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CHEMICAL INVESTIGATION OF BIOACTIVE COMPOUNDS OF BLACK PEPPER

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ABSTRACT: Black pepper (*Piper nigrum*) is a spice vine crop and which was used as a food preservative and as an essential component in traditional medicines. Moreover it is used also as a spice for curry cooking. The fruits and leaves may be both contains the medicinal activities. First time our concentration on to the fruits only. The fruits were collected and normally dried in the sunlight. The dried fruit was then converted into powder material. Then the powder of black pepper was extracted with ethanol in a soxhlet's apparatus. The concentrated filtrate was isolated by column chromatography using solvent system-benzene: ethyl acetate (3:1). Again, recrystallization was performed by benzene with a minimum volume of chloroform to give pale yellow crystals of MP. 128-129^{o}C (Reported 130^{o}C). The IR, ^{1}HNR and mass spectra of the pure crystalline compound (A_2) were recorded. The mass spectra shows molecular ion M+· 284.5 which indicates that the molecular weight of the compound A_2 is equal to that of *piperine*molecular weight 285.

INTRODUCTION: Black pepper (*Piper nigrum*) is a spice vine crop. It belongs to the family Piperaceae. It is cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black pepper is known as king of spices and widely and extensively used all over the world. It is a perennial climber, climbing by means of ivy-like roots which adhere to the support tree.

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The sessile, small white flowers are borne in pendulous, dense, slender spikes of about 50 blossoms each. The berry-like fruits, or peppercorns, are round, about 0.5–1.0cm in diameter and contain a single seed. They become yellowish-red at maturity and bear a single seed. Spike length varies greatly, based on cultivar. The young berries are green, whitish green or light purple, while mature ones are green, pale purple or pale yellow and change to red on ripening.

South West India is the traditional home of this important spice, particularly the Western coastal regions of South Peninsular India (the Malabar Coast). Black pepper was the first oriental spice to be introduced into the Western world, and was well

known among the Romans and Greeks. Black pepper [1] has multiple uses in the processed food industry, in kitchens, in perfumery, in traditional medicine and even in beauty care. Pepper is valued for its pungency and flavour, which is attributed by the alkaloid piperine and the volatile oil (Ravindran, 2000). By stimulating the digestive enzymes of the pancreas, piperine enhances digestive capacity and significantly reduces gastrointestinal food transit time. Piperine [2] has been documented to enhance the bioavailability of a number of therapeutic drugs as well as phytochemicals through its inhibitory influence on enzymatic drug bio-transforming reactions in liver and intestine. It strongly inhibits hepatic and intestinal aryl hydrocarbon hydroxylase and glucuronyl transferase.

Most of the clinical studies on piperine have focused on its effect on drug metabolism. Black pepper oil can be used to help in the treatment [3] of pain relief, rheumatism, chills, flu, colds, exhaustion, muscular aches, physical and emotional coldness, fevers, as a 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 (Pruthi, 1993). The pepper based compounds have also seen commercial use as chemical carminatives; they have also been used for their ability to reduce excess gas in the intestines and the stomach. Aside from these, these compounds have also been used to stimulate the activity in the human heart and kidneys in medicine.

The specific compound in pepper known as pipperine [4] is commercially utilized to prepare different insecticides to be used against houseflies and other insect pests, for example, many gardeners use commercially made pepper sprays to fight several kinds of agricultural pests on useful plants. Recently, Vietnam has become the world's largest producer and exporter of pepper (116,000 t in 2006).

Other major producers include Indonesia (67,000 t), India (65,000 t), Brazil (35,000 t), Malaysia (22,000 t), Sri Lanka (12,750 t), Thailand and China. Vietnam dominates the export market, exporting almost the entire produce. So far Bangladesh has to import a lot of spice from abroad to meet up the demand of spice. Though black pepper [5] is being cultivated in few areas of Bangladesh, it is not enough. Two hundred farmers of Vadeshar union of Bahubal upazilla under Habiganj district were dependent on the cultivation of betel leaf, pine apple, and lemon. But now they are cultivating black pepper commercially as it is profitable.

A crop of such economic importance is being neglected in our country as mere research has been done so far in our country. Besides the economic importance, the multipurpose use of black pepper can be known through research. Then it may possible to extend the commercial production of this spice throughout the country. So, the present study was undertaken for the following objectives:

I. To prepare the extracts of black pepper fruits by soxhlet's apparatus using ethanol.

II. To identify different spots by thin layer chromatographic (TLC) technique.

III. To isolate and purify major compounds by column chromatography.

IV. To perform spectroscopic analysis of the pure compound.

MATERIALS AND METHODS:

The experiment was conducted at Research Laboratory, Department of Applied Chemistry & Chemical Engineering, Rajshahi University, Rajshahi; Department of Agricultural Chemistry, Bangladesh Agricultural University, Mymensingh and BCSIR laboratory, Dhaka during the period from July 2009 to December, 2010.

Preparation of powdered black pepper fruit:

Dried black pepper fruits were collected from a local market in Mymensingh used in the present study. The fruits for the study were cleaned to free from stone and other undesirable matter and sun dried. Then the sun dried fruits were ground in an electrically operated grinder to fine particle size.

Hot ethanolic extraction of black pepper fruit powder:

The finely powder was extracted in a soxhlet apparatus. Exactly 250g of black pepper fruit powder was taken in thimble and around 800ml of

95% ethanol was added to it. The extraction was continued for 24 hours. The extract was filtered by whatman No.1 filter paper. A yellowish brown filtrate (600 ml) was obtained. The yellowish brown filtrate was concentrated to a minimum volume by rotary flash evaporator. An oily viscous mass was obtained.

Thin Layer Chromatography analysis of crude material:

The crude was subjected to TLC for separating the components present in it to the best possible extent. The minimum of mass was dissolved in a minimum volume of chloroform and was spotted on to a TLC plate. The plates were run at room temperature in a glass chamber containing benzene-ethyl acetate mixture (3:1) gave the best resolution and hence necessitated its use in TLC. The plate was then removed, air dried and placed in iodine chamber. There three spots with corresponding R f values of 0.88, 0.54 and 0.30 were obtained.

Isolation of compound by column chromatography:

exactly 50 ml black pepper fruit extract was percolated through a 39.5×5.5 inch column of alumina and washed with n-hexane first, then benzene : ethyl acetate (3:1). Fractions (20 ml) were collected at a flow rate 4-5 ml per minute, the elution being followed by periodic testing of the eluent by circular paper chromatographic technique using solvent system benzene : ethyl acetate (3:1) and locating the compound by placing the TLC plate in the iodine chamber. The pooled extracts (benzene: ethyl acetate-3:1) containing the expected compound were then concentrated to about 50 ml by rotary flash evaporator and kept in the freezer overnight. The pale yellow crystal was obtained.

Analytical techniques:

I. Melting point of the crystalline compound was recorded with Fisher-John's electrothermal melting point apparatus

II. Thin Layer Chromatography (TLC) was performed on glass plates coated with silica gel 60 (E. Mark, India Ltd.)

III. IR spectra were recorded on infrared spectrophotometer (Model no-8900)

IV. ^{1}H NMR spectra were recorded in deuterated CDCl3 (0.5 ml) on a Bruker 400 MHz NMR spectrophotometer (spectral range: 0-14ppm) with TMS as internal standard.

V. Mass spectra were recorded on Liquid Chromatography Mass Spectrophotometer (Model-LCT premier, UK)

RESULTS AND DISCUSSION:

TABLE 1: TLC REFLUXING EXTRACT OF BLACK PEPPER AND R_f VALUES OF THE SPOTS

Sample	Extracting solvent	Eluting solvent	R_f values		
			A_1	A_2	A_3
Black pepper	Ethanol	Benzene : Ethyl acetate = 3:1	0.88	0.54	0.30

The R_f value of the pure compound was obtained 0.54 which matches with the R_f value of the refluxing extract that was 0.54.

IR Spectra of the compound A_2

The IR data of compound (A_2) was shown in (**Fig.1**) and the characteristic peaks with corresponding wave number were presented in **Table 2**. All of the absorption was expected for molecular structure of piperine.

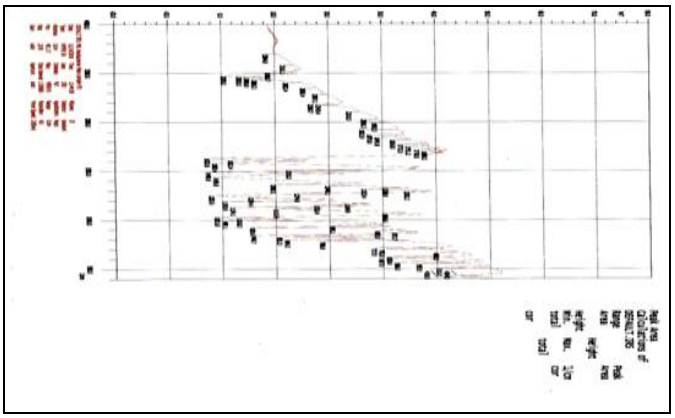

FIG. 1: INFRA-RED (IR) SPECTRUM OF COMPOUND A_2

The compound (A_2) showed infrared (IR) absorption band at 2920 cm^{-1} for aromatic ring (aromatic C-H stretching) which was similar to wave number 2922 cm^{-1} (Pall, 2008). The compound (A_2) showed infrared absorption band at 1633 cm^{-1}, 1612 cm^{-1}, 1699 cm^{-1}, 1018 cm^{-1}, 1253 cm^{-1} and 1134 cm^{-1}, and 997 cm^{-1} for symmetric stretching of conjugated diene, asymmetric stretching of conjugated dine, -CO-N stretching,

symmetric stretching of =C-O-C, asymmetric stretching of =C-O-C, C-H banding of trans-CH=CH- is nearly similar to the infrared absorption band at 1628 cm^{-1} 1608 cm^{-1}, 1654 cm^{-1}, 1019 cm^{-1}, 1250 cm^{-1} and 1130 cm^{-1}, and 994 cm^{-1} (Pall, 2008).

TABLE 2: IR SPECTRAL DATA OF COMPOUND A_2

Characteristics Bond Stretching	Wave No.	Reference Wave No. (Pall, 2008)
Aromatic ring (Aromatic C-H stretching)	2920 cm^{-1}	2922 cm^{-1}
Symmetric stretching of conjucateddiene	1633 cm^{-1}	1628 cm^{-1}
Asymmetric stretching of conjucateddiene	1612 cm^{-1}	1608 cm^{-1}
-CO-N stretching	1699 cm^{-1}	1654 cm^{-1}
Symmetric stretching of =C-O-C	1018 cm^{-1}	1019 cm^{-1}
Asymmetric stretching of =C-O-C	1253 and 1134 cm^{-1}	1250 and 1130 cm^{-1}
C-H bending of trans –CH=CH-	997 cm^{-1}	994 cm^{-1}

^{1}H -NMR spectrum of compound A2

^{1}H-NMR Spectrum was recorded in 400MHz spectrometer as showed in **Fig. 2**. The NMR results again confirmed the purity and identity of the compound and it also indicated nineteen protons within the molecule of which twelve are in aliphatic region.

The ^{1}H-HMR Spectrum of the isolated compound (A_2) showed characteristic signals (^{1}H-HMR, CDCl3): δ 1.54-1.62 (m, br, 6H), 3.49-3.59 (s, br, 4H), 5.92 (s, 2H), 6.65-6.93 (m, 5H, aromt-H), 7.25 (d, 1H) which are similar to (Pall, 2008). The ^{1}H-HMR Spectrum of the isolated compound showed characteristic signals (^{1}H-HMR, CDCl3): δ 1.59-1.67 (m, br, 6H) 3.53-3.62 (s, br, 4H), 5.57 (s, 2H), 6.63-6.95 (m, 5H, aromt-H), 7.27 (d, 1H) (Pall, 2008). Thus NMR data is shown in **Table 3** and it matches with the reference standard of piperine in the literature. But from this NMR data we cannot conclude whether extracted compounds are E and or Z isomer.

TABLE 3: ^{1}H-NMR DATA OF A_2

Characteristic ^{1}H	δ in ppm	δ in ppm (Pall, 2008)
m, br, 6H	1.54-1.62	1.59-1.67
s, br, 4H	3.49-3.59	3.53-3.62
s, 2H	5.92	5.97
d, 1H	6.38	6.39
m, 5H, aromat-H, δ, γ-H	6.65-6.93	6.63-6.95
d, 1H	7.25	7.27

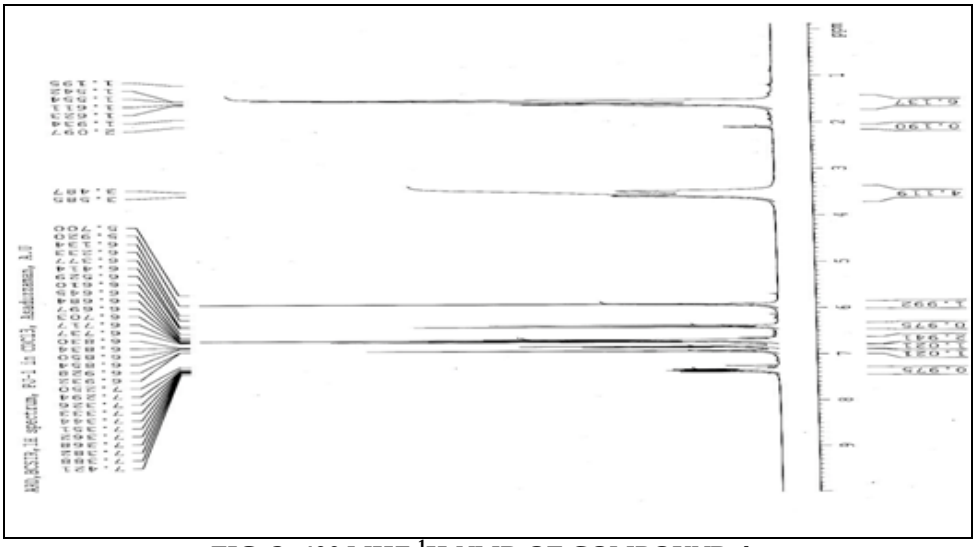

FIG. 2: 400 MHZ ^{1}H-NMR OF COMPOUND A_2

The above signals are similar to the characteristics of piperine as shown in **Fig 3**. The symbols α, β, γ, δ represents the position of protons in the piperine structures. The ^{1}H-NMR spectrum of the isolated compound did not contain signals characteristics of the geometric isomers of the species confirming the purity of the compound.

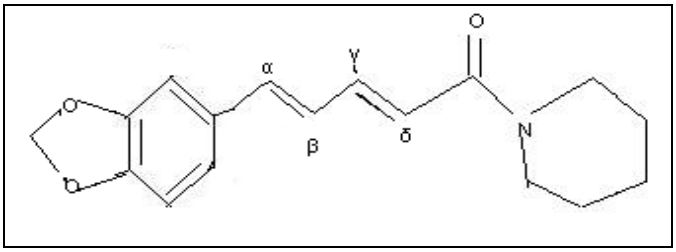

FIG. 3: STRUCTURE OF PIPERINE

Mass spectra of the compound A_2:

The mass spectra of compound A_2 (**Fig. 4**) and probable mass fragmentation are shown in **Table 4**. The mass spectra clearly showed a molecular ion peak at m/z 285 corresponding to molecular formula, $C_{17}H_{19}NO_3$ from which the mass identification of the peaks obtained is represented in (**Table 4**). The mass spectral data of compound A_2 is nearly similar to the mass spectral data shown (Pall, 2008) in the **Table 5.** Mass analysis is directed one to identify the component of A_2 tentatively as piperine, which has been further compared with the structure available in MS-library.

TABLE 4: MASS SPECTRAL DATA OF COMPOUND A_2

m/z	Fragment unit
285	$C_{17}H_{19}NO_3$
209	$C_{12}H_{17}O_3$
163	$C_{11}H_{15}O$
122	C_9H_{14}

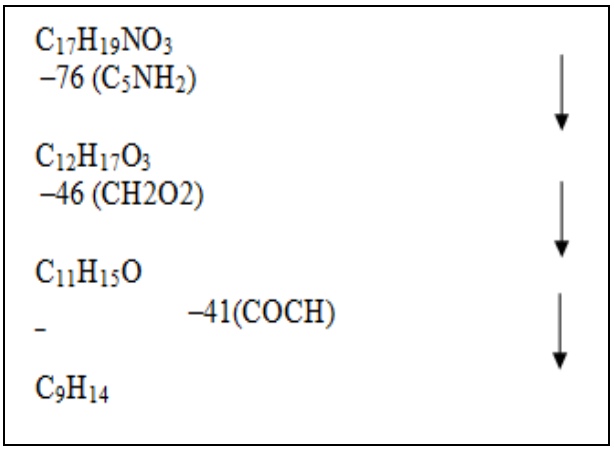

FIG. 5: FLOW CHART OF MASS FRAGMENTATION

TABLE 5: MASS SPECTRAL DATA (PALL, 2008)

m/z	Reference fragment unit
285	$C_{17}H_{19}NO_3$
201	$C_{12}H_9O_3$
173	$C_{11}H_9O_2$
143	$C_{10}H_7O$
115	C_9H_7

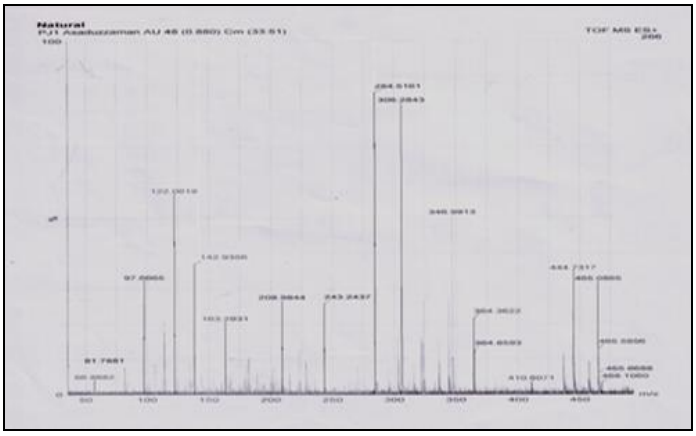

FIG. 4: MASS SPECTRA OF COMPOUND A_2

CONCLUSION: For isolation and identification of bioactive compounds of *Piper nigrum*, we extracted powdered *Piper nigrum* by soxhlet apparatus using ethanol as solvent. Using TLC and allowing column chromatographic technique, we isolated a pale yellow crystalline compound. It was purified by recrystallization process. Its melting point indicates that it is piperine. Different spectroscopic studies of this compound were carried out. These are Infrared, ^{1}H-NMR and Mass. The IR absorption of this compound indicates that it is piperine. Also all the ^{1}H-NMR absorption for different protons are expected as for piperine. The base peak in mass spectra indicates its molecular weight (m/z 285). Thus we concluded that the isolated compound is piperine. It is an important compound of piperine. Further research work is going on for exploring the bioactivity of this compound on agricultural field particularly as organic insecticide.

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