International Journal of Herbal Medicine
Available online at www.florajournal.com

IJHM International Journal of Herbal Medicine

E-ISSN: 2321-2187
P-ISSN: 2394-0514
IJHM 2016; 4(2): 36-40
Received: 27-01-2016
Accepted: 28-02-2016

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Phytochemical screening and evaluation of tuber extract of *Plectranthus rotundifolius* Spreng. By GC-MS and FT-IR spectrum analysis

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Abstract
The present study was aimed to examine the phytochemical constituents present in the tuber of *P. rotundifolius*. The GC-MS analysis determined the presence of forty different phytochemical compounds in methanolic extract of tuber of *P. rotundifolius*. Based on percentage of area, Cis-Vaccenic acid was identified as an active phyto component. FT-IR results proved the presence of Alcohols, Phenols, Amines, alkanes, aldehydes, carboxylic acid, iso cyanides, alkyne, isocyanate, ketones, aromatics, phenols, tertiary and primary alcohols and Chloro compounds which shows major peaks at 3966.15, 3769.76, 3394.56, 2947.66, 2835.85, 2524.80, 2362.84, 2214.45, 2043.33, 1656.15, 1451.82, 1413.08, 1112.70, 1024.79 and 668.99 respectively. The result of the present study help to enhance the usage of *P. rotundifolius* for its medicinal uses which is greatly depends on the diversified chemical substances present in the tuber of *P. rotundifolius*.

Keywords: *P. rotundifolius*; Phytochemicals; GC-MS; FT-IR; Spectroscopy

1. Introduction
Herbs are mine of medicinal agents and large number of medicinal herbs are found to be efficacious, cheap and safe in preventing various diseases. Moreover, the use of herbal medicines for the treatment of different ailments is very important in developing countries where the cost of conventional medicines is a burden to the population. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. India has one of the oldest, richest and most diverse cultural traditions associated with the use of medicinal plants. This knowledge is accessible from thousands of medical texts and manuscripts. The use of medicinal plants as traditional medicine is well known in rural areas of the many developing countries.

Plectranthus rotundifolius (Spreng) is a perennial herbaceous plant of the mint family native to topical Africa. *P. rotundifolius* is called as Chinese potato, coleus potato or Hausa potato. It is cultivated in Africa and south East Asia for its edible tubers (lost crops of Africa). Flesh colour is white, reddish yellow dark brown and light grey [1]. *Plectranthus rotundifolius* is an erect, semi-succulent annual herb. It is bushy from the base up to 30cm tall, has a succulent stem and thick leaves. The flowers are blue pinkish white or pale violet in a distal inflorescence [2]. Plant is highly tolerant of more drought and rainfall. It grows well in loose or sandy soil and direct sunlight. The tubers are harvested about four to five months after planting flowering and aerial parts of plant have died. Tubers of *P. rotundifolius* can be used as edible potatoes in Tamil Nadu. This tuber is oval shaped and smaller than commercial potatoes. They are usually cooked by baking and frying. The taste of potato is fairly bland than sweet potato. This potato leaves can be used as treatment for dysentery [3].

Identification of individual components of complex mixtures such as terpenes/terpenoids in root hexane extracts requires the use of several techniques. GC-MS and FT-IR are useful tools in medicine and biological research aiming for the identification of mixtures and this method has already been applied successfully for the analysis of terpenoids. Especially mono- and sesquiterpenes, in various plant extracts. Identification of the bio molecules found in an extracts by comparing their relative retention times/indices and their mass spectra. Therefore, the identified chemical constituents are used in folk medicine for a variety of diseases including infectious conditions.

2. Materials and Methods

2.1 Collection & Preparation of Plant Material

The tubers of *P. rotundifolius* (AUBOT0296) were collected from the January – March season with their natural habitats of Virudhunagar, Tamil Nadu, India. The species have been identified with help of standard flora [4, 6]. And the herbarium specimen was deposited in the Department of Botany, Annamalai University, Tamil Nadu. Tubers were washed three times thoroughly with running tap water to remove soil particles and adhered debris and finally with sterile distilled water. The tubers were cut, shade dried, ground into fine powder and stored in air tight container for further use.

2.2 Chemicals

The chemicals were purchased from Himedia, Mumbai, India and the solvents used were of analytical grade.

2.3 Equipment

Equipment's used in this experiment include GC-MS and FT-IR. GC-MS was used for the comparison of samples and FT-IR was used for identification of functional groups presented in the species of *P. rotundifolius.*

2.4 Extraction

Shade dried and powdered plant material of *P. rotundifolius* (Spreng) was successively extracted with Petroleum ether, Chloroform, Ethyl acetate and Methanol with gentle stirring for 72 hrs separately. The extracts were filtered with Whatman No.1 filter paper and concentrated using vacuum distillation. Now the resultant samples are subjected to analysis [7].

2.4.1 GC-MS analysis

10g of powdered sample is extracted with 30ml methanol overnight and filtered in ashless filter paper with sodium sulphate (2g) and the extract is concentrated to 1ml by bubbling nitrogen into the solution. The Clarus 500 GC used in the analysis employed a column packed with Elite-1(100% Dimethyl poly siloxane, 3nm X 0.25nm ID X 1um df) and the components were separated using helium (1ml/min) as the carrier gas. The 2ul sample extract injected into the instrument was detected by the Turbo mass gold mass detector (Perkin Elmer) with the aid of the Turbo mass 5.1 software. During the 36th minute GC extraction process, the over was maintained at a temperature of 110 °C with a 2miin holding. The injector temperature was set at 25 °C (Mass analyser). The different parameters involved in the operation of Clarus 500 MS, were also standardized (inlet line temperature: 200 °C; Source temperature 200 °C; Electron energy 70eV; Mass scan (m/2) 45-450). The MS detection was completed in 36 min. the relative percentage of each component was calculated by comparing its average peak to the total areas. The detection employed the NIST (National Institute of Standards and Technology) Version 2.0 year 2010 library. The compound prediction is based on Dr. Duke's Phytochemical and Ethno botanical databases by Dr. Jim Duke of the agricultural Research Science/USDA [8].

2.4.2 FT-IR Analysis

The FT-IR analysis was performed using Perkin Elmer Spectrum Version 10.03.09 system, which was used to detect the functional groups of the compound. A small amount of compound was placed directly on the zinc solenoid piece and constant pressure. Data of infrared absorbent, collected over the wave number ranged from 3500 cm-1 to 500 cm-1 using spectra software. Samples were run in triplicate and all of them were undertaken within a day period.

3. Results and Discussion

The phytochemical constituents present in the tuber of *P. rotundifolius* were reported in Table 1. *P. rotundifolius* tuber extract was contains forty phytochemical components according to GC-MS spectra. (Fig.1). Cis-Vaccenic acid, Ergost-5-En-3-Ol, Stigmasta-5, 22-Dien-3-Ol, hexadecanoic acid and 9-decanoic acid were majorly presented components from methanolic tuber extract. (Fig.2). The retention time of these components are 26.808, 42.341, 43.301, 24.632 and 26.351 have been confirmed by spectra. Based on percentage of area, Cis-Vaccenic acid was identified with molecular formula of $C_{18}H_{34}O_2$. (Table.1). Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute of Standards and Technology (NIST08s) and WILEY8 and FAME having more patterns. The spectrum of unknown component was compared with the spectrum of known components stored in the NIST08s, WILEY8 and FAME library. The name, molecular formula, molecular weight and structure of the component of the test material were determined.

P. rotundifolius revealed the different functional groups based on the FT-IR spectrum such as Alcohols, Phenols, Amines, alkanes, aldehydes, carboxylic acid, iso cyanides, alkyne, isocyanate, ketones, aromatics, phenols, tertiary and primary alcohols and Chloro compounds (Table.2) which shows major peaks at 3966.15, 3769.76, 3394.56, 2947.66, 2835.85, 2524.80, 2362.84, 2214.45, 2043.33, 1656.15, 1451.82, 1413.08, 1112.70, 1024.79 and 668.99 respectively (Fig.3). Therefore, comparison of chemical constituents and pharmacological activities of these phytochemical constituents is helpful to elucidate the mechanism of therapeutic effects and active components from *Plectranthus* species

Table 1: Phytochemical constituents present in the Methanolic tuber extract of *P. rotundifolius* (Spreng) based on GC-MS analysis

Peak	R. Time	% of Area	Formula	Molecular weight	Name of the Compounds
1	11.690	0.20	$C_{13}H_{28}$	184	Nonane, 5-(2-methylpropyl)-
2	12.264	0.34	$C_{10}H_{14}O$	150	2-ETHYL-4,5-DIMETHYLPHENOL
3	12.586	0.58	$C_9H_{10}O_2$	150	2-Methoxy-4-vinylphenol
4	14.161	0.19	$C_{14}H_{28}$	196	1-Tetradecene
5	16.379	0.13	$C_{13}H_{28}$	184	Nonane, 5-(2-methylpropyl)-
6	16.903	0.22	$C_{15}H_{26}O$	222	sesquicineole
7	17.867	0.41	$C_{10}H_{12}O_3$	180	Ethanone, 1-(3,4-dimethoxyphenyl)-
8	19.492	0.38	$C_{15}H_{26}O$	222	.tau.-Cadinol
9	21.475	0.55	$C_{14}H_{28}O_2$	228	Tetradecanoic acid
10	24.137	3.04	$C_{17}H_{34}O_2$	270	Hexadecanoic acid, methyl ester
11	24.632	5.45	$C_{16}H_{32}O_2$	256	HEXADECANOIC ACID
12	24.718	0.28	$C_{20}H_{30}O_4$	334	1,2-BENZENEDICARBOXYLIC ACID, BUTYL OCTYL
13	25.177	0.41	$C_{15}H_{14}O_3$	242	2-Methoxybenzoic acid, benzyl ester

14	26.074	1.15	$C_{18}H_{32}O_2$	280	13-Hexyloxacyclotridec-10-en-2-one
15	26.289	2.69	$C_{19}H_{34}O_2$	294	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTE
16	26.351	5.38	$C_{19}H_{36}O_2$	296	9-OCTADECENOIC ACID (Z)-, METHYL ESTER
17	26.413	0.54	$C_{19}H_{36}O_2$	296	9-OCTADECENOIC ACID (Z)-, METHYL ESTER
18	26.620	1.65	$C_{19}H_{38}O_2$	298	Methyl stearate
19	26.808	31.45	$C_{18}H_{34}O_2$	282	cis-Vaccenic acid
20	27.026	1.48	$C_{18}H_{36}O_2$	284	Octadecanoic acid
21	27.286	3.39	$C_{19}H_{34}O_2$	294	9,12-OCTADECADIENOIC ACID (Z,Z)-, METHYL ESTE
22	27.366	5.29	$C_9H_9ClO_3$	200	2,6-Dimethoxybenzoyl chloride
23	27.666	0.78	$C_{18}H_{32}O_2$	280	9,12-OCTADECADIENOIC ACID
24	28.220	0.83	$C_{22}H_{42}O_2$	338	Phytol, acetate
25	28.352	0.37	$C_{19}H_{36}O_3$	312	METHYL (9Z)-12-HYDROXY-9-OCTADECENOATE #
26	28.478	0.38	$C_{19}H_{36}O_3$	312	Octadecanoic acid, 12-oxo-, methyl ester
27	28.531	0.17	$C_{14}H_7F_5O_2$	302	Pentafluorobenzoic acid, benzyl ester
28	29.028	0.26	$C_{18}H_{35}NO$	281	9-OCTADECENAMIDE
29	30.113	0.24	$C_{39}H_{73}F_5O_2$	668	Hexatriacontane Pentafluoropropionate
30	30.190	0.39	$C_6H_{11}BO_3$	142	ERYTHRIT, 1,4-ANHYDRO-2,3-O-(ETHYLBORANDIYL
31	30.329	1.55	$C_{32}H_{52}O_2$	468	LUP-20(29)-EN-3-YL ACETATE
32	41.720	1.63	$C_{28}H_{44}O$	396	Ergosterol
33	42.341	7.56	$C_{28}H_{48}O$	400	ERGOST-5-EN-3-OL
34	43.301	5.50	$C_{29}H_{48}O$	412	STIGMASTA-5,22-DIEN-3-OL
35	45.214	3.84	$C_{29}H_{50}O$	414	.gamma.-Sitosterol
36	46.767	2.20	$C_{27}H_{44}O$	384	Cholest-4-en-3-one
37	47.276	1.65	$C_{30}H_{48}O$	424	4,4,6A,6B,8A,11,11,14B-OCTAMETHYL-1,4,4A,5,6,6A,6
38	47.998	3.11	$C_{29}H_{46}O$	410	4,22-Stigmastadiene-3-one
39	50.533	3.83	$C_{29}H_{48}O$	412	STIGMAST-4-EN-3-ONE
		100.00			

Table 2: FT-IR absorption and functional groups of tuber of *P. rotundifolius* (Spreng).

S No	Wave No	Molecular Motion	Functional group	Absorption intensity
1	3966.15	O-H stretch	Alcohols	Strong
2	3769.76	O-H stretch	Phenols	Medium
3	3394.56	N-H stretch	Amines	Medium
4	2947.66	C-H stretch	Alkanes	Medium
5	2835.85	C-H stretch	Aldehydes	Weak
6	2524.80	Hydroxyl stretch	Carboxylic acid	Weak
7	2362.84	C=N stretch	Isocyanides	Medium
8	2214.45	C=N stretch	Alkyne	Weak
9	2043.33	C=N stretch	Isocyanate	Medium
10	1656.15	C-O stretch	Ketones	Strong
11	1451.82	C-C stretch	Aromatics	Medium
12	1413.08	C-O stretch	Phenols	Strong
13	1112.70	C-O stretch	Tertiary alcohols	Strong
14	1024.79	C-O stretch	Primary alcohols	Strong
15	668.99	C-Cl stretch	Chloro compounds	Strong

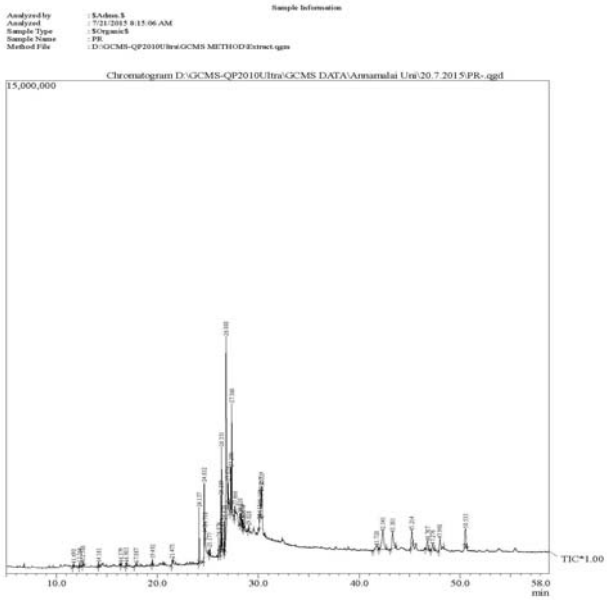

Fig 1: GC-MS chromatogram of methanolic tuber extract of *P. rotundifolius* (Spreng).

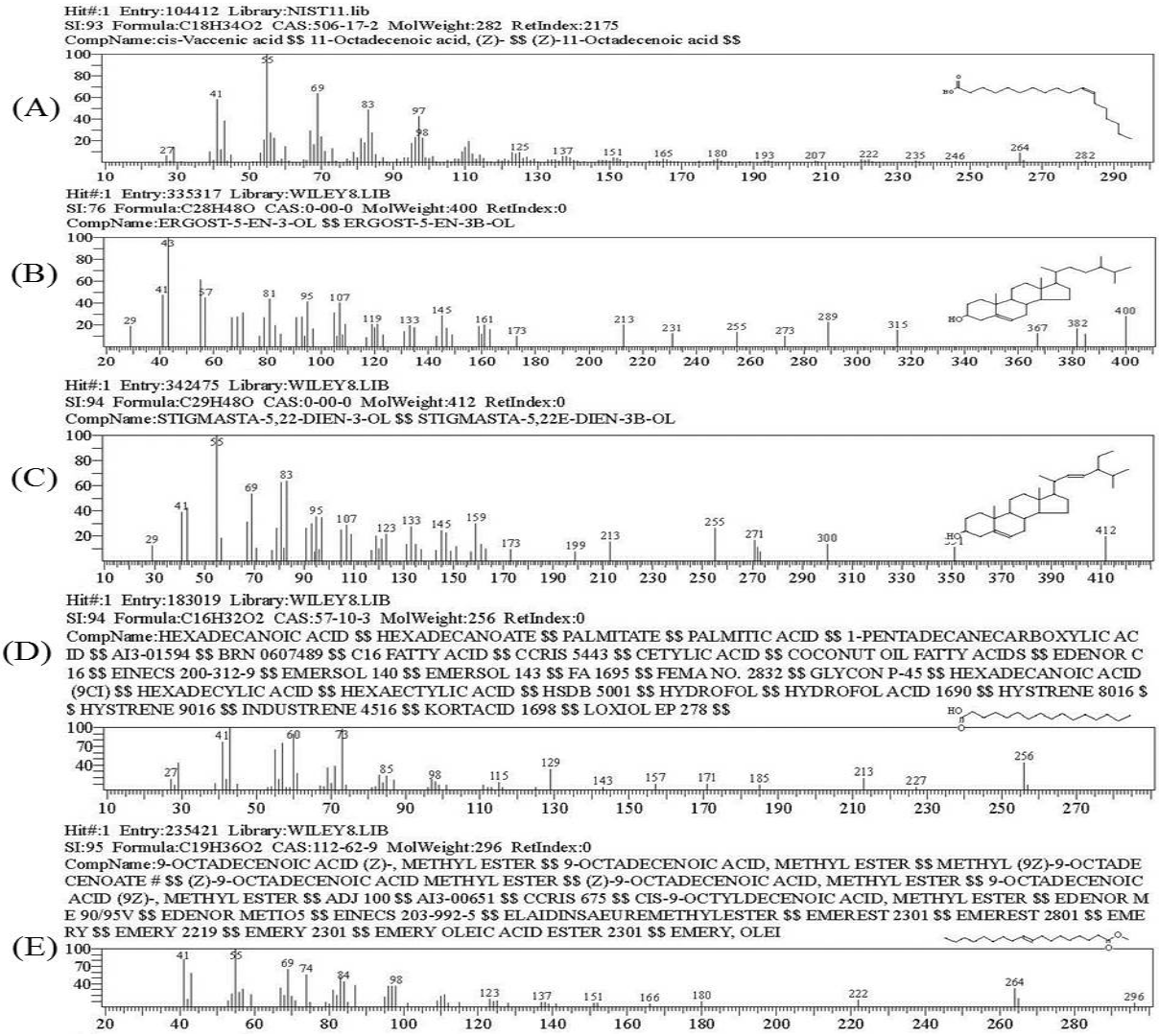

Fig 2: Spectrum structure of major components of methanolic extract of *P. rotundifolius* (Spreng).

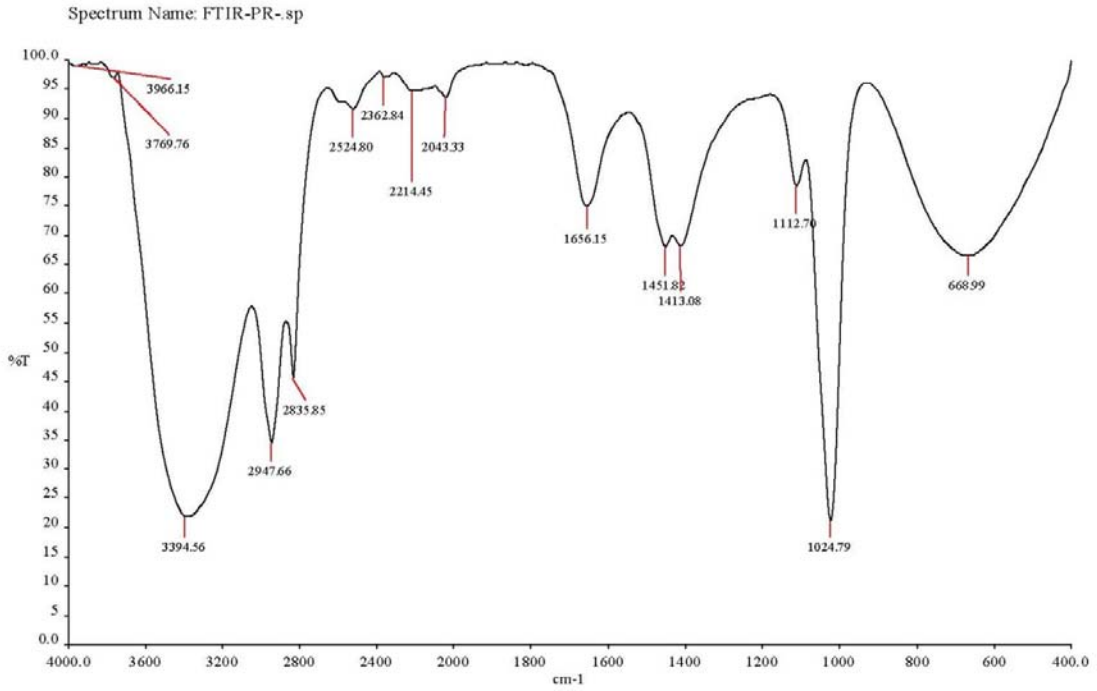

Fig 3: FT-IR Spectrum of methanolic tuber extract of *P. rotundifolius* (Spreng).

4. Conclusion

In the present study, we observed forty compounds from methanolic extract of tuber of *P. rotundifolius*. The result of the present study conform traditional applications of the medicinal plant *P. rotundifolius*. The tuber of *P. rotundifolius* can be used as a food or food additive. The plants studied here can be seen as a potential source of useful drugs. Further studies are going on these plants in order to isolate, identify, characterize and elucidate the structure of the bioactive compounds.

By using FT-IR spectrum, we can conform the functional constituents from given extract, identify the medicinal materials from the adulterate and even evaluate the quantities of medicinal materials. Many researchers applied the FT-IR spectrum as tool for distinguish closely associated plants and other organisms. The results of the present study developed novel phytochemical marker to identify the medicinally important plant. Further advanced spectroscopic studies are required for the structural elucidation and identification of active principles present in the tuber of *P. rotundifolius*.

5. Acknowledgement

The authors are thankful to Botanical Survey of India, Southern Circle and Department of Botany for species identification and technical support.

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