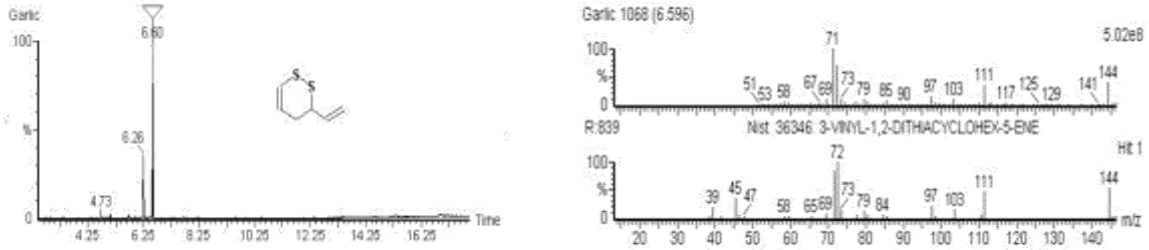


Figure 10. Gas chromatogram and mass spectra of 3-vinyl-1,2-dithiacyclohex-5-ene

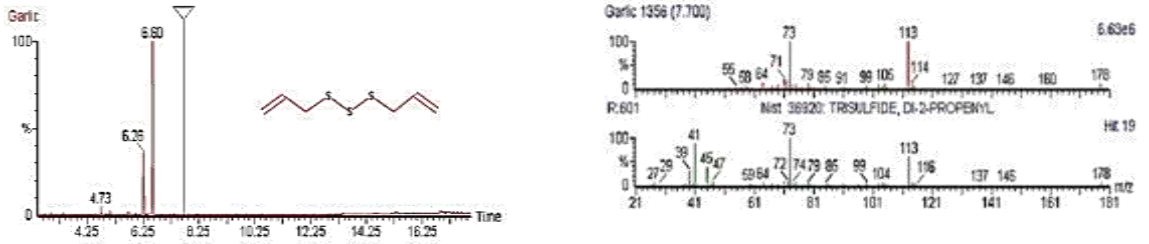


Figure 11. Gas chromatogram and mass spectra of di-2-propenyl trisulphide

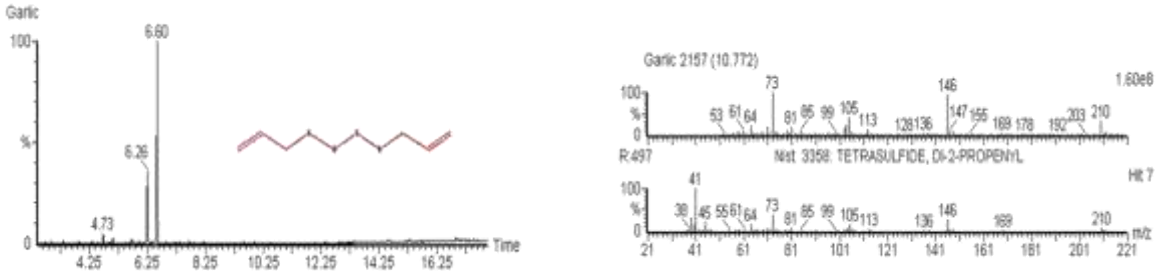


Figure 12. Gas chromatogram and mass spectra of di-2-propenyl tetrasulphide

Conclusion

The bulbs of garlic were chosen to be studied in the present work because these bulbs have many biological activities. Alkaloids, α- amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, tannins, hydrolysable tannins, flavonoids, and steroids are present in garlic bulbs, but starch is not. From the study of the elemental analysis of the *Allium sativum* L. (garlic) bulbs, the essential metals potassium (1.321%), sulphur (1.176%), calcium (0.081%), and iron (0.006%) were found in the garlic sample. The toxic heavy metals such as arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) were not detected in the bulb sample. The essential oil (21.3% yield) was extracted from *Allium sativum* L. (garlic) by steam distillation. The antioxidant activity of extracted essential oil, ethanol extract, and watery extract was screened by the DPPH free radical scavenging assay method. The IC_{50} value of extracted essential oil was found to be 514.79 µg/mL, 95% EtOH extract was 55.70 µg/mL and watery extract was 147.55 µg/mL. So, 95% EtOH has the most potent antioxidant activity. Seven sulphur containing compounds were detected in garlic essential oil by GC-MS analysis: methyl-1-propenyl disulphide, diallyl disulphide, 2-vinyl-1, 3-dithiane, 3-vinyl-1, 2-dithicyclohex-4-ene, 3-vinyl-1, 2-dithicyclohex-5-ene, di-2-propenyl trisulphide, and di-2-propenyl tetrasulphide. Therefore, it may be deduced from all of these experimental results that *Allium sativum* L. (garlic) has Therefore, it may be deduced from all of these experimental results that Allium sativum L. (garlic) has medicinal value.

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