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(Research Article)

Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of the Two Varieties of the Aniseed (*Pimpinella anisum* Linn.) in Bangladesh

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ABSTRACT

Pimpinella anisum Linn. is locally known as Mohuri in Bangladesh. It is used as spices all over the world. In this study essential oil of the two varieties of aniseed from two different places of Bangladesh were investigated by GC-MS. Aniseed (*Pimpinella anisum* Linn.) essential oil has been widely used in aromatherapy for breathing difficulties as well as has a good effect on asthma as a natural asthma remedy. Total 9 chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from the essential oil of Bangladeshi Dhaka aniseed. The oil rich in cis-Anethole (69.404%), D-Limonene (13.273%), Bicyclo[2,2,1]heptan-2-one,1,3,3-trimethyl(11.413%), trans-Anethole (1.977%), Benzene,1 methyl-3-(1-methylethyl)- (1.447%), 1R-alpha-pinene (0.946%), alpha-pinene (0.943%), Eucalyptol (0.447%), Hexaborane (0.150%). On the other hand total 8 chemical constituents were found from the Bangladeshi Bogra aniseed essential oil. The oil contains cis-Anethole (85.321%), Cyclobutane,1,2-bis(1-methylethenyl) (6.492%), Bicyclo[2,2,1]heptan-2-one,1,3,3-trimethyl (5.440%), 1R-alpha-pinene (0.873%), trans-Anethole (0.697%), 1,3,8-p-Menthatriene(0.462%), 1,3,6-octatriene,3,7-dimethyl-,(E)- (0.414%), Eucalyptol (0.300%). 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 aniseed 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 aniseed by GC-MS methods.

Key Words: Gas chromatography and mass spectrometry (GC-MS), *Pimpinella anisum* Linn., Quantification, Active principle, Analytical tool, Essential oil, Chemical constituents.

INTRODUCTION

Aniseed plant (*Pimpinella anisum* Linn.) is a flowering medicinal plant and is annual important spices belonging to the family of Apiaceae native to eastern Mediterranean region and Southwest Asia. Today, aniseeds are an important raw material which is used for pharmaceuticals, perfumery, food and cosmetic industries¹. Recently, this spice plant has drawn more consideration of consumers due to the antimicrobial, antifungal, insecticidal and antioxidative effect of this herb on human health²⁻⁶. The world production of aniseed essential oil amounts to 40-50 tons per annum.

Because of aniseed plant favors warm climatic conditions throughout the growing season it is cultivated particularly subtropical regions^{7,8}. The quantity of aniseed is determined mainly by the essential oil content and its composition. In Bangladesh because of warm climate aniseed plant can grow well and it has been cultivated and consumed mostly in the north-east part. The production of aniseed in Bangladesh is approximately 1,00,000 tones per annum.

Aniseed plant is a herbaceous annual plant growing to 3 ft (0.91 m) tall. The leaves at the base of the plant are simple, 0.5-2 in (1.3-5.1 cm) long and shallowly lobed, while leaves higher on the stems are feathery pinnate, divided into numerous leaves. The flowers are white, approximately 3 mm diameter, produced in dense umbels. The fruit is an oblong dry schizocarp, 3-5 mm long⁹. the essential oil is located in the schizogenic oil ducts of anise fruit and shoots¹⁰.

Essential oil of the genus *Pimpinella* is a complex mixture of various components that contain sesquiterpens, phenolic compounds (C₆-C₃) and alkenes. The essential oil is located in the schizogenic oil ducts of fruits, shoots and roots. According to European Pharmacopoeia aniseed as drugs must have an essential oil concentration higher than 2%⁶. It is also clear that the concentration of essential oil can significantly vary among aniseed from different origins¹¹⁻¹³. Aniseed as well as aniseed essential oil has medicinal values. The aniseed tea is used for children's flatulence, upper respiratory tract problems and bronchial asthmatic attacks¹⁴. Trans-anethole (4-methoxyphenyl-1-propane), the

major component of aniseed oil, is precursor that can produce 2,5-dimethoxybenzaldehyde which is used in the synthesis of psychedelic drugs such as DOB (2,5 dimethoxy-4-bromoamphetamine)¹⁵. Aniseed is useful in destroying body lice¹⁶, head lice and itching insects¹⁴ and the oil can be used by itself¹⁷, which makes it helpful for pediculosis, the skin conditions caused by lice¹⁸. It can also be used for scabies¹⁹, where it may be applied is an externally in an ointment base¹⁷.

Essential oil of aniseed is used in perfumery, soaps and other toilet-articles and for flavoring culinary preparations, confectionery, beverages and liquor anisette. It is used in perfuming sachets, dental preparations and mouth washes; it is also used in the manufacture of liquors. It has medicinal values also. Oil of aniseed is also reported to be used as an aromatic carminative to relieve flatulence and as an ingredient of cough-lozenges in combination with liquorice. It is a mild expectorant and is used as an antiseptic, and for the treatment of cholera. It may be used in the preparation of gripe water²⁰.

However many researchers have been carried out on aniseed (*Pimpinella anisum* Linn.), but no systematic research on comparative studies has been reported on the essential oil of aniseed in Bangladesh. 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 *Pimpinella anisum* Linn. of two varieties from two different places of Bangladesh including its physical & chemical properties along with GC-MS analysis.

MATERIALS AND METHODS

The fresh Bangladeshi aniseed is available in the local markets from both the place Dhaka and Bogra. The collected samples were washed clearly by water to remove dust materials. Then they were dried. Finally the dried aniseed were 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.

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 itself could also provide a valuable means of producing flavor extracts of two varieties from two different places of Bangladeshi aniseed under mild conditions which preserve the natural characteristics of the fresh product. In the process, definite amount of sample (dirt free powder aniseed) 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 mantle 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 Na₂SO₄ and then filtered. It was collected in vial. The ether or chloroform was removed in vacuum condition. Thus the essential oil of fresh aniseed was collected.

GC-MS Analysis

The essential oil of *Pimpinella anisum* Linn. (Aniseed) 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 split ratio 10, carrier gas- helium gas at constant pressure 90 kPa, sample dissolved- in chloroform, range of linear temperature increase- 10°C per min.

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.

Identification of the Components

The physicochemical properties of the essential oil of *Pimpinella anisum* Linn. (Aniseed) of two varieties from two different places of Bangladesh 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 (the essential oil of *Pimpinella anisum* Linn. (Aniseed) of two varieties from two different places of Bangladesh.)

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 methods. The result of the physical properties of *Pimpinella anisum* Linn. (Aniseed) of two varieties from two different places of Bangladesh are presented in Table-1. Chemical characteristics of the oil such as acid value, ester value were

determined by the conventional methods. The comparative results are shown in Table-1.

The slight variation of this oil content and the composition of the essential oil depend on several factors such as 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 species and variety. So far we are aware till now no systemic investigation on the *Pimpinella anisum* Linn. (Aniseed) has 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 *Pimpinella anisum* Linn. (Aniseed) of two varieties from two different places of Bangladesh are presented in Table-2. Total 9 chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from the essential oil of Bangladesh, Dhaka aniseed essential oil. The oil rich in *cis*-Anethole (69.404%), *D*-Limonene (13.273%), Bicyclo[2,2,1]heptan-2-one, 1,3,3-trimethyl (11.413%), *trans*-Anethole (1.977%), Benzene, 1-methyl-3-(1-methylethyl)- (1.447%), 1*R*-alpha-pinene (0.946%), alpha-

pinene (0.943%), Eucalyptol (0.447%), Hexaborane (0.150%). On the other hand total 8 chemical constituents were found from the Bangladeshi Bogra aniseed. The oil contains *cis*-Anethole (85.321%), Cyclobutane, 1,2-bis (1-methylethenyl) (6.492%), Bicyclo[2,2,1]heptan-2-one, 1,3,3-trimethyl (5.440%), 1*R*-alpha-pinene (0.873%), *trans*-Anethole (0.697%), 1,3,8-p-Menthatriene (0.462%), 1,3,6-octatriene, 3,7-dimethyl-(*E*)- (0.414%), Eucalyptol (0.300%).

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 amounts. It is worth monitoring that there is a great variation in the chemical composition of these two regions oil of *Pimpinella anisum* Linn. (Aniseed). This confirms that the reported variation in oil is due to geographic divergence and ecological conditions.

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Table 1: Comparative studies on physical properties and chemical properties of essential oil of Bangladeshi Dhaka Aniseed and Bangladeshi Bogra Aniseed.

Physical properties		Bangladeshi Dhaka Aniseed essential oil	Bangladeshi Bogra Aniseed essential oil
Oil yield (%) g/100g		1.9759	2.0493
Organoleptic	Taste	Bitter in taste	Bitter in taste
	Odor	Spicy	Spicy
	Color	Slight yellowish	Slight yellowish
	Appearance at room temperature (30° C)	Homogeneous, transparent liquid, lighter than water	Homogeneous, transparent liquid, lighter than water
Specific gravity at 30° C		0.824	0.828
Refractive index [n_D^{25}]		1.559	1.554
Optical rotation [α] ²⁶		+1°	+1°
Solubility in different % of alcoholic solution and other solvents	60% alcohol	Slightly hazy up to 20 volume	Slightly hazy up to 20 volume
	70% alcohol	Clearly soluble at 9.5 volume	Clearly soluble at 9.5 volume
	80% alcohol	Clearly soluble at 5.0 volume	Clearly soluble at 5.0 volume
	90% alcohol	Clearly soluble at any volume	Clearly soluble at any volume
	Distilled water	Not Soluble	Not Soluble
	Chloroform	Soluble at any Volume	Soluble at any Volume
	CCl ₄	Soluble at any Volume	Soluble at any Volume
	Pet-ether	Soluble at any Volume	Soluble at any Volume
	Diethylether	Soluble at any Volume	Soluble at any Volume
	n-hexane	Soluble at any Volume	Soluble at any Volume
Chemical properties			
Acid Value		8.0909	10.2237
Ester value		44.2576	40.3072

Table 2: Chemical constituents of the essential oil of Aniseed from Dhaka and Bogra of Bangladesh

No.	Retention time	Name of the compound	Molecular weight	Molecular formula	Composition (%) of Bangladeshi Aniseed essential oil	
					From Dhaka	From Bogra
1.	948	1R-alpha-pinene	136	C ₁₀ H ₁₆	0.946	0.873
2.	1042	Benzene,1methyl-3-(1-methylethyl)-	134	C ₁₀ H ₁₄	1.447	Not detected
3.	1018	D-Limonene	136	C ₁₀ H ₁₆	13.273	Not detected
4.	1059	Eucalyptol	154	C ₁₀ H ₁₈ O	0.447	0.300
5.	948	Alpha-pinene	136	C ₁₀ H ₁₆	0.943	Not detected
6.	1121	Bycyclo[2,2,1]heptan-2-one,1,3,3-trimethyl,	152	C ₁₀ H ₁₆ O	11.413	5.440
7.	1190	Benzene,1methoxy-4-(1-prpenyl)-, <i>cis</i> -Anethole	148	C ₁₀ H ₁₂ O	69.404	85.321
8.	0	Hexaborane	76	B ₆ H ₁₀	0.150	Not detected
9.	1190	Benzene,1methoxy-4-(1-prpenyl)-, <i>trans</i> -Anethole	148	C ₁₀ H ₁₂ O	1.977	C ₁₀ H ₁₂ O
10.	1029	1,3,8-p-menthatriene	134	C ₁₀ H ₁₄	Not detected	0.462
11.	934	Cyclobutane,1,2-bis(1-methyethenyl)	136	C ₁₀ H ₁₆	Not detected	6.492
12.	976	1,3,6-Octatriene,3,7-dimethyl-, (E)	136	C ₁₀ H ₁₆	Not detected	0.414

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