ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 7, Suppl 2, 2014

ISSN - 0974-2441

Research Article

PHYTOCHEMICAL SCREENING, HPTLC AND GC-MS PROFILING IN THE RHIZOMES OF *ZINGIBER NIMMONII* (J. GRAHAM) DALZELL

ASSAMAKANTAKATH FINOSE[1,2], VELLIYUR KANNIAPPAN GOPALAKRISHNAN[1]*

[1]Department of Biochemistry, Karpagam University, Coimbatore, Tamil Nadu, India-641021, [2]MIMS Research Foundation, Calicut, Kerala, India-673007

Email: vkgopalakrishnan@gmail.com.

Received: 27 February 2014, Revised and Accepted: 31 March 2014

ABSTRACT

Objectives: The preliminary phytochemical screening was carried out in the hexane and methanolic extracts of *Z.nimmonii* rhizomes. HPTLC and GCMS profiling were done to develop the fingerprint of the plant to detect the major chemical constituents of the plant.

Methods: Preliminary screening involved the qualitative methods to detect the presence of carbohydrates, flavonoids, alkaloids, terpenoids, tannins, oils and fats. HPTLC fingerprint was developed for the hexane extract using hexane, ethyl acetate and isopropanol as the solvent system in the ratio 7:2:1.GCMS profiling was done using triple axis detector.

Results: The study revealed the presence of flavonoids, oils and fats as the major components. Terpenoids and tannins were present in moderate amounts. HPTLC profiling revealed 6 peaks in 254nm wavelength and 2 peaks in 366nm.GCMS profiling of the hexane extract gave the presence of 23 components with benzoic acid(35%),3,5-dicyclohexyl-4-hydroxy-,methyl ester and 1-chloroeicosane(29%) as the major components.

Conclusion: *Z.nimmonii* contains many important components which has significant role in the drug research and isolation of the compounds will be of therapeutic usage.

Keywords: *Z.nimmonii*. phytochemical screening, HPTLC, GCMS, therapeutic.

INTRODUCTION

World Health Organization in 2003 reported that around 80% of the people in the developing countries depend on plant based traditional medicines for their health care needs. The traditional medicine is based on theories, beliefs and experiences.

It is also used to diagnose, prevent and treat both mental and physical illness [1].Ayurveda is totally based on the usage of plant based medicine and treatment[2]. Application of plants in treatment of different diseases including cancers is inevitable and it seems to be the basis for modern medical science, as they are considered to be great sources of new drugs [3,4,5,6,7,].Chemicals which impart color and smell to the plants are known as secondary metabolites or phytochemicals. Phytochemical term is given to those chemicals which possess significant biological potential, but not have been established as essential nutrients. Identification as well as isolation of new compounds with medicinal importance is a recent and promising development in recent research[8,9,10,11].

Zingiber nimmonii (J. Graham) Dalzell, also called as wild ginger is an endemic Zingiber species from the Western Ghats in South India. It usually grows both at low and high altitudes, in moist areas under the shades of trees[12,13].The oil showed significant activities against the human pathogenic fungi,Candida glabrata,C.albicans and Aspergillus niger[14].

Phytochemical evaluation includes preliminary screening, chemoprofiling and compound analysis using modern analytical instrumentation.WHO accepted chromatography as a strategy for the evaluation and identification of quality of plants used as medicines [15,16].HPTLC is a widely used technique since it is highly accurate, precise and reproducible in case of results. High sample throughput at low operating cost,ease of sample preparation and short analysis time are a few advantages of HPTLC [17,18].

Gas Chromatography Mass Spectroscopy (GCMS),is a very compatible technique and the most commonly used technique for the identification and quantification purposes. The unknown compounds in a complex mixture can be determined by interpretation and also by matching the spectra with the spectra of the reference [19].

Preliminary screening in methanol solvent revealed the presence of major class of compounds. HPTLC profile along with the fingerprint and GCMS profile was developed for the hexane extract of *Z.nimmonii* rhizome.

MATERIALS AND METHODS

Plant Material

Zingiber nimmonii (J. Graham) Dalzell, was collected from Calicut University Campus,Kerala, India and taxonomically identified by Dr.A.K Pradeep, Herbarium curator,University of Calicut, Kerala,India.The voucher specimen (95954) has been retained in Department of Botany, University of Calicut, Kerala, India.

Plant Extraction

The collected rhizomes were dried under shade and then powdered with mechanical grinder. The dried powder material was extracted with Hexane,and Methanol in a soxhlet apparatus sequentially.25gms of the dried sample was extracted in 250ml of the respective solvents.The excess of the solvents were concentrated using a rotary flash evaporator.The crude extracts obtained were free from solvents.These extracts were further used for phytochemical screening, HPTLC and GCMS profiling.

Chemicals

Hexane,Ethyl acetate,Isopropanol and Methanol were purchased from Merck, India. All the chemicals and solvents used were of

analytical grade and purchased from Merck Chemicals, India.

Preliminary screening

The phytochemical screening of the plant extract was carries out by following the method of Paech and Tracey[20,21,22]

Test for alkaloids

2 ml aliquot of the extract was treated with Dragendorff's and Mayer's reagents to test the presence or absence of alkaloid. Orange red precipitate in case of Dragendorff's and White turbidity in case of Mayer's test indicate the presence of alkaloids.

Test for Flavonoids

Shinoda test:One ml of the extract was treated with magnesium turnings and 1-2 drops of concentrated HCI.Formation of pink or red colour shows the presence of flavonoids.

Ferric Chloride test:One ml of the extract was treated with 1 ml of ferric chloride.The formation of brown color confirms the presence of flavonoids.

Test for Tannins

One ml of the extract was treated with few ml of 15 % neutral ferric chloride.A dark blue or bluish black colour product shows the presence of tannins.

Lead acetate test:One ml of the extract was treated with few ml of lead tetra acetate solution.A precipitate production shows the presence of tannins and phenolic compounds.

Test for Carbohydrates

Fehling's test:The extract was treated with 5.0 ml of Fehling's solution and kept at boiling water bath.Formation of yellow or red colour precipitate indicates the presence of reducing sugars.

Benedicts test:To 1 ml of the extract added 5 ml of Benedict's solution and kept at boiling water bath. Red, yellow or green precipitate indicates the presence of reducing sugars.

Test for saponins

Frothing test:About 1 ml of alcoholic extract was diluted separately with 20 ml of distilled water and shaken in a graduated cylinder for 15 minutes.One cm layer of foam indicates the presence of saponins.

Test for fixed oils and fats

Spot Test:Press small quantity of extract between two-filter papers.Oil stains on the filter paper indicates the presence of fixed oil.

Test for Terpenoids

Liebermann test:To 1 ml of extract, 3 ml of acetic acid and few drops of concentrated sulphuric acid were added.Colour change from red to blue indicates the presence of terpenoids.

HPTLC Profiling

HPTLC was performed on 4.0x10.0cm aluminium backed plates coated with silica gel $60F_{254}$ (Merck,Mumbai,India).The samples were applied at a bandwidth of 6mm.The samples were applied in duplicates.The distance between the two tracks were 18.0mm.The samples were applied approximately 10.0mm from the bottom of the plate using CAMAG Linomat 5"-171118"S/N 171118(1.00.12).The solvent front position was 80.0 mm.The solvent system used was Hexane,Ethylacetate and Isopropanol in the ratio 7:2:1(v/v/v) in a Camag glass twin-trough chamber, previously saturated with mobile phase vapour for 20 mins.After development the plates were dried with a hair dryer and then scanned at wavelengths 254nm and 366nm with a Camag TLC scanner with WINCAT Software along with a deuterium lamp. The method was validated according to the International Conference on Harmonization(ICH) guidelines[23].

GCMS Profiling

GCMS profiling was done using Agilent 7890A series Gas chromatograph with 5975C triple Axis detector. The column used was Agilent J and W GC Column DB-5 with dimensions 30mmX0.25mmX0.25µm.The flow rate was 1mL/min.The oven temperature was on 55°C hold for initial 2 mins,then slowly increased to 55°C-125°C for 6 mins.Further the temperature was increased to 200°C for 2mins followed by 250°C for 6 mins.The software employed was Chemstation(Agilent).

RESULTS AND DISCUSSION

Preliminary phytochemical screening in methanolic extract revealed the presence of certain secondary metabolites which is demonstrated below(Table 1).

Table 1: Preliminary phytochemical screening results.

Sl No.	Compounds	Results
1	Alkaloids	---
2	Flavonoids	+++
3	Carbohydrates	+
4	Tannins	++
5	Terpenoids	++
6	Oils & fats	+++
7	Saponins	+

+:Traces,++:Moderate,+++:Major,---:Absence

HPTLC profiling

HPTLC profiling of the hexane extract gave six separate peaks with distinct Rf values(Table 2) of 0.06,0.34,0.55,0.62,and 0.65.Two distinct peaks were obtained at the wavelength of 254nm(0.06,0.65).An overlaid chromatogram was developed for *Z.nimmonii* rhizomes at both 254 and 366nm(Fig 1).The TLC plates was further visualized.(Fig 2).There was only two distinct peaks visible when scanned at 366nm which gave Rf values of 0.06 and 0.65.This plate was also further visualized and documented(Fig 2).

Table 2: Evaluation results of peaks at 254nm and 366nm showing specific retention factors and area %.

Evaluation results of peaks at 254nm									
Peak	**Start Rf**	**Start height**	**Max Rf**	**Max height**	**Max %**	**End Rf**	**End height**	**Area**	**Area %**
1	0.04	0.4	0.06	102.1	8.14	0.09	5.0	941.0	2.03
2	0.30	15.9	0.34	25.0	2.00	0.35	16.6	844.3	1.82
3	0.45	26.3	0.55	134.7	10.75	0.56	125.9	5299.0	11.43
4	0.56	126.3	0.62	497.8	39.70	0.64	453.3	17991.1	38.82
5	0.64	454.2	0.65	466.0	37.16	0.79	59.1	20744.6	44.76
6	0.91	23.6	0.93	28.3	2.26	0.96	0.5	524.3	1.13
Evaluation results of peaks at 366nm									
1	0.03	3.1	0.06	66.0	13.79	0.08	5.8	616.4	2.95
2	0.48	16.0	0.65	412.2	86.21	0.78	10.7	20284.8	97.05

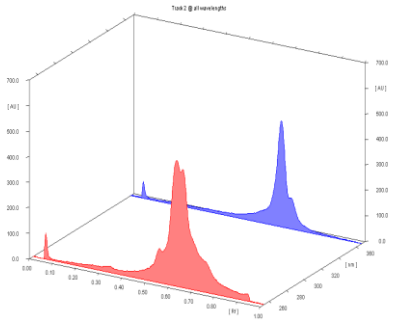

Fig 1: Overlaid chromatogram at 254nm(red) and 366nm(blue) wavelengths respectively.

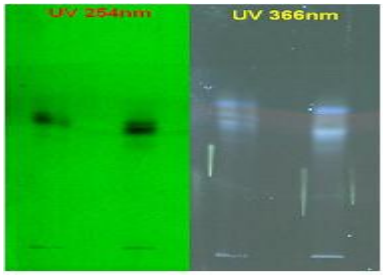

Fig 2: TLC Plates after visualization in 254nm and 366nm(in duplicates)

GCMS PROFILING

GCMS Profiling (Fig 3) of the n-Hexane extract revealed the presence of 23 compounds. Benzoic acid,3,5-dicyclohexyl-4-hydroxy-,methylester(35.670%),1-Chloroeicosane (29.474%), Campesterol(7.946%),Heptatriacotanol(7.637%) and γ-Sitosterol(5.094%) as the major constituents(Table 3).

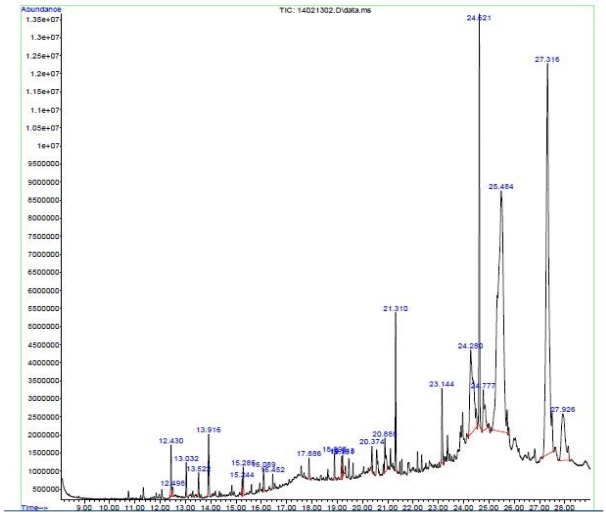

Fig 3: Gas Chromatographic profile of *Z.nimmonii* hexane extract

Table 3: Compound list and their composition percentage in the hexane extract of *Z.nimmonii*.

Peak#	RT min	Corr % max	% of total	Compound
1	12.430	2.80%	0.999%	Tetradecane
2	12.498	0.61%	0.216%	n-pentadecane
3	13.032	1.52%	0.542%	β-Caryophyllene
4	13.522	1.12%	0.400%	α-Caryophyllene
5	13.916	3085%	1.372%	Octadecane, 1,1'-[1,3-propanediylbis(oxy)]bis-
6	15.244	0.82%	0.292%	Tetradecane, 2,6,10-trimethyl-
7	15.285	1.30%	0.463%	Caryophyllene oxide
8	16.089	1.36%	0.484%	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-
9	16.452	0.62%	0.222%	Heptadecane
10	17.886	1.09%	0.391%	Heptasiloxane, hexadecamethyl-
11	18.892	1.23%	0.438%	Hexadecanoic acid, methyl ester
12	19.181	1.29%	0.459%	Benzothiazole, 2-(2-hydroxyethylthio)-
13	19.213	1.08%	0.384%	n-Hexadecanoic acid
14	20.374	1.32%	0.471%	Cyclic octaatomic sulfur
15	20.888	3.12%	0.113%	1-Monolinoleoylglycerol trimethylsilyl ether
16	21.310	6.72%	2.399%	1,6,10,14-Hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-
17	23.144	4.15%	1.481%	Methenolone
18	24.280	22.28%	7.946%	Campesterol
19	24.621	21.41%	7.637%	1-Heptatriacotanol
20	24.777	5.76%	2.053%	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-
21	25.484	100.00%	35.670%	Benzoic acid, 3,5-dicyclohexyl-4-hydroxy-, methyl ester
22	27.316	82.63%	29.474%	1-Chloroeicosane
23	27.926	14.28%	5.094%	γ-Sitosterol

CONCLUSION

Pharmaceutical industry has been supported by chromatographic methods[24].So these methods are to be used routinely in each and every raw drug analysis so that a specific chromatographic profile can be developed for each plants with specific medicinal properties.

The preliminary phytochemical screening in both polar(methanol) and nonpolar(n-hexane) extracts revealed the presence of major secondary metabolites in *Z.nimmonii*.HPTLC profiling was performed in two different wavelengths 254nm and 366nm.Six major peaks were obtained in the 254nm wavelength and two significant peaks in 366nm.GCMS profiling gave the composition of 23 major chemical constituents in the hexane extract of the plant with immense potential.

Therefore isolation of these components from the plant will be of great significance in pharmaceutical and natural product industry.

ACKNOWLEDGEMENT

The authors are thankful to our Chancellor, Chief Executive Officer, Vice Chancellor and Registrar of Karpagam University and MIMS Research Foundation for providing facilities and encouragement.

REFERENCES

1. Aneesh TP, Mohamed Hisham, Sonal Sekhar M, Manjusree Madhu and Deepa TV. International Market Scenario of Traditional Indian Herbal Drugs. Int. J. Green Pharm. 2009; 3 suppl 3: 184-190.
2. Jones W, Chin Y and Kinghorn A. The role of pharmacognosy in modern medicine and pharmacy.Curr Drug Target 2006; **7**:247-264.
3. Kim J.Protective effects of Asian dietary items on cancers- soy and ginseng. Asian Pac J Cancer Prev 2008;9:543-548.
4. Aune D, De Stefani E and Ronco A.Fruits, vegetables and the risk of cancer: a multisite case-control study in Uruguay. Asian Pac J Cancer Prev 2009;10: 419-428.
5. Hasan TN, B LG, Shafi G, Al-Hazzani AA and Alshatwi AA.Anti-proliferative effects of organic extracts from root bark of Juglans Regia L. (RBJR) on MDA-MB-231 human breast cancer cells: role of Bcl-2/Bax, caspases and Tp53.Asian Pac J Cancer Prev 2011; 12: 525-530.
6. Woo.H.D and Kim J.Nutritional epidemiology of cancer in Korea: recent accomplishments and future directions. Asian Pac J Cancer Prev 2011;12:2377-2383.
7. Khan MR, Rizvi W, Khan GN, Khan RA and Sheen S.Carbon tetrachloride induced nephrotoxicity in rats: Protective role of *Digera muricata*. J. Ethnopharmacol 2009;122: 91-99.
8. Khan RA, Khan MR and Sahreen S.Evaluation of *Launaea procumbens* use in renal disorders: A rat model. J. Ethnopharmacol 2010; 128:452-461.
9. Khan RA, Khan MR, Sahreen S and Bukhari J.Prevention of CCl4 induced nephrotoxicity with Sonchus asper in rat. Food Chem. Toxicol 2010; 23: 1304-1321.
10. Sahreen S, Khan MR and Khan RA.Evaluation of antioxidant activities of various solvent extracts of *Carissa opaca* fruits. Food Chem 2010; 122: 1205-1211.
11. Bhat, G, Studies on Zingiberaceae of Karnataka. In: Higher Plants of Indian Subcontinent, Bishen Singh Mahendra Pal Singh, Dehradun, Vol. 4;39–102,1993.
12. Sabu M.Revision of the genus Zingiber in South India. Folia Malaysiana;4: 25–52,2003.
13. Baby S, Mathew D, Anil J, Rajani K, Pradeep N S, Renju K.V and Varughese G.Caryophyllene-rich rhizome oil of *Zingiber nimmonii* from South India: Chemical characterization and antimicrobial activity.Phytochemistry2006;67:2469–2473.
14. Anonymous.Quality control methods for medicinal plant material. WHO/PHARM, Geneva. WHO/PHARM/92.559.1992.
15. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD and Guo ZG.Medicinal plants in therapy. Bull. WHO63: 965; 1985
16. Di X, Chan KKC, Leung HW and Huie CW. Fingerprint profiling of acid hydrolyzates of polysaccharides extracted from the fruiting bodies and spores of Lingzhi by high--performance thin-layer chromatography. J. Chromatogr. A.2003; 1018: 85-89.
17. Larsen T, Axelsen J and Ravn HW. Simplified and rapid method for extraction of ergosterol from natural samples and detection with quantitative and semi-quantitative methods using thin layer chromatography. J. Chromatogr. A.2004; 1026: 301-304.
18. Ronald Hites A. Gas Chromatography Mass Spectroscopy: Handbook of Instrumental Techniques for Analytical Chemistry;p609-611, 1997
19. Paech, D. and M.V. Tracey. Modern methods of plant analysis. Springer Verlag, 1st Edition, Berlin, 3: p64-65.1955.
20. Priya G and Chellaram C. Antiproliferative effect of ethanolic leaf extract of *Solanum trilobatum* on hep2 cancer cell lines. Asian J Pharm Clin Res 2014;7:258-61.
21. Paramasivam Ragavendran, Dominic Sophia, Chinthamony Arul raj, Thangarajan Starlin and Velliyur Kanniappan Gopalakrishnan.Phytochemical screening, antioxidant activity of *aerva lanata* (l) – an *invitro* study. Asian J Pharm Clin Res 2012;5:77-81.
22. International Conference on Harmonization.ICH Harmonised tripartite guideline validation of analytical procedures:test and methodology Q2(R1)ICH, Geneva, Nov 2005.
23. Kamlesh Dhalwal, Vaibhav M. Shinde and Kakasaheb R. Mahadik. Optimization and validation of reverse phase HPLC and HPTLC method for simultaneous quantification of vasicine and vasicinone in *Sida* Species. Journal of Medicinal Plants Research 2010;4(13): 1289-1296.
24. World Health Organization ,http://www.who.int/mediacentre/factsheets/fs134/en/,2003