

Phyto-Chemical Profiling of Leaf Extract of Black Pepper, *Piper Nigrum* L. Through GCMS Analysis

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Abstract

Piper nigrum L. is a widely used plant in traditional treatments and recognized for its several biological properties. Seeds of *P. nigrum* were studied drastically, however, leaf is found lacking. Gass chromatography-mass spectrometry (GC-MS) analysis confirmed the presence of various phytochemicals belong to Mono and sesquiterpenes, fragrant hydrocarbon, cardiac glycoside, steroid, steroid lactone and pentacyclic terpenoids with Anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic, and anti-mutagenic, anti-insect, contraceptive, cardiotoxic, anti-allergenic, anti-fungal, anti-viral houses. Resibufogenin, (steroid lactone) and Lupeol (pentacyclic terpenoid) had been discovered to be extra with high therapeutic values.

Keywords: *Piper nigrum*, Phytochemicals, GC-MS analysis, therapeutic values, anti-viral, anti-mutagenic, cardiotoxic

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Introduction

Medicinal plants and plant derived merchandise are regarded to play giant position in number one healthcare and in the control of numerous illnesses when you consider that long times [1]. Bioactive plant extracts are the vital a part of conventional system of drugs and often exhibit more than one biological activity together with Anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-mutagenic, anti-insect, anti-allergenic, anti-fungal, anti-viral activities. This has led the researchers for the delineation of energetic compounds of medicinal plants with therapeutic values. *Piper nigrum*, generally known as black pepper, is used as a spice, grown in tropical areas which include Brazil, Indonesia, and India. Seeds of *P. nigrum* and its biologically active constituents are used in exceptional traditional structures of medicine (Ayurvedic and Unani) as a stimulant, carminative, diuretic, anticholera, sialagogue, anti-asthmatic, etc. [2], medical investigations discovered the antimicrobial, antioxidant [3], [4], hepatoprotective [5], anti-depressant [6] and immunomodulatory [7] seeds of the black pepper. Piperine (1-peperoyl piperidine), and different fundamental alkaloids of *P. nigrum* has been proven to own several pharmacological properties [8, 9]. But, investigation of leaf of *P. nigrum* is determined lacking and therefore the present study gains importance.

Materials and Methods

Collection of plant material

Fresh leaves of *P. nigrum* were collected from Madikeri, Karnataka (India) located between $11^{\circ} 42' 34.0056''$ N latitudes and $76^{\circ} 5' 43.9332$ E longitudes. It is situated at an altitude of 1000 metres above sea level. The collected plant material was authenticated by a taxonomist at ICFRE-Institute of Forest genetics and Tree Breeding, Coimbatore.

Preparation of plant extracts

The collected plant materials were air dried and ground into uniform powder. Dry powder of plant sample was extracted with methanol using soxhlet apparatus for 6 hours. The extract was filtered over anhydrous sodium sulphate followed by concentrated using rotary evaporator. The concentrated extract was subjected to freeze drying in a lyophilizer till dry powder was obtained. Finally, the extracted powder was resuspended with the methanol at the concentration of 100mg/ml (w/v) followed by filtration through Varian Bond Elut C₁₈ solid phase extraction to remove impurities. 1μl of this solution was employed for GC-MS-MS analysis.

GC-MS-MS analysis

The GC-MS-MS analysis was carried out using Varian 4000 Ion trap GC/MS/MS with Fused silica 15m x 0.2 mm ID x 1μm of capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period the oven temperature was rose up to 280 °C, at the rate of an increase of 5 °C/min, and maintained for 9 min. Injection port temperature was ensured as 250 °C and Helium flow rate as 1 ml/min. The ionization voltage was 70eV. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45-

450 (m/z) [10]. Using computer searches on a NIST Ver.2.1 MS data library and comparing the spectrum obtained through GC-MS-MS compounds present in the plants sample were identified.

Identification of phytocompounds

Interpretation on mass-spectrum GC-MS-MS was conducted using the database of National institute Standard and Technology (NIST) having more 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

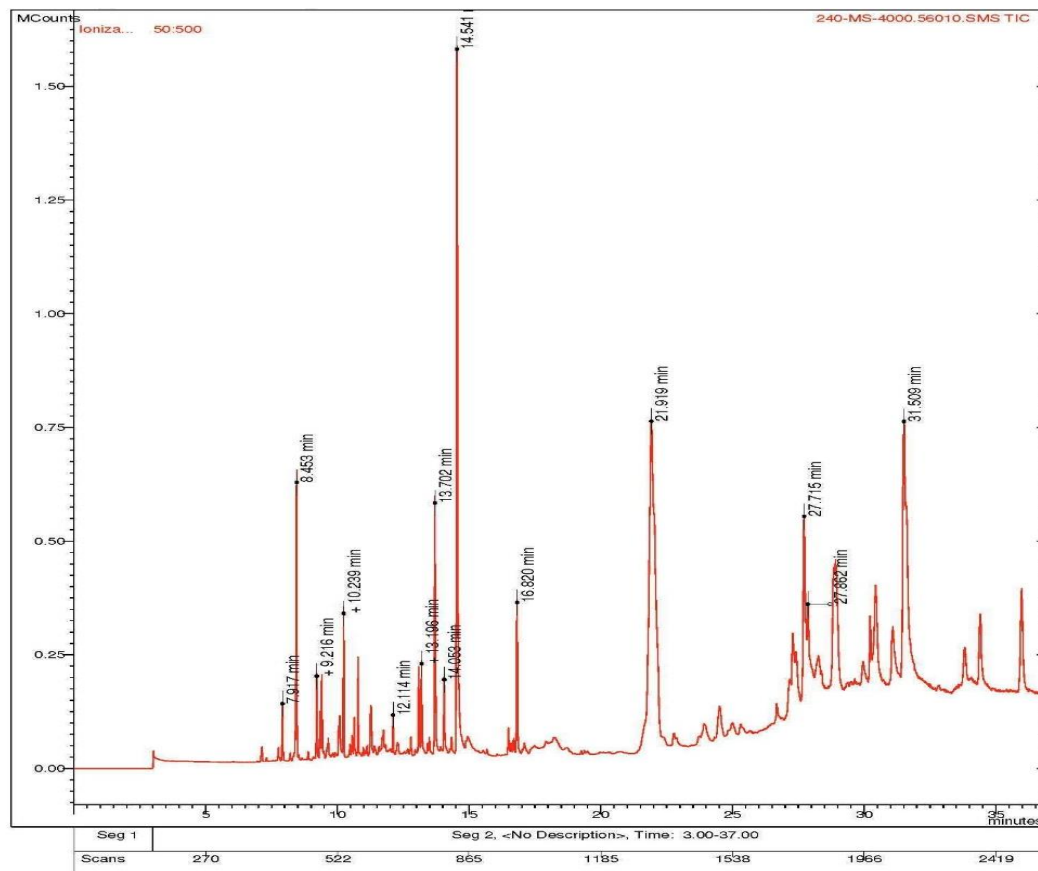


Figure 1 GC-MS chromatogram of methanol extract of leaf of *P. nigrum*

Results and Discussion

The GC/MS/MS evaluation of methanol extract of leaf of *P. nigrum* gave us nineteen essential compounds (**Figure 1**). GCMS analysis separated and identified compounds from different classes including esters, ketones, acids, hydrocarbons, alcohols, aldehydes, amino compounds, etc. Resibufogenin, Lupeol, Elemene, Azulene, Gitoxigenin and Germacrene are major phytochemicals identified. Ling Liu *et al.*, (2007) [11] reported that 30 compounds were identified in the essential oil of *P. nigrum* L. and β -caryophyllene (23.49%) was reported high followed by 3-carene (22.20%), and D-limonene (18.68%). Resibufogenin having molecular weight of 384 was separated at retention time 14.541min in maximum quantity of 25.805% followed by 14.531% Lupeol having molecular weight of 426 separated at 21.919 min. All compounds diagnosed by way of GC/MS/MS screening have been assessed for biological property using physico-chemical assets calculations according to Tice policies. As in keeping with Tice rule compounds are much more likely to have properties of antimicrobial, anticancerous antioxidants and anti-insect if molecular weight is within ≥ 150 and ≤ 500 ; theoretical logarithm of the noctanol/water partition coefficient ($\log P$), is less than or same to 5; hydrogen bond acceptor is inside 1-8; hydrogen bond donor is less than or equal to 2 and the range of rotatable bond is less than or same to twelve. The compounds those are strictly following the Tice guidelines were considered as antimicrobial, anti-cancerous, antioxidant and anti-insect potential compounds for brand new or novel pills. The phytochemicals including Resibufogenin, Lupeol, Elemene, Azulene, Gitoxigenin and Germacrene identified in the present study were found to have Anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic, and anti-mutagenic, anti-insect, contraceptive, cardiotoxic, anti-allergenic, anti-fungal, anti-viral properties [12-14].

Table 1 Phyto-chemical constituents of methanol extract of leaf of *P. nigrum* by GC-MS analysis

S. No.	Chemical name	Retention Time	Molecular Formula	Molecular Weight (g/mol)	Abundance (%)	Biological property
1	Germacrene B	7.917	C ₁₅ H ₂₄	204	1.442	Anti-microbial
2	gamma.-Elemene	8.453	C ₁₅ H ₂₄	204	7.437	Anti-microbial, Anti insect
3	Germacrene D	9.216	C ₁₅ H ₂₄	204	1.900	Anti-mosquitoes
4	Azulene, 1,2,3,4,5,6,7,8-oct	9.344	C ₁₅ H ₂₄	204	1.101	Anti-allergenic
5	Humulen-(v1)	9.408	C ₁₅ H ₂₄	204	2.130	Anti-microbial, Anti insect
6	Azulene, 1,2,3,4,5,6,7,8-oct	10.239	C ₁₅ H ₂₄	204	3.041	Anti-allergenic
7	Aristolene epoxide	10.644	C ₁₅ H ₂₄ O	220	0.980	Anti-oxidant, Anti-microbial, Anti insect
8	Norethynodrel	10.785	C ₂₀ H ₂₆ O ₂	298	2.254	Contraceptive
9	Card-20(22)-enolide, 3,5,14,19-tetrahydroxy-, (3. beta.,5. beta.)-	12.144	C ₂₃ H ₃₄ O ₆	406	0.922	Anti-fungal
10	Strophanthidin	13.092	C ₂₃ H ₃₂ O ₆	404	2.164	Cardiotonic
11	7-Octadecyne, 2-methyl-	13.196	C ₁₉ H ₃₆	264	2.691	Anti-oxidant
12	Gitoxigenin	13.702	C ₂₃ H ₃₄ O ₅	390	7.678	anti-cancer, Antiviral
13	Androstan-17-one, 3-ethyl-3-	14.053	C ₂₁ H ₃₄ O	318	2.003	Immunomodulator
14	Resibufogenin	14.541	C ₂₄ H ₃₂ O ₄	384	25.805	Cardiotonic, anti-cancer, Antiviral (MERS)
15	Cyclohexanol, 5-methyl-2-(1-	16.820	C ₁₀ H ₂₀ O	156	4.821	Analgesic
16	Lupeol	21.919	C ₃₀ H ₅₀ O	426	14.531	Anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic, and anti-mutagenic
17	Benzoic acid, 3-methoxy-	27.715	C ₅ H ₈ O ₃	152	8.514	Cosmetics and dye industry
18	3-Furanmethanol, .alpha.-(3,4-dimethoxyphenyl) tetrahydro-3-hydroxy-4-veratryl-	27.862	C ₂₂ H ₂₈ O ₇	404	0.568	Hepatitis
19	2',3'-O-p-Anisylidene-guanosine	31.509	C ₁₈ H ₁₉ N ₅ O ₆	401	10.019	Cytotoxic.

Resibufogenin, (steroid lactone) and Lupeol (pentacyclic terpenoid) were found to be extra with high therapeutic values reported that lupeol act as clinically active anti-inflammatory agent. Elemene, a sesquiterpene compound was isolated from *Curcuma zedoaria* with anticancer activity [15]. Lupeol a plant marker compound reported in *Crateva adansonii* found to inhibit NF- κ B the transcriptional factor and molecular targets of inflammation [16] and also and suppress tumor necrosis factor- α (TNF- α) and IL-1 β [17]. Zahin *et al.*, (2021) [18] said the anti-oxidant, anti-cancers, anti-bacterial, and anti-mutagenic activities of methanol extract of seeds of *P. nigrum*. Nearly all the compounds detected in GC-MS analysis follow Tice rule. As a result, isolation and bioassay research with recognize to aforementioned compounds can be an insight into broaden novel pills for application in pharmacology.

Conclusion

Piper nigrum, normally known referred to as black pepper, is used as a spice, grown in lots of south India states viz., Kerala, Karnataka and Tamil Nadu found to possess many medicinal values. Seeds of *P. nigrum* has been studied extensively, however, phytochemical investigation of leaf of *P. nigrum* is found missing and consequently a strive has been made on this aspect. GC-MS analysis of leaf extract of *P. nigrum* revealed the presence of secondary metabolites belong to mono and sesquiterpenes, aromatic hydrocarbon, cardiac glycoside, steroid, steroid lactone and pentacyclic

terpenoid with anti-oxidant, anti-inflammatory, anti-hyperglycemic, anti-dyslipidemic, and anti-mutagenic, anti-insect, contraceptive, cardiogenic, anti-allergenic, anti-fungal, anti-viral properties. Resibufogenin, (steroid lactone) and Lupeol (pentacyclic terpenoid) have been found to be more with high healing values, and hence leaf of *P. nigrum* can be a potential source of raw material for use in pharmacology enterprises for growing useful drugs.

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