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Secondary Metabolites obtained from Cold and Hot DCM Extracts of *Piper nigrum* Using GC-MS Analysis and their antifungal activity –A Comparative study

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ABSTRACT

A variety of secondary metabolites (Caryophyllene, δ–Cadinene, Elemol, Nerolidol, c-murrolene, α- Eudesmol, Ethyl linoleate , Carvacrol, dimethoxy phenol, β–bisabolene) have been obtained from the DCM extracts of Piper nigrum. The essential oil isolated from the cold and hot dichloromethane extracts of Piper nigrum seeds were subjected to GC-MS analysis. The components isolated from its oil are meant for aroma. A comparative study of the various phytochemicals obtained from cold and hot DCM extracts was done and their antifungal activity against Aspergillus niger was found out. These phytochemicals have various pharmaceutical properties.

Keywords: Piper nigrum, Phytoconstituents, DCM Extracts, GC-MS Analysis.

1. INTRODUCTION

The family Piperaceae has shown tremendous effects in therapeutics. *Piper nigrum* (Black Pepper), a member of family piperaceae, is a perennial herb and has various medicinal values.(Reshmi, S. K et al). It has been used as a spice and in folk medicine due to its physiological activities and thus having 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 "The King of Spices" contributes its major share in Indian Spice Export Scenario (Zachariah T.J et al., 2010). The various pharmaceutical properties of Piper nigrum are CNS depressant, antioxidant, radical scavenging, anti insecticidal, antibacterial, allelopathy, anticonvulsant, antitubercular, antipyretic, antiinflammatory, exterofective (C.F.Su, Helen, et al., 1981; Dorman, H.J.D., 2008; Siddiqui, Z.S., 2007; Daniel, M., 2006).

Phytoconstituents has obtained great attention in the recent years as they can prevent and cure various human ailments and considered more boifriendly. Plant produces these chemicals to protect itself from predators but researchers have demonstrated their use to treat various diseases(Veerachari, U et al., 2011). The main objective of entire study is to compare the secondary metabolites obtained from cold and hot DCM extracts and their antifungal activity.

2. MATERIALS AND METHODS

2.1 **General:** The essential oils obtained from the hot and cold DCM extracts were analyzed by using Varian 4000 GC-MS. The instrument operates at 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 0.25µm, the Helium carrier gas had a delivery rate of 1 ml/min, a capillary injector operating at 280°C in the split mode (1:150),flame ionization detector (FID) running at 300°C, the column oven temperature programming was 50°C for 5 min and then increased from 50 to 250°C at the rate of 3°C/min and hold for 7 min.

2.2 **Plant Material:** Theseeds of *Piper nigrum* were purchased from an authentic seed shop at Jammu's district and classified systematically by Dr. Rajesh Manhas of Botany Department at University of Jammu.

2.3**Extraction and Isolation: Cold Extraction:** The dried and crushed seeds (1 Kg) of *Piper nigrum* were taken in muslin cloth and dipped in dichloromethane for three days. The cold extractsobtained were treated with ethanol repeatedly to obtain the essential oil which is further subjected to GC-MS analysis.

Hot Extraction: The dried and crushed seeds of *Piper nigrum* were soxhlated and distilled with dichloromethane to obtain DCM extracts which is thenrepeatedly treated with ethanol to obtain essential oil whose components were identified using GC-MS analysis.

2.4 Antifungal Activity

2.4.1 Fungal Culture: *Aspergillus niger* culture was obtained from NCIM Pune and maintained at Biotech Research Laboratory, Lovely Professional University.

2.4.2 Chemicals: Potato dextrose agar and broth for fungal cultivation and standard antibiotic like gentamicin were purchased from Hi – Media Laboratories Pvt. Ltd., Mumbai.1 ml of essential oil is dissolved in 1 ml of dichloromethane solvent and various discs of Whatman No. 1filter paper were then dipped in it.

2.4.3 Disc Diffusion Method: The *invitro* antifungal activity of the hot and cold essential oils of pepper were checked by disc diffusion method (Elgavyar. M et al, 2001). Fungal culture was inoculated in potato dextrose agar and plated. The Whatman filter paper discs were dried and then placed on to the petriplates containing fungal cultures and incubated in BOD incubator for 7 days. The diameter of zone of inhibition was measured.

3. RESULTS AND DISCUSSION

3.1 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 IIIM Library. The GC-MS spectral analysis of oily fraction obtained from hot and cold DCM extracts of *Piper nigrum* seeds revealed the presence of following phytoconstituents.

Table 1: Various Secondary metabolites obtained from cold DCM essential oil:

Compound No.	RT (min)	Peak Name	Area	Amount/Rf
1	5.946	Methyl benzene	505274	99.129
8	38.362	Caryophyllene	4441	0.871

Table 2: Various Secondary metabolites obtained from hot DCM essential oil:

Compound number	RT (min)	Compound Name	Area	Amount/Rf
1	25.176	Formylpiperidine	2658	0.446
2	27.230	4-Terpeneol	633	0.106
3	28.362	A-phellandrene epoxide	1213	0.204
4	32.761	Carvacrol	1852	0.311
5	33.629	2-Methyl naphthalene	618	0.104
6	34.732	Piperonal	15416	2.587
7	35.000	Dimethoxyphenol	2359	0.396
8	38.367	Caryophyllene	3855	0.647
9	41.686	2,4-di-t-butylphenol	3216	0.540
10	41.832	β–bisabolene	1590	0.267
11	42.351	δ –Cadinene	1359	0.228
12	43.573	Elemol	3059	0.513
13	43.853	Nerolidol	992	0.166
14	46.763	4,4-dimethyl-3-(3-methylbut-3-enylidene)methylenebicyclo[4.1.0] heptane	2047	0.343
15	47.471	c-murrolene	6958	1.168
16	47.901	α- Eudesmol	1436	0.241
17	56.881	Cyclopropanebutanoic acid	776	0.130
18	57.384	Bicyclo(3.3.1) Nonan-2-one	1044	0.175
19	59.074	Ethyl palmitate	1897	0.318
20	64.226	Ethyl linoleate	1202	0.202

Table 3: *Invitro* antifungal activity of hot and cold essential oils from DCM extracts:

S. No.	Essential oil	Micro organism	Zone of Inhibition
1	Oil from cold DCM extracts	*Aspergillus niger*	(-)
2	Oil from hot DCM extracts	*Aspergillus niger*	38mm

4. CONCLUSIONS

Phytochemical analysis of DCM extracts has shown the presence of large number of alkaloids, terpenoids and other phytochemicals that are having great pharmaceutical activities. Phytoconstituents obtained from cold and hot DCM extracts are different. These Secondary metabolites are used to combat many diseases and also to boost the immune system. The phytochemical characterization of the extracts, the identification of responsible bioactive components and quality standards are necessary for future study. Different phytoconstituents were obtained from cold and hot essential oils of DCM extracts and also having different biological activity.

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REFERENCES

1. S. K Reshmi, E Sathya, S Devi, Isolation of piperidine from *Piper nigrum* and its antiproliferative activity,*J. of Medicinal Plants Research., 4(15),* 2010, 1535-1546.
2. A Krishnamurthi,,*The Wealth of India*. Counsil of Scientific and Industrial Research, New Delhi 1969.
3. VS Parmar, S.C Jain, et.al. Phytochemistry of the genus *piper.Phytochemistry.,46,* 1997, 597-673.
4. F Kiuchi, et.al. Amides from Piper nigrum L. with dissimilar effects on melanocyte proliferation in-vitro. *Chem.Pharm. Bull.,36,* 1998, 2452—2465.
5. B S Siddiqui et.al. An Amide from fruits of *Piper nigrum. Phytochemistry*, *45*, 1997, 1617—1619.
6. Nadkarni, A.K. *Indian Materia Medica*. Popular Book Depot, Bombay, 1954.
7. T. J. Zachariah, et.al. Correlations of metabolites in the leaf and berries of selected black pepper varieties. *Scientia Horticulturae., 123*,2010; 418-422.
8. C. F. Su, Helen., R Horvat,. Isolation, Identification and Insecticidals Properties of *Piper nigrum* Amides. *J.Agric.Food.chem*., 29(1), 1981, 115-118.
9. H .J. D Dorman, S. C. Deans, Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *J.of Applied Microbiology., 2(88),*2008; 308-316.

10. Z S Siddiqu, Allelopathic effects of black pepper leachings on Vigna mungo(L) Hepper. *Acta Physiologiae Plantarum*., 29(4), 2007, 303-308.

11. Daniel, M. *Medicinal Plants: Chemistry and Properties*. Science Publisher.USA, 2006.

12. U Veerachari, A. k Bopaiah,. Preliminary phyto-chemical evaluation of the leaf extract of five Cassia Species. *J. Chem. Pharm. Res.*,3(5), 2011, 574-583.

13. M Elgavyar, F. A Draughon, et.al. Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. *J Food Prot*., 64(7), 2001,1019-1024.