

## Isolation of Piperine and Few Sesquiterpenes from the Cold Petroleum Ether Extract of *Piper nigrum* (Black Pepper) and its Antibacterial Activity

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### ABSTRACT

A crystalline compound [(1,5-(1,3)-benzodioxol-5yl)-1-oxo-2,4 pentadienyl]-piperidine and fifteen sesquiterpenes (linalool, 4-terpinol, alpha terpinol, delta elemene, alpha copane, beta elemene, caryophyllene, alpha caryophyllene, tau gurjunene, beta bisabolene, delta cadinene, elemol, caryophyllene oxide, murrolene, beta eudesmol) have been isolated from the petroleum ether extract of *Piper nigrum* seeds. Piperine is the compound, mainly responsible for the pungent taste of *Piper nigrum* where as the components isolated from its oil is meant for aroma. The oil separated from petroleum ether extract was analyzed by GC-MS spectroscopy. The components identified by GC-MS spectra, show that caryophyllene was present as major component and linalool as minor component. Their structures were fully elucidated assuming that detailed spectroscopic study and chemical analysis. Antibacterial activity of petroleum ether extracts is checked against Gram (+) and gram (-) bacterias.

**Keywords:** *Piper nigrum*, Piperaceae, fruits, amide and alkaloid.

### INTRODUCTION

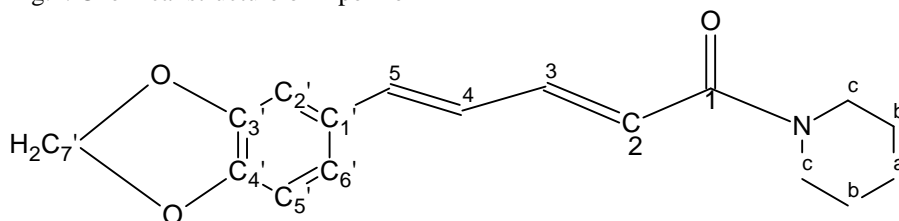
The genus piper a member of family piperaceae, is an perennial herb, which has over 700 species distributed in both hemispheres. This family has shown a promising effect in therapeutics (Reshmi S.K et al., 2010). Various piper species have been used as spice and in folk medicine due to attributed physiological activities and thus bear a great commercial, economic and medicinal potential (Krishnamurthi A., 1969; Parmar V.S et al., 1997; Kiuchi F et al., 1998; Siddiqui B.S et al., 1997; Nadkarni., 1954). Black pepper (*Piper nigrum* L.) "The king of spices" contributes its major share in Indian Spice Export scenario (Zachariah T.J et al., 2010). The fruit of *Piper nigrum* is one of the oldest and most popular spices in the world, indigenous to Malabar coast of India and used as an aromatic stimulant in cholera, dyspepsia, flatulence, anti periodic in malarial fever and arthritis disease (Nayar et al., 1956; Nadkarni, 1954; Warriar, 1989). *Piper nigrum* is more well known species because of its high commercial, economic and medicinal properties (Ee G.C.L et al., 2010). The phytochemistry of the genus piper is rich where the studies have revealed the adequate presence of terpenes, amides and alkaloids (Sengupta and Ray, 1987; Parmar et al., 1993; Siddiqui et al., 2004). It is known that *Piper nigrum* has biological activity such as CNS depressant, antioxidant, radical scavenging, anti-insecticidals, antibacterial, allelopathy, anticonvulsant, anti-tubercular, antipyretic, anti-inflammatory, antioxidant and exteroffective (C.F.Su, Helen, et al., 1981; Dorman, H.J.D., 2008; Siddiqui, Z.S., 2007; M.Daniel, 2006). The pungency of black pepper attributed to the alkaloid piperine, aroma and flavour by components such as and pinene, sabinene, myrcene, limonene, -caryophyllene,

camphene etc, (Gopal Krishnan et al., 1993; Menon et al., 2000, 2002, 2003; Martins et al., 1998) reported the most important constituents in *Piper nigrum* essential oils as limonene, -caryophyllene, sabinene and -pinene. Many authors have reported the presence of various secondary metabolites of black pepper. The main objective of entire study is to isolate some new compounds, during our research for novel, bioactive natural products, the seeds from the plant were successively extracted with the light petroleum ether, chloroform and alcohol. All the extracts were showing the potential for further treatment. The petroleum ether extract kept for around two months, shows two different layers the upper oily layer and lower thick portion as a precipitate. The oily fraction on GC-MS analysis shown to be mixture of around fifteen components and the precipitate portion on the repeated process of crystallization gave shiny, pale yellow crystals of melting point 132°C.

### RESULTS AND DISCUSSION

The shiny pale yellow crystals (m. p. 132°C) obtained from petroleum ether extract of *Piper nigrum* fruits is found to be an alkaloid on performing Dragendroff test (De, S et al., 2010). The alkaloid framework was also supported by UV and IR spectroscopy. TLC of the crystals which were recrystallised using ethanol showed R<sub>f</sub> value equals to 0.23 that is similar to R<sub>f</sub> value of piperine observed in literature was 0.25 (Madhavi, B.B et al., 2006) thus crystals obtained may be of piperine. The molecular formula was established as C<sub>17</sub>H<sub>19</sub>O<sub>3</sub>N by Agilent, 6540 Q-TOF (HRMS) mass spectrometer. The presence of an alkaloid framework was suggested by the UV spectrum, showing absorption at

Fig.1. Chemical structure of Piperine

Table 1: GC-MS of the various components extracted from the petroleum ether Extracts of *Piper nigrum*

Compound number	RT (min)	Peak Name	Area	Amount/RF
1.	22.948	Linalool	2200	0.120
2.	27.240	4-terpinol	8915	0.485
3.	27.902	-terpinol	7085	0.386
4.	34.456	-elemene	38616	2.101
5.	34.367	-copane	185422	10.091
6.	36.884	-elemene	20814	1.133
7.	38.362	Caryophyllene	898540	48.8998
8.	39.896	-caryophyllene	118490	6.448
9.	41.819	-bisabolene	214102	11.651
10.	42.348	-cadinene	140509	7.646
11.	43.572	Elemol	44625	2.428
12.	45.112	Caryophyllene oxide	26166	1.424
13.	47.477	Murrolene	53433	2.908
14.	47.905	-eudesmol	7264	0.395
15.	41.572	-gurjunene	71396	3.885

342nm (Berger, S., 2009). The IR(neat) spectra showed the presence of absorption bands at 3421  $\text{cm}^{-1}$  (intermolecular H-bonding), 3000 $\text{cm}^{-1}$  (aromatic C-H stretch), 1633 and 1610  $\text{cm}^{-1}$  (symmetric and asymmetric stretching of c=c of dienes), 1610,1585,1492  $\text{cm}^{-1}$  (aromatic stretching of c=c of phenyl ring), 1633  $\text{cm}^{-1}$  (stretching of-CO-N group), 2941 and 2859  $\text{cm}^{-1}$  ( $\text{CH}_2$  asymmetric and symmetric stretching), 1448  $\text{cm}^{-1}$  ( $\text{CH}_2$  bending), 1253 and 1193  $\text{cm}^{-1}$  (asymmetric stretching of =C-O-C), 929  $\text{cm}^{-1}$  (C-O stretching), 1134  $\text{cm}^{-1}$  (in plane bending of phenyl CH), 997  $\text{cm}^{-1}$  (CH bending for trans -CH=CH-), 848,830 and 804  $\text{cm}^{-1}$  (out of plane C-H bending). It resembles to the IR spectra of piperine given in literature (Berger, S., 2009). The 400 MHz  $^1\text{H}$  NMR spectrum showed methylene dioxy signal at 5.9 (2H, s, O- $\text{CH}_2$ -O) and other hydrogen atoms signals at 7.3 (1H, ddd, J=10.5, 4.12, 10.53 Hz, H-3), 6.63 (1H, d J=15 Hz, H-2), 3.3-3.6 (4H,m, H-c), 1.6- 1.7 (5H,m,H-a,b). The  $^{13}\text{C}$  NMR spectra showed the peaks at 167.7 for C-1(C=O), 120.6, 140.2 for C-2 and C-3(C=C), 123.9, 132.4 for C-4 and C-5(C=C), 106.7 (C-2'), 149.7-149.8 for C-3 and C-4'(C=C), 109.4, 207 for C-5'and C-6'(C=C), 102.7 (C-7') giving the information of C=O, C=C and other carbon atoms. The HR-mass spectrum showed a [M+H] $^+$  peak at 286.14,[2M+H] $^+$  peak at 571.2 and [2M+Na] $^+$  peak at 593.2. Based on above result's structure of the compound is assigned as piperine.

#### Experimental

General: The melting point was determined on Lab fit melting point apparatus. A UV spectrum in ethanol was obtained on SHIMADZU UV-1800 UV

spectrophotometer. An IR spectrum was recorded on SHIMADZU FTIR-8400S (Fourier Transforms infrared spectrophotometer).  $^1\text{H}$ -NMR (400MHz) and  $^{13}\text{C}$ -NMR were recorded in MeOD on Bruker, Avance 400 MHz NMR spectrometer. Chemical shifts are given as with TMS as internal standard. A HR-mass spectrum was recorded on Agilent, 6540, Q-TOF (HR-MS) mass spectrometer. The oil was analyzed by using Varian 4000 GC-MS. The instrument operates at the following conditions: equipped with fused silica 30m (CP-Sil-8,Varian) capillary column with an internal diameter of 0.25 mm and a film thickness of 0.25 $\mu\text{m}$ , the Helium carrier gas had a delivery rate of 1 ml/min, a capillary injector operating at 280 $^\circ\text{C}$  in the split mode (1:150),flame ionization detector (FID) running at 300 $^\circ\text{C}$ , the column oven temperature programming was 50 $^\circ\text{C}$  for 5 min and then increased from 50 to 250 $^\circ\text{C}$  at the rate of 3 $^\circ\text{C}/\text{min}$  and hold for 7 min.

Plant material: Seeds of *Piper nigrum* were purchased from a specific seed shop of Jammu's district and classified systematically by Dr. Gurdev Singh of the botany department at lovely professional university.

Extraction and isolation: The dried and crushed seeds (one kg) of *piper nigrum* were soaked in ethanol for 120 hours. The crude extract of ethanol was successively distilled with different solvents according to their polarity gradient. Around after 142 hours, the petroleum ether extracts get separated into shiny, pale yellow crystals and an oily portion. Crystals thus separated and recrystallised with ethanol thrice and oily layer was subjected to GC-MS for identification of components.

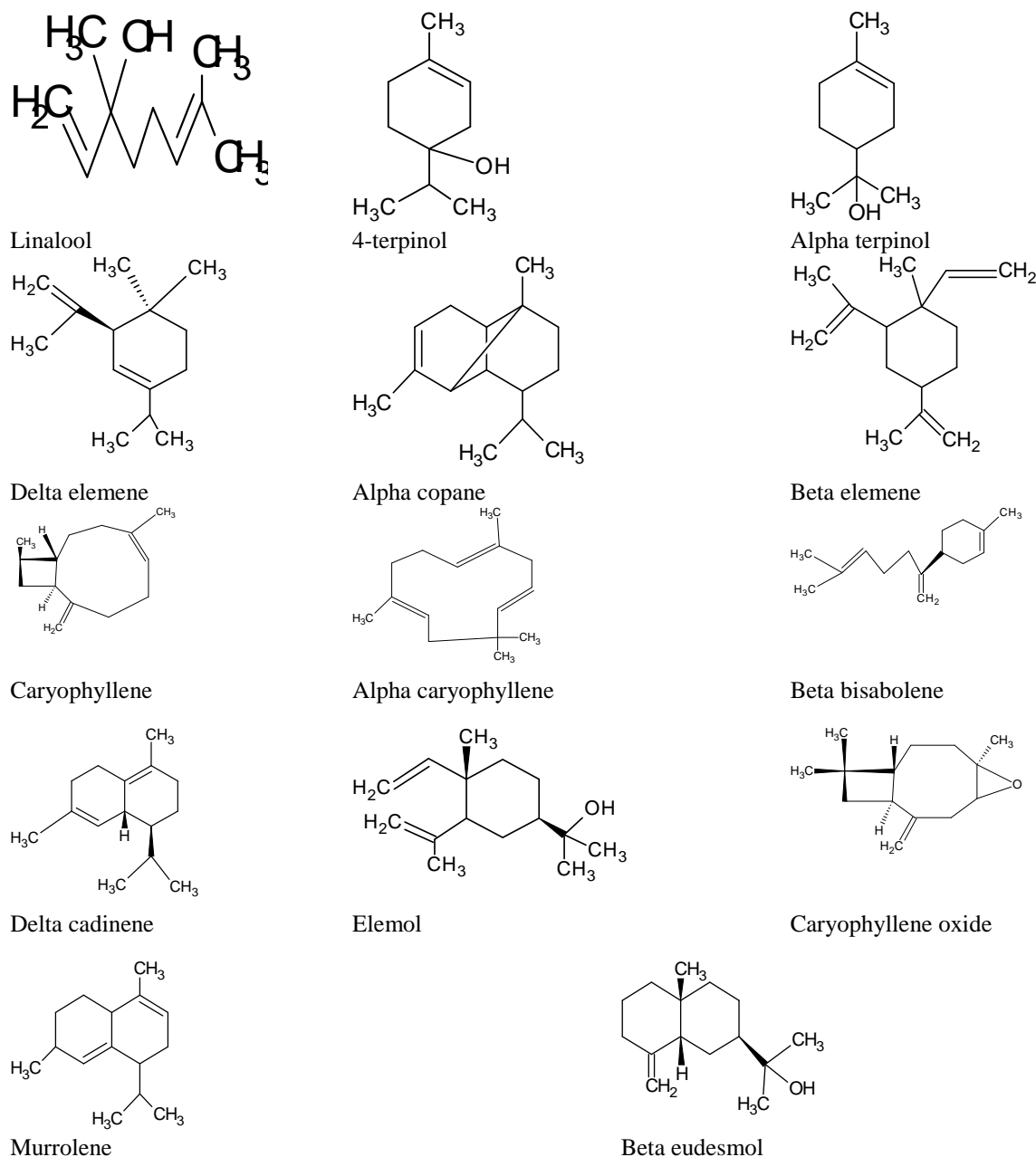


Fig.2 Structures of various components isolated from Petroleum ether extracts of *Piper nigrum*

Table 2: In vitro antibacterial activity of Petroleum ether Extracts

Bacterias	Diameter in (mm)
Gram (+) <i>Bacillus subtilis</i>	(-)
Gram (-) <i>Escherichia coli</i>	(+)
Gram (-) <i>Pseudomonas aeruginosa</i>	(-)

Yellow Crystals: Shiny, pale yellow crystals (m. p. 132°C), HR-mass spectra shows the peak at 286.14 [M+H]<sup>+</sup>, max 342 nm, max cm<sup>-1</sup>: 3421, 2941 2859, 1633, 1610, 1585, 1492, 1253, 1193, 1134, 1031, 997, 929, 830, 804. The data was in accordance with the Mass spectra which showed a molecular ion peak at 286.14 [M+H]<sup>+</sup> and at 571.2 [2M+H]<sup>+</sup> and at 593.2 [2M+Na]<sup>+</sup>. The 1H NMR spectra showed the peaks at 5.9 (2H,7), 7.3 (1H,3), 6.63 (1H,2), 3.3-3.6 (4H,c), 1.6-1.7 (5H,a,b).

Analysis of oily fraction: Compounds were identified by their GC retention time relative to known compounds and by comparison of their mass spectra with those present in IIM library. The GC-MS spectra of the oily fraction of petroleum ether extracts of *Piper nigrum* unveiled the presence of following components. From structure 1 to 14, the name of the compounds obtained are (linalool, 4-terpinol, alpha terpinol, delta elemene, alpha copane, beta elemene, caryophyllene, alpha caryophyllene, tau

Fig 3 : Antibacterial activity of Pet. Ether Extracts against *E. coli*

gurjunene, beta bisabolene, delta cadinene, elemol, caryophyllene oxide, murrulene, beta eudesmol) displayed in table 1.

#### Antibacterial activity

Cultures: Gram positive bacteria: *Bacillus subtilis*, Gram negative bacteria: *Escherichia Coli* and *Pseudomonas aeruginosa* were obtained from Biotech research laboratory, Lovely Professional University.

Chemicals: Nutrient agar and nutrient broth for bacterial cultivation and standard antibiotic like gentamicin were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai.

One gram of the extracts was dissolved in same solvent in such a way that the final concentration of each extract would be 1gm/ml of respective solvent.

Disc diffusion method: The *in vitro* antimicrobial activity of petroleum ether extracts of pepper were checked by disc diffusion method (Elgavyar M et al., 2001). Bacterial culture in log phase was inoculated in nutrient agar and plated. The 5  $\mu$ l of various extracts were poured on to different discs prepared from whatman No: 1 filter paper. The 2 or 3 discs were then placed on the petriplates containing cultures and incubated bacterias for 24 hours at 37°C. The diameter of zone of inhibition was measured.

#### CONCLUSIONS

The results suggest that the petroleum ether extracts is found very much effective against *E.coli*. No inhibition zone is seen against the bacteria *Bacillus subtilis* and *Pseudomonas aeruginosa*

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