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Research Article

Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of Two Varieties of *Allium sativum Linn* (Garlic)

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Abstract

Allium sativum Linn. is locally known as Garlic in Bangladesh. It is used as spices all over the world. In this study essential oil of the two varieties of garlic from Bangladesh and China were investigated by GC-MS. The regular consumption of garlic oil can reduce blood pressure; prevent heart disease including atherosclerosis, high cholesterol and cancer[1]. Garlic oil is an effective antibiotic, anti-viral, anti-fungal agent, which could be used to prevent nausea, diarrhea, ease coughs, even treatment in conditions such as malaria and cholera probably an immune system enhancement, some studies have found lower rates of certain types of cancer in people[2]. Garlic is one of the most popular spices in the world. Total 11 chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from both the essential oil of Bangladeshi and China garlic. Among them Diallyl disulfide (28.99%); Trisulfide, methyl 2-propenyl (23.22%) and Trisulfide, di-2-propenyl (15.36%) are the principle components of Bangladeshi garlic. In China garlic, Trisulfide, methyl 2-propenyl (29.12%); Trisulfide, di-2-propenyl (21.98%) and Diallyl disulfide (17.24%) were the major components. Quantification of active principles through analytical tools is essential for establishing the authenticity and credibility. Steam distillation extraction combined with GC-MS has been shown to be a valuable tool for the analysis of garlic constituents and can provide a useful guide to component variation. The main objective of the present study was focused on identification and quantification of chemical constituents present in the essential oil of garlic using GC-MS method.

1. INTRODUCTION

Garlic (*Allium sativum Linn.*), is one of the edible plants which has generated a lot of interest throughout human history as a medicinal panacea. It is a perennial plant belonging to the genus *Allium. Allium* the Latin word given to garlic[3], a flowering plant with hundreds of distinct species; which many have been harvested through human history, but only about a dozen are still economically important today as crops or garden vegetables[4]. One of the species belong to this genus is the *Allium sativum Linn.* also known as the cultivated garlic, which belongs to the family *Alliaceae* (Formerly classified in the lily (Liliaceae) family)[5] and closely related to the onion, shallot, leek, chive, and rakkyo[6]. It has been used throughout recorded history for both culinary and medicinal propose [7]. Garlic plant is moderately tall (up to 3 feet) it is an erect herb normally grown as an annual that is a plant that only last for a year, it has adventitious roots and condensed, flattened stem and narrow flat leaves. The bulb consists of 6 to 35 bulblets called cloves various shapes and sizes[8-10] with glistering and transparent covering. They grow in various conditions from dry, well-drained mineral-based soil to moist organic soil, most grow in sunny locations but a number also grow in forests, or even in swap water areas [11]. Garlic is propagated by planting cloves or top bulblets. A classic ingredient in many national cuisines, garlic has a powerful onion like aroma and pungent taste [12]. Though Original abode of garlic in Central Asia [13], garlic has been cultivated in the Middle East for over 6000 years, making one of mankind's first cultivated plants. The medicinal benefits and claims for garlic have awarded it the name "wonder drug among all herbs". Garlic contains 0.1 to 0.36% essential oil, the principal components of which are diallyl disulfide, diallyl trisulfide, allyl propyl disulfide. European standards specify that garlic supplements contain not less than 0.45% Allicin[14]. Allicin is the active principal and a yellow liquid responsible for the odor of freshly crushed garlic[15]. When the clove is crushed, the enzyme alliinase is activated and breaks down alliin into allicin and other allyl thiosulfinates[16]. At least 35 different compounds have been identified in garlic[17]. These sulfur-containing compounds have been the focus of the many quantitative and qualitative studies on garlic and related species [18].

Commercially available garlic oil capsules generally contain vegetable oil and a small amount of garlic essential oil because of the pungent odors. Other garlic supplements fall into one of these categories; dehydrated garlic powder, garlic oil macerate and aged garlic extract[19]. The regular consumption of garlic oil can reduce blood pressure; prevent heart disease including atherosclerosis, high cholesterol and cancer [20]. Garlic oil is an effective antibiotic, anti-viral, anti-fungal agent, which could be used to prevent nausea, diarrhea, ease coughs, even treatment in conditions such as malaria and cholera probably an immune system enhancement, some studies have found lower rates of certain types of cancer in people [21]. In 2007, the BBC reported *Allium sativum* may have other beneficial properties, such as preventing and fighting the common cold [22].

However many researchers have been carried out on garlic (*Allium sativum Linn.*), but no systematic research on comparative studies has been reported on the essential oil of garlic in Bangladesh and China. Some disagreement about the presence of its constituents was observed. Therefore, present work was undertaken to carry out a complete investigation of the essential oil of *Allium sativum Linn.* of two varieties from Bangladesh and China including its physical properties & chemical constituents along with GC-MS analysis.

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2. MATERIALS AND METHODS

The fresh Bangladeshi and China garlic are available in the local markets of Dhaka. The collected samples were washed clearly by water to remove dust materials. Then they were dried. Finally the dried garlic was ground by Fritsch mortar grinder, Germany for one hour. Then the powder was sieved prior to the extraction process. The mean particle diameters obtained were 0.25 and 0.50mm.

2.1 Extraction of essential oil

There are a number of methods employed for the extraction of essential oil or volatile oil from the plant. In the present study steam distillation method was used. This extraction procedure was simple and it could also provide a valuable means of producing flavor extracts of two varieties from Bangladeshi and China garlic under mild conditions which preserve the natural characteristics of the fresh product. In the process, definite amount of sample (dirt free powdered garlic) were taken in a distillation flask (Clevenger's apparatus). Then distilled water was added two third of its volume to the flask. Then the flask was heated by electric heating mental for 4 hours. Volatile substances of aniseed and generated steam in the flask were condensed by water condenser. The essential oil was lighter than water and so could be separated out. The steam distilled essential oil layer which was collected over water, was extracted and washed with analytical grade ether or chloroform. The ether extract of the oil was dried over anhydrous Na_2SO_4 and then filtered. It was collected in vial. The ether or chloroform was removed in vacuum condition. Thus the essential oil of fresh garlic was collected.

2.2 GC-MS Analysis

The essential oil of *Allium sativum Linn.* (Garlic) of two varieties were analyzed by Electron Impact Ionization (EI) method on GC-17A gas chromatograph, coupled to a GC-MS 2010 plus mass spectrometer; fused silica capillary column temperature of 40˚ C (was held 2 min) was maintained with carrier gas helium at a constant pressure of 90kPa. Samples were injected by splitting with the split ratio 10. Essential oil sample was dissolved in chloroform. The operating condition were as follows: name of column- RTS-5MS, diameter 30 cm, length 0.25mm, temperature of the column-initial temperature 40˚C (was held 2 min) , injector temperature-220 ˚C, holding time 5 min, column packing- column packing was done with 10% diethylene glycol succinate on 100-120 mesh diatomic CAW, splitting- samples were injected by splitting with the spilt ratio 10, carrier gas- helium gas at constant pressure 90 kPa, sample dissolved- in chloroform, range of linear temperature increase- 10˚C per min.

2.3 Preparation of essential oil samples for GC-MS analysis

Essential oil was diluted to 7% by chloroform. An inert gas (i.e. nitrogen) was introduced, from a large gas cylinder through the injection part, the column and the detector. The flow rate of the carrier gas was adjusted to ensure reproducible retention time and to minimize detector dirt. The sample was then injected by a micro syringe through a heated injection part when it was vaporized and carried into the column. The long tube of the column was tightly packed with solid particles. The solid support was uniformly covered with a thin film of a high boiling liquid (the stationary phase). The mobile and stationary phases were then partitioned by the samples and it was separated into the individual components. The carrier gas and sample component was then emerging from the column and passed through a detector. The amount of each component as concentration by the device and generates a signal which was registered electrically. The signal passed to a detector.

2.4 Identification of the components

The physical properties of the essential oil of *Allium sativum Linn.* (Garlic) of two varieties of Bangladesh and China are presented in Table-1. Interpretation of mass spectroscopy (GC-MS) was conducted using data base of National Institute Standard and Technology (NIST) having more than 62000 patterns. The spectrum of the unknown component was compared with the spectrum of the known component stored in the NIST library. The retention time, molecular weight, molecular formula and composition percentage of the sample material was recorded and presented in Table -2 and Table-3.

3. RESULTS AND DISCUSSION

The physical characteristics such as color, appearance, specific gravity, optical rotation, solubility, refractive index of the essential oil were determined by conventional method. The result of the physical properties of *Allium sativum Linn.* (Garlic) of two varieties from Bangladesh and China are presented in Table-1.The percentage yield was found to be Bangladeshi garlic contained essential oil 0.1249 % and China garlic contained 0.1364 %. The colour of the essential oil was light yellow with a pungent odour. Refractive Index of essential oil was 1.56678 and 1.56470 of Bangladeshi and China garlic. The slight variation of this oil content and the composition of the essential oil depend on several factors such genotype, stage of maturity, cultivation peculiarities, soil composition and climate differences in various geographical locations. Fluctuation of the oil composition can impart change in the organoleptic properties of the plant belonging to the botanical spices and variety. So far we aware till now no systemic investigation on the *Allium sativum L.*(Garlic) have not been investigated in Bangladesh by using modern analytical techniques.

GC-MS analyzed results which include the active principles with their retention time, molecular formula, molecular weight and composition of the essential oil of *Allium sativum L.*(Garlic) of two varieties from two different countries of Bangladesh and China are presented in Table-2 and Table-3. 11 major components of essential oils were found from both the samples by using GC-MS. Among them Bangladeshi garlic bulb (clove) contained 1-Propene, 3, 3' –thiobis (0.89%); Disulfide, methyl 2-propenyl (3.27%); β-Phenylethyl butyrate (1.85%); Diallyl disulfide (28.99%); Trisulfide, methyl 2-propenyl (23.22%); Hydroxylamine, methyl-(1-phenylethyl) (1.45%); Naphthalene, decahydro (2.91%); 3-Vinyl-1,2-dithiacyclohex-4-ene (5.47%); Trisulfide, di-2-propenyl (15.36%); 3H-1,2,4-Triazole-3-thione,2,4-dihydro-4-methyl (3.57%); 2-Chloro-4,4-dimethyl-1,3,2-oxathiaphospholane (9.11%). China garlic bulb (clove) contained 1-Propene, 3, 3' –thiobis (0.89%); Disulfide, methyl 2-propenyl (4.94%); Diallyl disulfide (17.24%); Trisulfide, methyl 2-propenyl (29.12%); Benzene, 1-methyl-3-(methylthio) (4.01%); 2,4-Dithiahex-5-ene 2,2-dioxide (1.01%); 3-Vinyl-1,2-dithiacyclohex-4-ene (5.65%); Trisulfide, di-2-propenyl (21.98%); 3H-1,2,4-Triazole-3-thione,2,4-dihydro-4-methyl (1.64%); Trimethyl silyldiazomethane (4.48%); 1,2,4,6-Tetrathiepane (9.05%).

Results show that essential oil from both of the two varieties from two different places of Bangladesh oils are a complex mixture of numerous compounds, many of which are found in trace amount. It is worth monitoring that there is a great variation in the chemical composition of these two regions oil of *Allium sativum Linn.* (Garlic). This confirms that the reported variation in oil is due to geographic divergence and ecological conditions.

Table 1: Comparative studies on physical properties of essential oil of Bangladeshi and China garlic (*Allium sativum L.)*

Parameters	Results	
	Bangladeshi garlic	**China garlic**
Colour of oil	Light yellow	Light yellow
Taste	Pungent	Pungent
Smell	Spicy	Spicy
Refractive Index	1.56678	1.56470
Oil yield	0.1249%	0.1364%
Moisture	62.824%	65.016%
Total ash	1.21%	1.33%
Crude fiber	2.08%	1.91%
Protein	3.49%	3.84%
Carbohydrates	26.70%	24.30%
Food energy (cal/gm)	144.474	139.896

Table 2: Chemical constituents of the essential oil of garlic from Bangladesh

Peak No:	Retention Time	Area (%)	**Name of the Compound**	M.W	Formula
1	3.83	0.89	1-Propene, 3,3' –thiobis	114	$C_6H_{10}S$
2	5.080	3.27	Disulfide, methyl 2-propenyl	120	$C_4H_8S_2$
3	6.240	1.85	β-Phenylethyl butyrate	192	$C_{12}H_{16}O_2$
4	11.130	28.99	Diallyl disulfide	146	$C_6H_{10}S_2$
5	13.596	23.22	Trisulfide, methyl 2-propenyl	152	$C_4H_8S_3$
6	13.905	1.45	Hydroxylamine, methyl-(1-phenylethyl)-	151	$C_9H_{13}NO$
7	14.091	2.91	Naphthalene, decahydro-	138	$C_{10}H_{18}$
8	15.205	5.47	3-Vinyl-1, 2-dithiacyclohex-4-ene	144	$C_6H_8S_2$
9	20.381	15.36	Trisulfide, di-2-propenyl	178	$C_6H_{10}S_3$
10	20.899	3.57	3H-1,2,4-Triazole-3-thione,2,4-dihydro-4-methyl	115	$C_3H_5N_3S$
11	22.933	9.11	2-Chloro-4,4-dimethyl-1,3,2-oxathiaphospholane	170	C_4H_8ClOPS

Table 3: Chemical constituents of the essential oil of garlic from China

Peak No:	**Retention Time**	**Area (%)**	**Name of the Compound**	**M.W**	**Formula**
1	3.845	0.89	1-Propene, 3,3' –thiobis	114	$C_6H_{10}S$
2	5.133	4.94	Disulfide, methyl 2-propenyl	120	$C_4H_8S_2$
3	10.839	17.24	Diallyl disulfide	146	$C_6H_{10}S_2$
4	13.870	29.12	Trisulfide, methyl 2-propenyl	152	$C_4H_8S_3$
5	14.319	4.01	Benzene, 1-methyl-3-(methylthio)-	138	$C_8H_{10}S$
6	14.406	1.01	2,4-Dithiahex-5-ene 2,2-dioxide	152	$C_4H_8O_2S_2$
7	15.356	5.65	3-Vinyl-1, 2-dithiacyclohex-4-ene	144	$C_6H_8S_2$
8	20.637	21.98	Trisulfide, di-2-propenyl	178	$C_6H_{10}S_3$
9	20.975	1.64	3H-1,2,4-Triazole-3-thione,2,4-dihydro-4-methyl	115	$C_3H_5N_3S$
10	21.383	4.48	Trimethyl silyldiazomethane	114	$C_4H_{10}N_2Si$
11	23.079	9.05	1,2,4,6-Tetrathiepane	170	$C_3H_6S_4$

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