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Metabolite profile and radical scavenging activity of alcoholic extract of *Ophiorrhiza tirunelvelica* Henry & Subram. (Rubiaceae) – An endemic plants of southern Western Ghats of India.

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Abstract

The presence of diverse secondary metabolites have been reported from *Ophiorrhiza tirunelvelica* by preliminary screening and GC-MS analysis of ethanolic plant extracts. From the qualitative screening, alkaloids, flavonoids and triterpenes are reported positively. GC-MS analysis showed that the presence of 11 different phytoconstituents with 91.6% of extracts and Methyl-E,E-2,13-Octadecandien-1-ol is a major constituents with 36.9%. DPPH radical scavenging *in vitro* model resulted the moderate radical scavenging capacity of ethanol extract of *O. tirunelvelica* and IC_{50} value is comparable with ascorbic acid standard. Further investigation on isolation, purification, charaacterization of phytochemicals and clinical studies of the consitituents will support to develop the novel and safe medicine for postrity.

Key words: *Ophiorrhiza tirunelvelica*, endemic plant, phytochemicals, antioxidant.

Introduction

The genus *Ophiorrhiza* L. is comprised about 307 species (The Plant List, 2013) distributed in wet tropical forests of South-East Asia and extends from Sri Lanka and Eastern India to China. In India, it is represented with 49 species (Deb and Mondal, 1997). Peninsular India, especially the Western Ghats is one of the diversity centre of *Opiorrhiza* species following

the Western Himalayas. Nearly about 22 taxa are distributed in the evergreen forests of Western Ghats (Sasidharan, 2013). Among these, 18 are reported from Tamil Nadu state, and 14 are endemic to Western Ghats. Some species of *Ophiorrhiza* namely *O. mungos* and *O. pumila* are reported to have medicinal properties both in traditional and conventional medicine for treating snake bite, stomatitis, ulcers and wound healing (Kirtikar and Basu, 1975). Recently *Ophiorrhiza* species has reported as a source of the anticancer drug camptothecin in particularly from Western Ghats (Gharpure et al., 2010: Rajan et al., 2013), which has drawn great attention world wide as an anticancer drug and potential inhibitor of DNA topoisomerase-1 (Bodley *et al*., 1998; Uady and Kondapi, 2010; Krishnakumar et al., 2012). Camptothecin is also possessed clinical applications against anticancer, HIV-1 (Priel *et al*., 1991; Li et al., 2010), HSV-2 (Liu et al., 2010), parasitic trypanosomas and leishmania (Bodley and Shapiro, 1995). It is an indole alkaloid group of phytochemical reported from taxonomically unrelated families of Apocynaceae, Gelsemiaceae, Icacinaceae, Loganicaceae, Nyssaceae and Rubiaceae (Kurtan *et al*., 2014).

Antioxidants, molecules with a radical-scavenging capacity, are thought to exert a protective effect against free radical intercellular damage. Several phytomolecules may contribute to the prevention of many chronic diseases such as cancer, cardiovascular diseases, atherosclerosis, diabetes, asthma, hepatitis and arthritis (Formagio et al., 2014). The species of the genus *Ophiorrhiza* are not well explored so far that to establish the phytomedicine for challenging diseases like cancer and diabetes. In order to evaluate the phytochemical investigation and radical scavenging property of an endemic *Ophiorriza tirunelvelica* is aimed to carry out in the present study.

Materials and Methods

Plant materials

Plant materials of *Ophiorrhiza tirunelvelica* was collected (Fig.1) from Naraikaadu valley of Thirukarungudi range in Tirunelveli hills of Tamil Nadu and botanical identification authenticated from Botanical Survey of India (BSI), Southern Circle, Coimbatore, Tamil Nadu, India. The voucher specimen (SK3217) was kept at the Sriganesan Herbarium (SGH), Department of Botany, The Madura College, Madurai, India. Aerial parts of the plant materials were air-tried in room temperature for two weeks before extraction.

Preparation of extracts

Shade tried plant materials was ground with electronic grinder and prepared coarse powder. 250 g of plant powder filled with Soxhlet's thimble and 250 ml of ethanol as solvent taken in bottom flask for extraction. The Soxhlet's apparatus runs with 50^0C for 8 hr continuous reflux. The extracts removed from the Soxhlet's apparatus and concentrated under reduced pressure using a rotary evaporator with a temperature set a 40^0C. The crude plant extract then air-tried and stored in refrigerator for 15 days. After the crude extracts were reconstituted with 95% ethanol, and filtered through a Whatman No. 1 paper for further bioassay.

Qualitative phytochemical profile

Ethanolic extracts derived from whole plant of *O. tirunelvelica* were subjected to phytochemical screening for the preence of alkaloids, flavonoids, tannins, sterols, triterpenes, saponins and glycosides by standard methods (Evans, 2002).

GC-MS analysis

GC-MS analysis of the ethanol extract of *O. tirunelvelica* was performed using a Perkin–Elmer GC Clarus 500 system comprising an AOC-20i auto-sampler and a Gas Chromatograph

interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl/95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.9%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature was 200 °C, the oven temperature was programmed from 110 °C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass-detector used in this analysis was Turbo-Mass Gold-Perkin-Elmer, and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver-5.2.

Identification of phytocomponents

Interpretation on mass-spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 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.

DPHH – radical scavenging activity

The DPPH radical scavenging activity of *O. tirunelvelica* extract was performed following the method of Chigayo et al., (2016) with a few modifications. A volume of 500 mL of test extract at various concentrations (1–30 mg/mL) was mixed with 375 mL of 99.5% ethanol and 125 mL of DPPH solution (0.02%) as a free radical source. After that, the preparation was incubated for 1 h in the dark at room temperature. At the end, scavenging capacity was

estimated spectrophotometrically by controlling the reduction in absorbance at 517 nm. In its radical form (purple color), DPPH has an absorption band at 517 nm which disappears upon reduction by an antiradical molecule (yellow colour). A good radical scavenging activity has been interpreted by decreasing it in mixture absorbance. Synthetic antioxidant, BHA was used as positive reference. DPPH radical scavenging activity was calculated as:

DPPH radical scavenging activity % = [(Ac−As)/Ac] × 100

Where, Ac is the absorbance of the control reaction, As is the absorbance of *O. tirunelvelica* ethanol extract. Tests were performed in duplicate. IC_{50} values were estimated by a linear regression.

Results

The phytochemical profile of the ethanolic extracts of *O. tirunelvelica* are presented in Table 1. The plant extract was found to contain a greater number of secondary metabolite chemical groups. Alkaloids and flavonoids are represented in abundance whereas triterpenes were detected modereate amount. Sterols and tannins are resulted in little amount. Saponin and glycosides were not detected in the test extract.

A total of 11 constituents, representing 91.6% of the extract were identified by GC-MS analysis (Fig. 2). It showed 12-Methyl-E, E-2, 13-Octadecadien-1-ol (36.9%), E-2-Tetradecen-1-ol (22.3%) and Cyclopropanedecanoic acid (12.3%) as major constituents. Moreover Phenol-2, 6-bis (1,1-diemthylethyl) (5.5%), 4-Methoxy-5,7-dihydroxy isoflavone (3.8%) and Ethyl oleate (2.9%) were reported in significant concentrations (3-6%). Whereas in other five phytochemical compounds were noted trace amounts in the extract (Table 2).

Radical scavenging activity of the ethanol extract of *O. tirunelvelica* were tested in free radical scavenging DPPH assay. The results are expressed as IC_{50} vlaues in µg/ml, the concentration of sample required to scavenge 50% free radicals (Table 2). The values exhibited

a concentration dependent antiradical activity by quenching DPPH radicals and this activity was comparable to that of ascarbic acid standard. The marked radical scavenging activity of plant extract seemed to be due to the presence of phytochemicals.

Discussion

Preliminary phytochemical screening revealed the presence of alkaloids and flavonoids whihc is similar to that of its related plant species *Ophiorrhiza mungos* (Madhavan et al., 2015). These secondary metabolites have been reported to exhibit several medicinal and physiological properties. GC-MS analysis provides significant additional information on the chemical composition of plant extract which are showing major chemical constituents possessed to have potential alkaloid and flavonoid precursors (Table 2). Many other species of *Opiorrhiza* have already been identified camptothecin yielding plants in Western Ghats as *O. mungos* and *O. prostrata* (Krishnakumar et al., 2012), *O. pumila* (Roja, 2006) and *O. rugosa* (Gharpure et al., 2010). The present study is also provided possible occurence of camptothecin in *O. tirunelvelica*. The divergent bioactivities of plants belonging to the same genus may due to a difference in phytochemical profiles across geographical regions. It is known that variations in climatic conditions and soil types are major contributors to the differences in secondary metabolite profile of the plant species. Climatic and edaphic variations of Western Ghats served the habitat for potential metabolite producing plant species.

DPPH is a stable free radical at room temperature, which produces a violet solution in ethanol (Orech et al., 2005). It is widely used to evaluate the free radical scavenging effects of natural antioxidants. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet colour). As the electron became paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stochiometrically coincides with the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the test drugs to

scavenge free radicals independently. It has been found that tocopherol, flavonoids, tannins and aromatic amines reduce and decolorize DPPH radicals by their hydrogen donating ability (Yokozawa et a*l*., 1988). In the present study, the DPPH radical scavenging activity of plant extract were dose dependent (Table 3). Based on the mechanism of reduction of DPPH radical described in the literature, that is correlated with the presence of hydroxyl groups on the antioxidant molecule, the present investigation can infer that *O. tirunelvelica* extract significantly scavenge the free radicals comparable to ascorbic acid (Table 3). Antioxidants with high scavenging activity should have a low IC_{50} value. This is supported by lowest value being exhibited by ascorbic acid, a well kown antioxidant. This result is to be expected as crude extracts were used before purification. Results for purified extracts are expected to be much more closely related to those of ascorbic acid.

Conclusion

In our present investigation revealed that extracts of *O. tirunelvelica* are rich in phytochemicals particularly alkaloids and flavonoids. The results of radical scavenging activity that this plant have a powerful radical scavenging property. Our findings reinforce the potentials of *O. tirunelvelica* as a valuable source of natural antioxidants and support its medicinal uses in the treatment of many diseases.

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Table 1. Phytochemical profile of ethanol extract of *O. tirunelvelica*.

Phytochemicals	Sample extract
Sterols	+
Triterpenes	++
Flavonoids	+++
Alkaloids	+++
Saponins	-
Glycosides	-
Tannins	+

+ present in little amout; ++ present in moderate amount; +++ present in abundance: - constituents not detected.

Fig. 1 Plant twig of *Ophiorrhiza tirunelvelica* collected from the Western Ghats

Fig. 2 Gas chromatogram of ethanol extract of *Ophiorrhiza tirunelvelica*

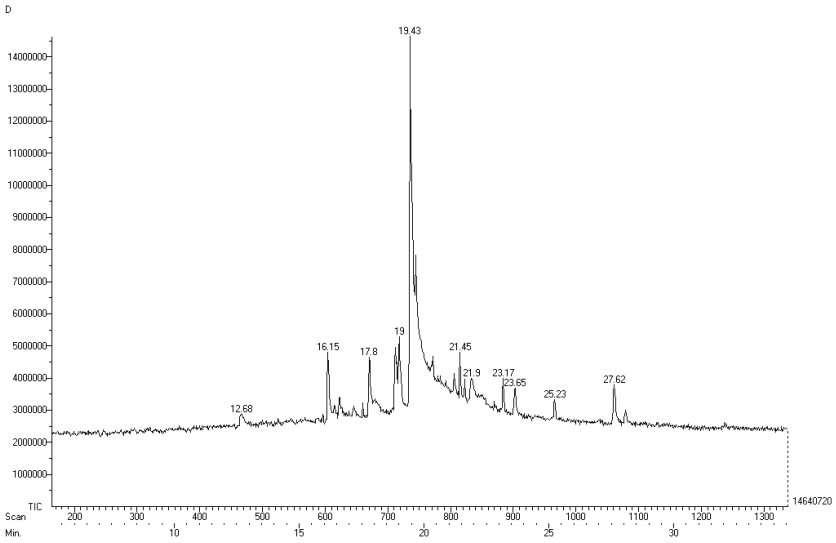

Table 2. Chemical composition of ethanol extract of *O. tirunelvelica* analysed by GC-MS

Sl.No.	Name of compound	RT	%	Chemical formula
1.	E-2-Tetradecen-1-ol	1615	22.3	$C_{14}H_{28}O$
2.	12-Methyl-E, E-2,13-Octadecadien-1-ol	1443	36.9	$C_{19}H_{36}O$
3.	Ethyl oleate	1228	2.9	$C_{20}H_{38}O_2$
4.	Cyclopropanedecanoic acid	1556	12.3	$C_{17}H_{15}O_4$
5.	9-Ocatadecanoic acid, 2 hydroxy ethyl	1394	1.6	$C_{20}H_{20}O_4$
6.	Isopropyl stearate	1541	1.3	$C_{21}H_{42}O_2$
7.	Docosa-Pentaen-22-al	1475	1.0	$C_{21}H_{40}O$
8.	Phenol-2,6-bis (1,1-diemethyethyl)	1441	5.5	$C_{14}H_{22}O$
9.	2,3-Dihydroxyprophyl elaidate	1394	1.6	$C_{21}H_{14}O_4$
10.	Eicosanoic acid ethyl ester	1452	2.4	$C_{22}H_{44}O_2$
11.	4-Methoxy-5,7-dihydroxy isoflavone	1402	3.8	$C_{16}H_{12}O_5$

Table 3. DPPH radical scavenging activity of the ethanolic extracts of *Ophiorrhiza tirunelvelica*

Sample	Concentration (µg/ml)	Absorbance at 517 nm	% inhibition	IC_{50} (µg/ml)
Plant extract	3.125	0.739 ± 0.003	47.90 ± 0.02	4.3 ± 0.88
	6.25	0.574 ± 0.009	61.07 ± 0.30	
	12.5	0.340 ± 0.002	76.83 ± .011	
	25	0.259 ± 0.001	91.90 ± 0.32	
	50	0.091 ± 0.002	98.62 ± 0.03	
Ascorbic acid (standard)	1	0.584 ± 0.003	42.98±0.003	3.2 ± 0.15
	2	0.549 ± 0.002	52.72±0.006	
	4	0.454 ± 0.003	65.64±0.002	
	8	0.310 ± 0.006	81.97±0.003	
	16	0.231 ± 0.009	95.46±0.005	