Available on line at : www.eijppr.com

International Journal of Pharmaceutical and Phytopharmacological Research

(ICV-5.09)

ISSN (Online) 2249 – 6084

ISSN (Print) 2250 – 1029

Int.J.Pharm.Phytopharmacol.Res. 2012, 2(2): 67-70

(Research Article)

Comparative Studies on Physicochemical Properties and GC-MS Analysis of Essential Oil of the Two Varieties of the Black Pepper (*Piper nigrum* Linn.)

Shahin Aziz[1], Shamsun Naher*[2], MD. Abukawsar[3], Sudhungshu Kumar Roy[4]

[1]*Senior Scienific Officer, Chemical ltesearch Division, BCSIR Laboratories, Dhaka 1205, Bangladesh*
[2]*Assistant Professor, Department of Chemistry, Jagannath University, Dhaka 1 100, Bangladesh*
[3]*Student, Department of Chemistry, Jagannath University, Dhaka 1100, Bangladesh*
[4]*Director (PRL), IFRD, BCSIR Laboratories, Dhaka 1205, Bangladesh*

Received on: 30/08/2012 ***Accepted on:*** 25/09/2012

ABSTRACT

Piper nigrum Linn. seed locally known as Gol Marich in Bangladesh. It is one of the oldest and the most widely used spices in the world. Gol Marich or black pepper is rightly considered the "King of Spices". In this study essential oil of the two varieties of black pepper from Bangladesh and India were investigated by GC-MS. Black pepper (Piper nigram L.). Essential oil has been widely used as a warming and engineering oil that is helpful at the onset of respiratory infections, and for soothing muscular aches and pains. Quantification of active principles through analytical tools is essential for establishing the authenticity and credibility. The main objective of the present study was focused on identification and quantification of chemical constituents present in the essential oil of black pepper by GC-MS methods.Total 18 chemical constituents were found by gas chromatography and mass spectrometry (GC-MS) analysis from the essential oil of Bangladeshi black pepper. On the other hand total 14 chemical constituents were found from the Indian black pepper . Steam distillation extraction combined with GC-MS has been shown to be a valuable tool for the analysis of black pepper constituents and can provide a useful guide to component variation.

Key Words: GC-MS, *Piper nigrum* Linn., Quantification, Active principle, Analytical tool, Essential oil, Chemical constituents.

INTRODUCTION

Black pepper (*Piper nigrum L.)* is a flowering vine in the family of Piperaceae cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black pepper is one of the oldest and most widely used spices in the world. It is produced from the unripe but fully developed fruits. Black pepper has the characteristics of pepper aroma and flavor due to their chemical substances especially the volatile oil. Black pepper is native to South East Asia and China and is extensively cultivated there and elsewhere in tropical regions. As of 2008, Vietnam is the world's largest producer and exporter of pepper, producing 34% of the world's piper nigrum. Other major producers include India (19%), Malaysia (8%), Sri Lanka (6%), China (6%) and Thailand (4%). In Bangladesh it has been cultivated and consumed mostly in the north-east part of Bangladesh.

The main pungent principle in the green berries of pepper (*Piper nigrum L.*) is piperine. At least five other alkaloids, structurally related to the piperine also occur in smaller amounts; "Black pepper" is obtained from unripe green berries on sun drying, whereas fully ripe dried fruits devoid of pericarp from the commercial "White pepper". Generally the piperine content of black or white peppercorns lies within the range of 3-8 g/100 g, whereas the content of minor alkaloids piperyline and piperettine have been estimated as 0.2-0.3 and 0.2-1.6 g/100g respectively[1]. The aroma of pepper is determined by the composition of the essential oil usually obtained by steam distillation of the black pepper corns [2].

Many essential oils of black pepper (*Piper nigrum L.*) seeds and leaves from various geographical origins have been analyzed for their aroma components and more than 250 volatiles have been identified in this valuable spice and flavoring material for food and perfumery products[3-7]. Using aroma extract dilution analysis in combination with gas chromatographic analysis[8-11] the flavor and off-flavor compounds were found to be monoterpens, minor aldehydes, and pyrazines. Pepper oil can be stored more easily and safely than ground pepper [12]. Therefore, there is much interest in its extraction procedure.

Black pepper herb and its seeds are used as folkloric medicine e.g. aromatic carminative especially useful in flatulence, colic and hiccups of infants and children. The extracts from black pepper have been found to have antioxidant properties[13] and anti-carcinogenic effects, especially when compared to chili[14]. The black pepper spice is used as flavoring agent in soups, table sauces and salads in Asian cuisine, especially Thai cuisine[15].

Black pepper oil has medicinal values. It can be used to help in treatment of pain relief, rheumatism, chills, flu, colds exhaustion, muscular aches, physical and emotional coldness, fever as nerve tonic and to increase circulation. Furthermore, it increases the flow of saliva, stimulates, appetite, encourages peristalsis, tones the colon muscles and is a general digestive tonic [16].

However, many researches have been carried out on black pepper (*Piper nigrum* L.), but no systematic research on comparative studies has been reported on the essential oil of black pepper in Bangladesh and India. 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 *Piper nigrum* L. of two varieties (Bangladeshi and Indian) including its physical and chemical properties along with GC-MS analysis.

MATERIALS AND METHODS

Collection and Processing of Plant Material

The fresh Bangladeshi black pepper was obtained from the local markets of Dhaka city (Moulvibazar, Shrimangal). The sample of Indian black pepper was also obtained from local markets in Bangladesh as a lot of Indian black pepper imported in Bangladesh in bulk quantities from Kerala, India. These samples were washed clearly by water to remove dust materials and then dried. Finally the dried black pepper 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 from Bangladeshi and Indian black pepper under mild conditions which preserve the natural characteristics of the fresh product. In the process, definite amount of sample (dirt free powder black pepper) 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 black pepper 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 black pepper was collected.

GC-MS Analysis

The essential oil of *Piper nigrum* Linn. (Black Pepper) of two varieties were analysed 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 30cm, 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 chloform, range of linear temperature increase-10˚C per min.

Sample Preparation 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 *Piper nigrum Linn.* (Black Pepper) of two varieties 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 the essential oil of *Piper nigrum Linn.* (Black Pepper) from Bangladesh and India was recorded and presented in table - 2.

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 *Piper nigrum* Linn. (Black Pepper) essential oil of Bangladesh and India appeared 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 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 *Piper nigrum* Linn. (Black

Pepper) 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 *Piper nigrum Linn.* (Black Pepper) of two varieties are presented in table-2. For Bangladeshi *Piper nigrum Linn.* (Black Pepper), 18 chemical constituents were found. The oil rich in Caryophyllene(18.393%), α-Pinene(16.685%), D-Limonene (16.168%), β-Pinene(13.618%), 3-Carene(9.228%), 1,4,7,-cycloundecatrien,1,5,9,9-tetramethyl-,Z,Z,Z-100(3.439%), beta Phellandrene(3.167%), Copaene(3.139%), 1-Napthalenol(3.009%), beta-Myrcene(2.896%), alpha Phellandrene(2.877%), Cyclohexene(2.871%), Napthalene (1.361%), 1-Napthalenol(1.023%), Naphthalene(0.925%) , (+)- 4-Carene (0.434%), 1-Napthalenol (0.419%), Linalol(0.348%) i.e. total monoterpene(65.421%) and sesquiterpene(34.579%).On the other hand total 14 chemical constituents were found from the Indian black pepper .The oil contains 3-Carene (32.611%), D-Limonene (5.222%), β-Pinene(13.204%), Caryophyllene (10.661%), α-Pinene (7.350%), β-Cymene(3.446%), beta-Myrcene(3.108%), 1,4,7,-cycloundecatrien,1,5,9,9-tetramethyl-,Z,Z,Z-100 (2.143%), Cyclohexene (0.903%), alpha Phellandrene (0.756%), (+)- 4-Carene (0.756%), Eudesma-4(14), 11 diene (0.720%), beta-Humulene (0.500%), Cyclohexene (0.488%) i.e. total monoterpene (77.673%) and sesquiterpene (14.195%).

Results showed that essential oil from both of the two countries 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 *Piper nigrum* Linn. (Black Pepper). This confirms that the reported variation in oil is due to geographic divergence and ecological conditions.

ACKNOWLEDGEMENTS

Authors are thankful to the Director, Dhaka laboratories, Abu Anis Jahangir, Dr. Ismat Ara Jahan, P.S.O, CRD, Dhaka labs. Mosarraf Hossain, (Jr.Tech.) of BCSIR Laboratories, Dhaka.

Table-1: Comparative studies on physical properties and chemical properties of essential oil of Black Pepper from Bangladesh and India.

Physical properties		**Bangladeshi black pepper**	**Indian black pepper**
Yield (%)		**2.16**	**1.99**
Organoleptic	Taste	Pungent	Pungent
	Odor	Spicy	Spicy
	Color	Colourless	Slightly Greenish
	Appearance at room temperature (30˚ C)	Homogeneous, transparent liquid, lighter than water	Homogeneous, transparent liquid, lighter than water
Specific gravity at 30˚ C		0.84437	0.84455
Refractive index [$\eta^{t^\circ C}$]		1.48033	1.48067
Optical rotation [α]^{26}		-10.67˚	-9.33˚
Solubility	60% Alcohol	Opaque	Opaque
	70%Alcohol	Hazy	Hazy
	80% Alcohol	Slightly hazy	Slightly hazy
	90% Alcohol	Soluble in 14Volume	Soluble in 14 Volume
	95% Alcohol	Soluble in 5Volume	Soluble in 5 Volume
	100% Alcohol	Soluble at any Volume	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		3.75	4.26
Ester value		30.00	25.25

Table-2: Chemical constituents of the essential oil of black Pepper from Bangladesh and India

Sr. No.	Retention time	Name of the compound	Molecular weight	Molecular formula	Composition (%)	
					Bangladeshi Black pepper	Indian Black pepper
1.	1579	1,4,7-Cycloundecatrien,1,5,9,9-tetramethyl-Z,Z,Z-100	204	$C_{15}H_{24}$	3.439	2.143
2.	1494	Caryophyllene	204	$C_{15}H_{24}$	18.393	10.661
3.	948	Alpha-Pinene	136	$C_{10}H_{16}$	16.685	7.350
4.	1018	D-Limonene	136	$C_{10}H_{16}$	16.168	15.222
5.	948	3-Carene	136	$C_{10}H_{16}$	9.228	32.611
6.	948	(-)-beta-Pinene	136	$C_{10}H_{16}$	13.618	13.204
7.	964	Beta-phellandrene	136	$C_{10}H_{16}$	3.167	- - - - -
8.	1221	Copaene	204	$C_{15}H_{24}$	3.139	- - - - -
9.	1580	1-Napthalenol	222	$C_{15}H_{26}O$	3.009	- - - - -
10.	958	Beta-Myrcene	136	$C_{10}H_{16}$	2.896	3.108
11.	969	alpha-phellandrene	136	$C_{10}H_{16}$	2.877	0.756
12.	1377	Cyclohexene	204	$C_{15}H_{24}$	2.871	0.488
13.	1440	Napthalene	204	$C_{15}H_{24}$	0.925	- - - - -
14.	1580	1-Napthalenol	222	$C_{15}H_{26}O$	1.023	- - - - -
15.	1440	Napthalene	204	$C_{15}H_{24}$	0.925	- - - - - -
16.	919	(+)-4-Carene	136	$C_{10}H_{16}$	0.434	0.756
17.	1580	1-Napthalenol	222	$C_{15}H_{26}O$	0.419	- - - - - -
18.	1082	Linalol	154	$C_{105}H_{18}O$	0.348	- - - - - -
19.	1574	Beta-Humulene	204	$C_{15}H_{24}$	- - - - - -	0.500
20.	1469	Eudesme-4(14),11 diene	204	$C_{15}H_{24}$	- - - - - -	0.720
21.	1377	Cyclohexene	204	$C_{15}H_{24}$	- - - - - -	0.903
22.	1042	Beta-Cymene	134	$C_{10}H_{14}$	- - - - - -	3.446
Total %					100	91.868

REFERENCES

1) Tainter D.R., Grenis A. T. Spices and Seasonings, A Food Technology Handbook; VCH Publishers; New York, 1993.
2) Purseglove J. W., Brown E. G. , Green C. L., Robbins S. R.J., Spice, Vol. 2., Longman: New York, 1981.
3) BACIS, Volatile Compounds in Food Database, Pepper, TNO, Zeist, Netherlands, 1999.
4) Menon A.N., Padmakumari K.P., A. Jayalekshmy A., M.Gopalakrishnan M., Narayanan C.S, Essential oil composition of four majour cultivars of black pepper (Piper nigrum L.), *J. Essent. Oil Res.*, 2000, 12: 431-434.
5) Menon A.N., S. Chacko S., C.S. Narayanan C.S., *J. Essent. Oil Res.*, 2001,13 : 166.
6) Sumathykutty M.A., Rao J.M., Padmakumari K.P.,Narayanan C.S;Essential oil constituents of some piper species, *Flav. Fragr. J.* , 1999,14 (5): 279-282.
7) Tewtrakul S., Hase K., Kadota S., Namba T., K. Komatsu K., K. Tanaka K.,Fruit oil corn position of piper chaba Hunt , P. Longum L., and P. Nigrum L., *J. Essent. Oil Res.*, 2000,12(5) : 603-8.
8) Jagella T., Grosch W., *Eur. Food Res. Technol*., 1999,209: 6.
9) Jagella T., Grosch W., Flavour and off-flavour compounds of black and white pepper II [Black pepper],*Eur. Food Res. Technol*., 1999,209 : 22-26.
10) Jagella T., Grosch W., Flavour and off-flavour compounds of black and white pepper III [white pepper],*Eur. Food Res. Technol*., 1999, 209: 27-31.
11) Pino J., Rodriguez G., Borges P., Rosado A., Chemical and sensory properties of black pepper oil., *Nahrung*, 1990, 34:555.
12) Govindarajan V. S., Pepper Chemistry, Technology and Quality Evaluation, CRC *Critical Review in Food Science and Nutrition*, 1977,9 : 115-225.
13) Gülçin I.,The antioxidant and radical scavenging activities of black pepper (Piper nigrum) seeds, *Int J Food Sci Nutr.* , 2005, 56(7):491-9.
14) Nalini N, Manju V, Menon VP., Effect of spices on lipid metabolism in 1,2-dimethylhydrazine-induced rat colon carcinogenesis, *J Med Food.* , 2006, Summer; 9(2):237-45.
15) Thai Ingredients Glossary (http://www.tempeofthai.com/cooking/thai-ingrediant-glossary.php). Retrieved 6 November 2005.
16) Pruthi, J. S. , Major Spices of India: Crop Management and Post Harvest Technology. Indian Council of Agriculture Research, New Delhi, 1993.

Corresponding Author:
Shamsun Naher,
Asst. Professor, Department of Chemistry,
Jagannath University, Dhaka 1100,Bangladesh.
Email: *shamsunnaher2002@yahoo.com*