



Phytochemical Analysis of Cold Toluene Extracts of *Piper nigrum* and its Antibacterial and Antifungal Activity

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ABSTRACT

A crystalline compound [(1,5-(1,3)benzodioxol-5yl)-1-oxo-2,4pentadienyl]-piperidine] and few sesquiterpenes (Caryophyllene, β -bisabolene, δ -Cadinene, Elemol, (-)- Cedreanol, α -Eudesmol) were obtained from the toluene extract of dried fruits of *Piper nigrum*. This is for the first time piperine has been extracted from the cold toluene extract of *Piper nigrum* along with few other sesquiterpenes. Piperine is an alkaloid found naturally in plants belonging to the pyridine group of piperaceae family, mainly responsible for the pungent taste of *Piper nigrum* where as the components isolated from its oil are meant for aroma. Structure of these compounds is examined and concluded by UV, IR, NMR and MS spectral analysis. The oil separated from toluene extract was analyzed by GC-MS spectroscopy. The components identified by GC-MS spectra, show that caryophyllene was present as major component and δ -Cadinene as minor component. The present study was aimed to extract the phytochemical compounds in toluene extract of *Piper nigrum* and to check the antibacterial and antifungal activity of the extracts.

Key words: *Piper nigrum*, dried fruits, GC-MS, amide, antibacterial activity and antifungal activity.

INTRODUCTION

Among many plants of family piperaceae, one showing tremendous potential in therapeutics is *Piper nigrum*¹. *Piper* has 700 species distributed in both hemispheres². As the plant has great adaptability towards various soils and climatic conditions so there will be a good inter species diversity³. *Piper nigrum* also known as black pepper is a climbing perennial shrub, can be used as a spice and folk medicine². Piperine, a piperidine derivative has many pharmacological activities like analgesic, antipyretic, CNS depressant, anti

inflammatory, antioxidant, anticonvulsant, antibacterial, antitumor and hepatoprotective². *Piper nigrum* originated in India⁵. Piperamides are the compounds with greatest insecticidal activities⁶. It has a great commercial, economical and medicinal value⁵.

MATERIALS AND METHODS

Seeds of *Piper nigrum* is purchased from the authorized seed shop at Jammu and identified by Dr. Gurdev Singh of botany department of Lovely professional University, Phagwara.

Extraction and Isolation

During the research for novel, bio active natural products, the dried and crushed seeds of the plant were successively soxhlated with the light petroleum ether, toluene, chloroform and alcohol on their polarity gradient. All the extracts were showing the potential for further treatment. Toluene extract kept for around three and half months, separated into upper brown coloured oily layer and rod shaped shiny crystals settled at bottom of the beaker. Shiny, pale yellow and rod shaped crystals settled at bottom of the beaker which were further identified on the basis of their spectral studies.

RESULTS AND DISCUSSION

The shiny pale yellow crystals (m. p. 132°C) obtained from toluene extract of *Piper nigrum* fruits is found to be an alkaloid on performing Mayer's reagent test⁸⁻¹⁰. The molecular formula was established as C₁₇H₁₉O₃N by Agilent, 6540 Q-TOF (HRMS) mass spectrometer⁸ and the TLC of the pure crystals showed Rf value 0.23 which is very close to standard Rf value of piperine, reported in literature that is 0.25⁴, thus crystals obtained may be of piperine. A UV spectrum shows λ_{\max} at 314.5 suggestive of alkaloid framework. IR spectra is

Table 1: Different components from cold toluene extract of *Piper nigrum*

Compound number	RT(min)	Peak name	Area	Amount/Rf
1	5.934	Toluene	3.929e+6	99.216
2	38.357	Caryophyllene	10878	0.275
3	41.815	β -bisabolene	2341	0.059
4	42.325	δ -Cadinene	1828	0.046
5	43.562	Elemol	2101	0.053
6	47.459	(-)- Cedreanol	3755	0.095
7	47.885	α - Eudesmol	905	0.023
8	63.175	Piperine	9249	0.234

Table 2: In vitro antibacterial activity of Petroleum ether Extracts

Bacterias	Zone of inhibition
Gram (+) <i>Bacillus subtilis</i>	(+) 3mm dia
Gram (-) <i>Escherichia coli</i>	(-)
Gram (-) <i>Pseudomonas aeruginosa</i>	(-)
Gram (+) <i>Staphylococcus aureus</i>	(-)
Gram (-) <i>Salmonella typhi</i>	(-)

Table 3: In vitro antifungal activity of toluene extracts

Fungus	Zone of inhibition
<i>Aspergillus niger</i>	(+) 5 mm dia

compared with the original spectra of piperine⁹ that showed absorption bands at 3000 cm⁻¹ corresponds to aromatic C-H stretch; at 1633 and 1610 cm⁻¹ corresponds to symmetric and asymmetric stretching of C=C (diene); 1610, 1583 and 1491 cm⁻¹ corresponds to aromatic stretching of C=C phenyl ring; 1633 cm⁻¹ corresponds to stretching of -CO-N, 2939, 2856 cm⁻¹ corresponds to CH₂ asymmetric and symmetric stretching, 1446 cm⁻¹ corresponds to CH₂ bending; 1251, 1193 cm⁻¹ corresponds to asymmetric stretching of =C-O-C; 1030 cm⁻¹ corresponds to symmetric stretching of =C-O-C; 927 cm⁻¹ corresponds to C-O stretching, 1132 cm⁻¹ corresponds to in plane bending of phenyl CH; 997 cm⁻¹ corresponds to C-H bending for trans -CH=CH-; 848, 830 and 804 cm⁻¹ corresponds to out of plane C-H bending. The 400 MHz ¹H-NMR spectra showed the peaks at δ 5.976(2H, 7'), δ 7.39 (1H, 3), δ 6.43 (1H, 2), δ 3.529-3.633 (4H, C) and δ 1.562-1.688 (5H, b, a). The ¹³C-NMR spectra

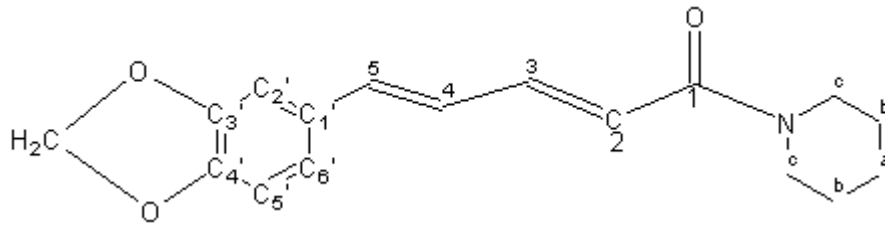
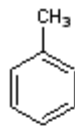
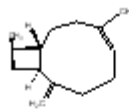


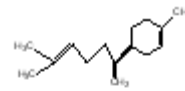
Fig. 1: Structure of piperine



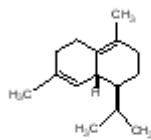
Toluene



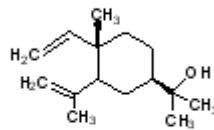
Caryophyllene



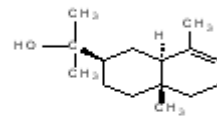
β -bisabolene



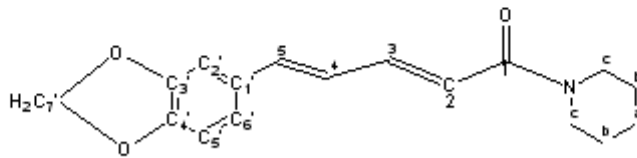
δ -Cadinene



Elemol



α -Eudesmol



Piperine

Fig. 2: Structures of various components present in Toluene extracts

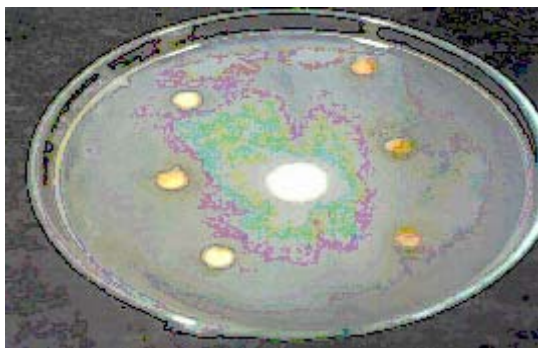


Fig. 3: Antibacterial activity of toluene extracts against the fungi *Bacillus subtilis*



Fig. 4: Antifungal activity of toluene extracts against the fungi *Aspergillus niger*

showed the peaks at δ 167.7 (C-1), δ 120.6 (C-2), δ 140.2 (C-3), δ 123.9 (C-4), δ 132.4 (C-1'), δ 106.7 (C-2'), δ 149.7-149.8 (C-3',C-4'), δ 109.4 (C-5'), δ 207 (C-6') and δ 102.7 (C-7'). The HR-mass spectrum showed a molecular ion peak at $[M+H]^+$ 286.14, $[2M+H]^+$ peak at 571.27 and $[2M+Na]^+$ peak at 593.25.

Piperine

Pale yellow, rod shaped crystals of toluene extract, exhibited melting point 132°C, UV λ_{max} at 314.5, IR ν_{max} cm^{-1} : 3000, 2939, 2856, 1633, 1610, 1583, 1491, 1446, 1251, 1193, 1132, 1030, 997, 927, 830, 804. The 1H -NMR spectra showed the peaks at δ 5.976, δ 7.39, δ 6.43, δ 3.529-3.633, δ 1.562-1.688. The ^{13}C -NMR spectra showed the peaks at δ 167.7, δ 120.6, δ 140.2, δ 123.9, δ 132.4, δ 106.7, δ 149.7-149.8, δ 109.4, δ 207, δ 102.7. The mass spectrum showed a molecular ion $[M+H]^+$ peak at 286.14, $[2M+H]^+$ peak at 571.27 and $[2M+Na]^+$ peak at 593.25.

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 toluene extracts of *Piper nigrum* unveiled the presence of following components.

Antimicrobial activity

Cultures

Gram positive bacteria: *Bacillus subtilis*, *Staphylococcus aureus*; Gram negative bacteria: *Escherichia Coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and Fungi *Aspergillus niger* were obtained from Biotech Research Laboratory, Lovely Professional University.

Chemicals

Nutrient agar and nutrient broth for bacterial cultivation; Potato dextrose agar and potato dextrose broth for fungal 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 toluene extracts of pepper were checked by disc diffusion method¹⁰. Bacterial culture in log phase was inoculated in nutrient agar and plated. The 5 μ 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 and fungi in B.O.D incubator for 7 days. The diameter of zone of inhibition was measured.

CONCLUSIONS

The results suggest that the toluene extracts is found very much effective against *Bacillus subtilis*, and fungus *Aspergillus niger*. No inhibition zone is seen against the bacteria *E.coli*, *Staphylococcus aureus*, *Salmonella typhi* and *Pseudomonas aeruginosa*.

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