INVESTIGATION OF ORGANIC CONSTITUENTS AND TOTAL PHENOL CONTENT OF EXTRACTED ESSENTIAL OIL FROM THE BULBS OF *ALLIUM SATIVUM* LINN. (GARLIC)

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Abstract

Natural products from plants have been used for thousands of years to treat many diseases. Garlic is one of those plants that is used to fight infectious diseases. In this research work, the essential oil was isolated from the bulbs of *Allium sativum* Linn. (Garlic) by steam distillation method. Then, the organic constituents in extracted essential oil of garlic were investigated by GC-MS at Department of Research and Innovation National Laboratory (DRI), Yangon. Seven compounds (disulfide methyl-1-propenyl, diallyl disulphide, 2-vinyl-1, 3-dithiane, 3-vinyl-1, 2-dithicyclohex-4-ene, 3-vinyl-1, 2-dithicyclohex-5-ene, trisulphide di-2-propenyl and tetrasulphide di-2-propenyl) were detected in essential oil of garlic. The total phenol content of extracted essential oil and two crude extracts were determined by Folin-Ciocalteu reagent method. Total phenol content was expressed as milligram gallic acid equivalent per gram (mg GAE/g) of extract. The higher total phenol content was detected in ethanol extract.

Keywords: *Allium sativum* Linn., DRI, Folin-Ciocalteu reagent, gallic acid equivalent.

INTRODUCTION

Natural products of animals, plants and microbial sources havebeen used for thousands of years either in the pure forms or crude extracts to treat many diseases. Garlic is one of those plants that isused for centuries to fight infectious diseases.Botanically, garlic is known as *Allium sativum* and is a member of Alliaceae family.

Garlic is used worldwide as a food additive, spice and medicine.Garlic has been proved to elicit antimicrobial, antihypertensive, hypolipidemic, hepatoprotective, antidiabetic and insecticidal properties. Immunomodulation and antitumor activities of garlic have been reported. Garlic extract has been shown to reduce serum cholesterol levels and increase blood coagulation time (Singh *et al*., 2001).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 medicinal plant since ancient times and it is still been used in folk medicine all over the world.

Experimental

Sampling

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

The collected fresh bulbs of sample were washed, sliced with knife and dried under shade for 7days. The dried samples were crushed into fine powder using a

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blender and stored in air tight 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)

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

The freshly prepared garlic bulbs paste (100 g) was added in round-bottomed flask and distilled water (500 mL) was added. The flask was flitted with steam distillation set. When the flask was heated at heating mantel for about 4 hr, the condensed mixture of oil and water was separation out and collected in the receiver flask. Form 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.

Determination of Total Phenol Content of Extracted Essential Oil by FCR Method

Construction of gallic acid standard curve

At first, 1 mL of different concentrations of gallic acid solution (1000, 100, 75, 50, 30, 25, 12.5, 6.25 and 3.125) µg/mL were mixed with 2.5 mL of FC reagent (FCR: H_2O; 1:10) and incubated for 5minutes. To each tube, 2 mL of 1 M sodium carbonate solution was added and the tubes were kept at room temperature for 15 minutes and the absorbance of reaction mixture was measured at λ_{max} 765 nm. A standard curve was constructed by plotting the absorbance against concentration of gallic acid.

Determination of total phenol content

Each sample solution 1 mL was mixed with 2.5 mL of FC reagent (1:10) and incubated for about 5 minutes. To each test tube, 2 mL of 1 M sodium carbonate was added and the test tubes were kept at room temperature for 15 minutes and absorbance of reaction mixture was measured at λ_{max} 765 nm. . The absorbance of blank solution using distilled water was measure. Total phenol content was estimated as microgram

gallic acid equivalent per gram (mg GAE/g) of extract. The total phenol content of test samples were calculated by the following formula.

$$C = \frac{c\,V}{m}$$

C = Total phenol content

c = concentration of gallic acid obtained from calibration curve

V = volume of extract

m = mass of extract

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 extracted essential oil of garlic, GC-MS method was used by Department of Research and Innovation National Laboratory (DRI), Yangon.

RESULTS AND DISCUSSION

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

Essential oil of *Allium sativum* L. (Garlic) was extracted by using the steam distillation method. The yield percent of essential oil was found to be 0.5 % based on the raw garlic bulbs sample.

Determination of Total Phenol Content of Extracted Essential Oil of *Allium sativum* L. (Garlic) Bulbs by FCR Method

The total phenol content of essential oil and two crude extracts (EtOH and H_2O) of *Allium sativum* L. (Garlic)were evaluated with spectrophotometric method using Folin-Ciocalteu reagents. The absorbance of standard gallic acid solution (difference conc :) were measured at different wavelengths (500-1000 nm). The maximum absorbance was observed at 765 nm. The principle of this method is the reduction ability of phenol functional group. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagents under alkaline conditions and result in the formation of blue coloured complex. The reduction of complex will be increased when the extracts contained more phenolic compounds. Thus, the colour will be darker and the absorbance will be higher. According to the results, the higher TPC (mg GAE/g) was detected in EtOH extract than watery extract of garlic. This means that phenolic compounds are more soluble in ethanol. The TPC value of extracted essential oil was found to be (0.241 mg GAE/g oil), 95% EtOH extract was (1.615 mg GAE/g extract) and H_2O extract was (0.518 mg GAE/g extract).

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 identification of unknown organic compounds by matching spectra with reference spectra. According to GC-MS chromatogram, the peak appears at the retention time 6.60 min with 100 % abundance.

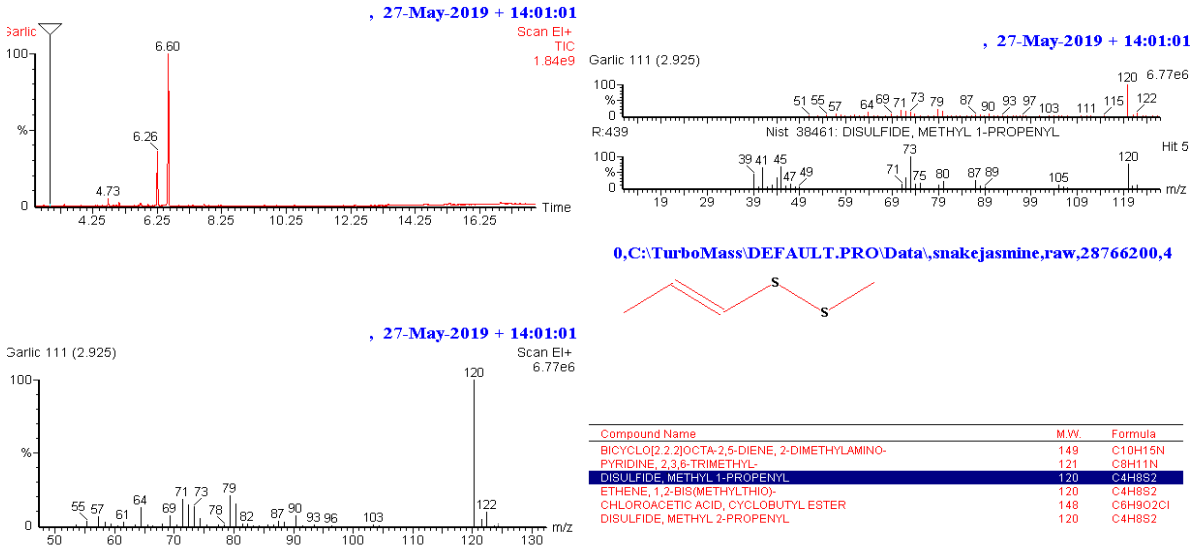

Figure 2.Gas chromatogram and mass spectrum of disulphide methyl-1-propenyl.

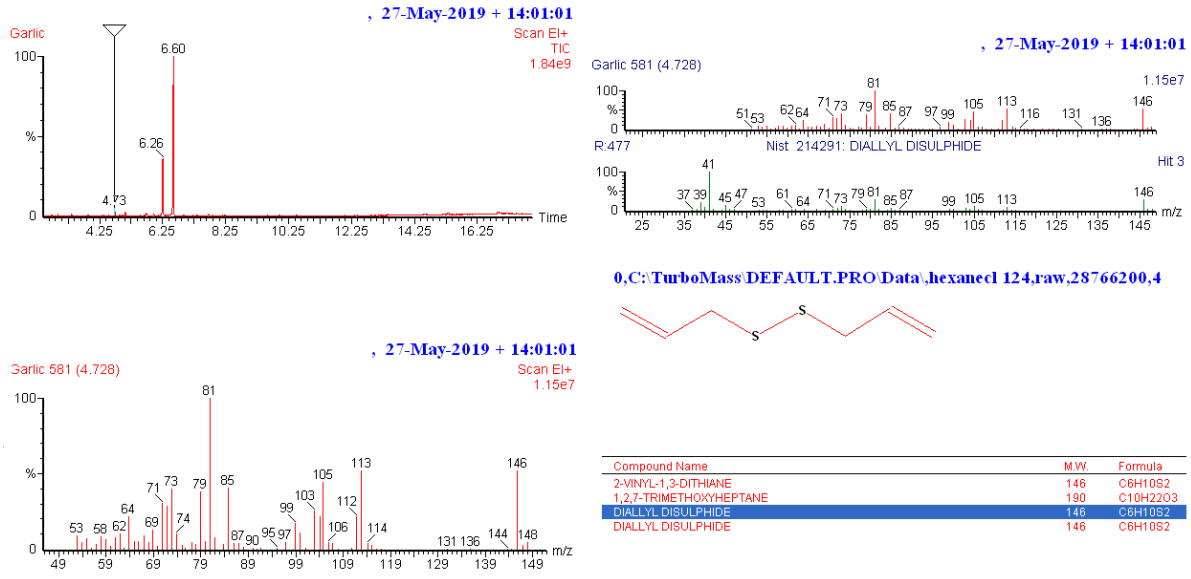

Figure 3.Gas chromatogram and mass spectrum of diallyl disulphide

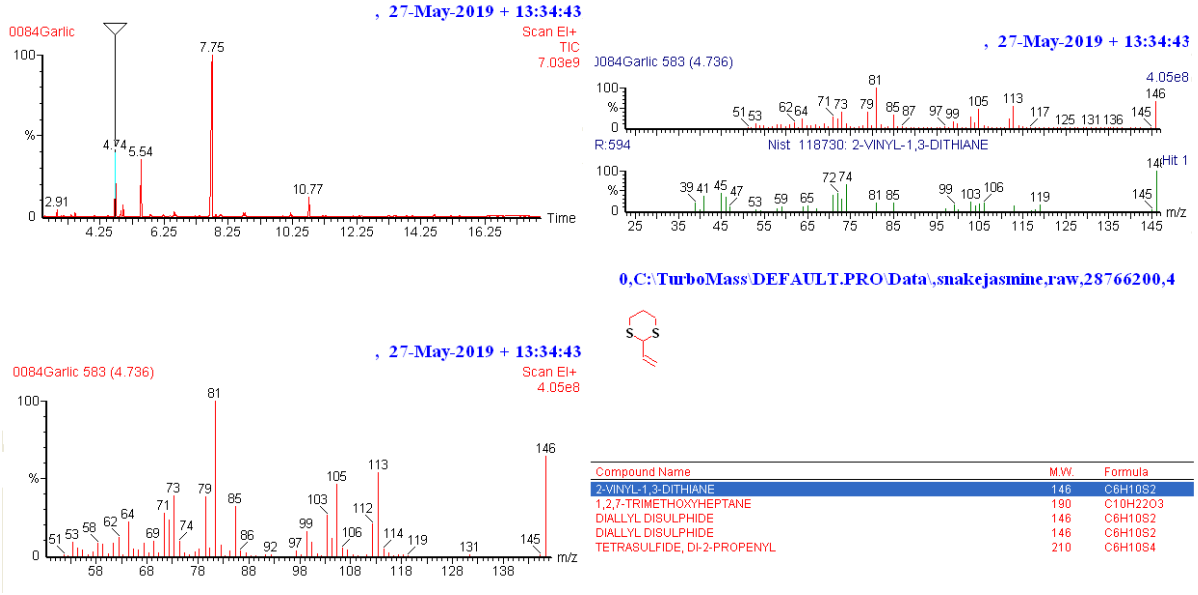

Figure 4.Gas chromatogram and mass spectrum of 2-vinyl-1, 3-dithiane

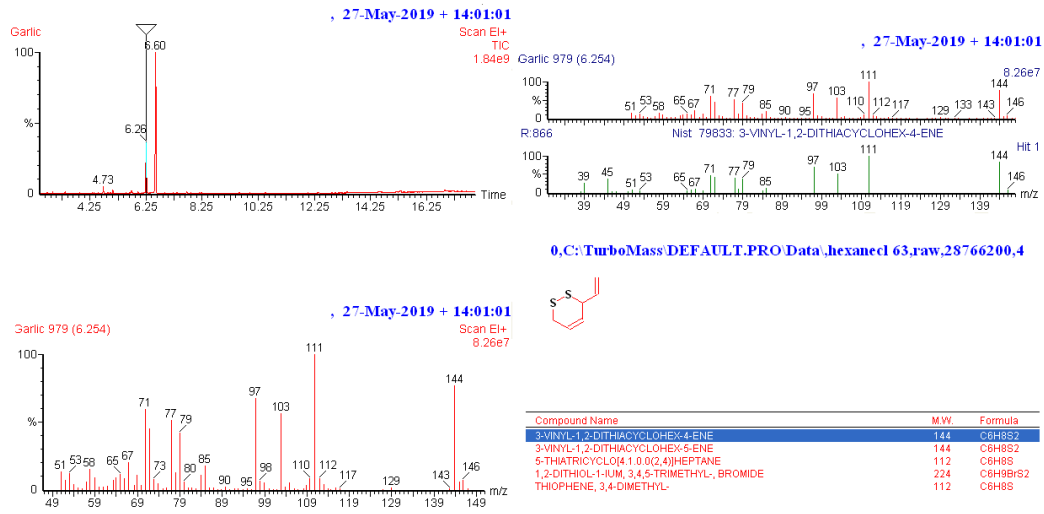

Figure 5.Gas chromatogram and mass spectrum of 3-vinyl-1, 2-dithiacyclohex-4-ene

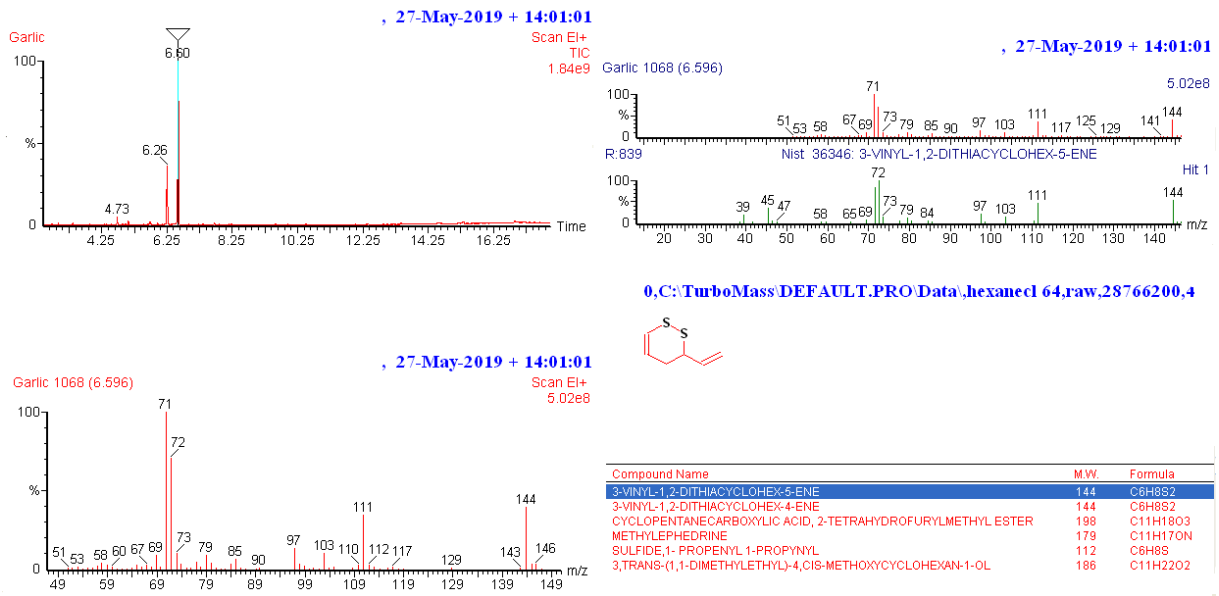

Figure 6.Gas chromatogram and mass spectrum of 3-vinyl-1, 2-dithiacyclohex-5-ene

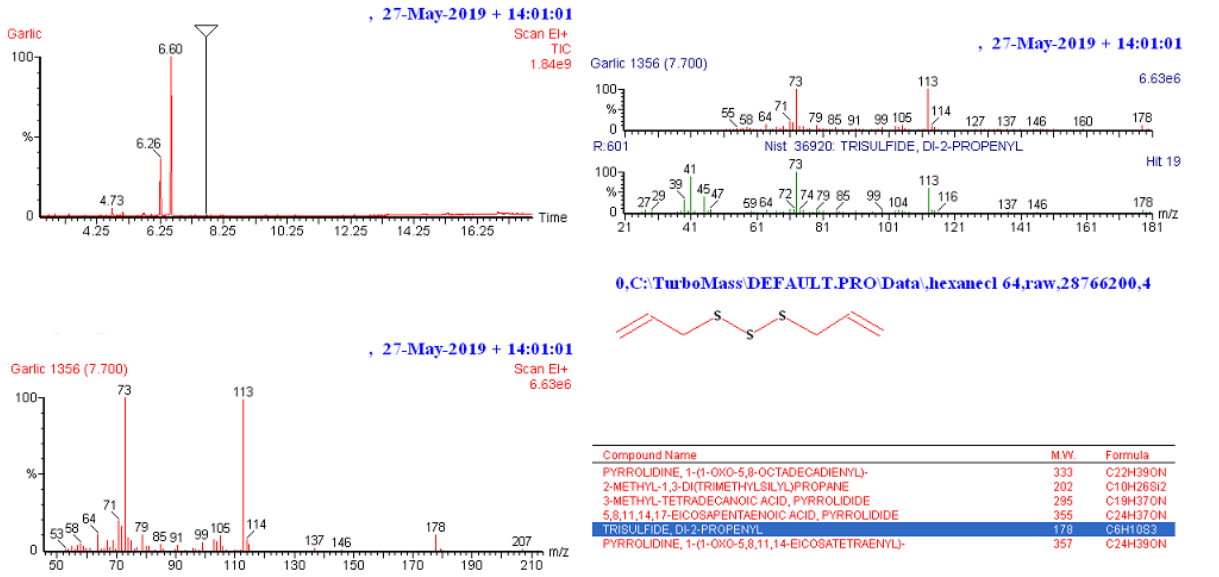

Figure 7.Gas chromatogram and mass spectrum of trisulphide di-2- propenyl.

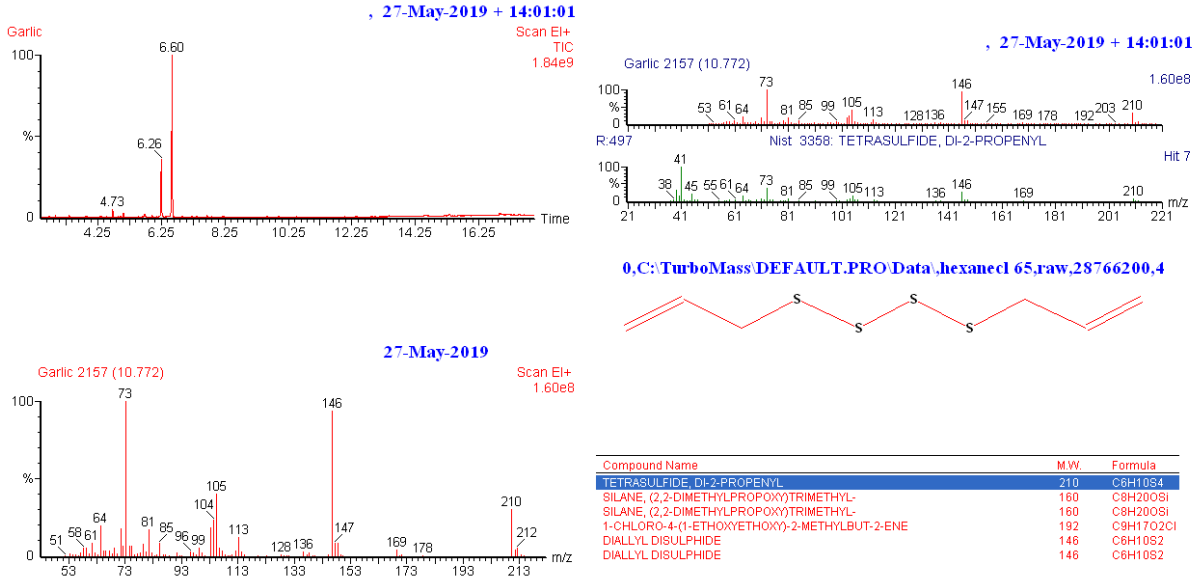

Figure 8.Gas chromatogram and mass spectrum of tetrasulphide di-2- propenyl

CONCLUSION

The essential oil was isolated from *Allium sativum* L. (Garlic) by steam distillation method. The mixture of essential oil with water was obtained and so, separation with n-hexane was carried out. The yield percent of essential oil was 0.5 % on the basic of bulb paste.Furthermore, the total phenol content of extracted essential oiland two crude extracts were determined spectrophotometrically by the Folin-Ciocalteu method.The higher total phenol content was detected in ethanol extract. From GC-MS of essential oil, seven sulphur containing compounds such as (disulfide methyl-1-propenyl, diallyl disulphide, 2-vinyl-1, 3-dithiane, 3-vinyl-1, 2-dithicyclohex-4-ene, 3-vinyl-1, 2-dithicyclohex-5-ene, trisulfide, di-2-propenyl and tetrasulfide, di-2-propenyl) were detected.*Allium sativum* L. (Garlic) is useful for medicinal purposes.

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