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Antioxidant Studies and GCMS Analysis of the Phytochemical Compounds of Some Endangered Plant Species Collected from the Western Ghats

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A B S T R A C T

Plant has an innate ability to produce non-enzymatic antioxidants which have an important role in the metabolism of Reactive oxygen species (ROS). Several plants serve as the source of therapeutic agents but the properties depend on the plant nature. Thus, an evaluation of antioxidant activity is essential to determine the importance of a plant. Three plants *Utleria salicifolia*, *Plectranthus vettiveroides* and *Nothapodytes nimmoniana* were selected to carry out the antioxidant study. Methanol assisted leaves extracts were prepared and subjected to antioxidant assay by means of DPPH radical scavenging assay, Metal ion Chelating Assay, Superoxide Anion Radical Scavenging Assay and Hydroxyl radical scavenging assay. All the extracts showed 20-80% inhibition depending on the concentration of extracts and the type of assay as well. The existence of crucial organic compounds in the leaf extracts of all the three plants were corroborated by Gas chromatography analysis. The present results offer supporting evidence for effective use of selected plant extracts.

Introduction

Free radicals play a crucial role in the development of tissue damage in pathological events. Antioxidants are chemical compounds which have the ability to quench the free radicals and thereby it prevents the human body against various diseases. Plants are the rich sources of antioxidants which contain secondary metabolites such as phenolic and flavonoid compounds commonly which act as antioxidants with redox and metal chelating properties (Karimi and Jaafar, 2011). Antioxidants are characterized as free radical which has an essential role to develop the damaged tissue in pathological field. Medicinal plants have been investigated from long time to evaluate their antioxidant properties. Natural antioxidants have potential to interrupt the destruction which is resulted from oxidative stress. These antioxidants may be either natural extracts or as an essential chemical compound of the extract (Zengin *et al.*, 2011). Though medicinal plants have been

carefully assessed for their toxicity profile, still the plant derived medicines are safer as compared to synthetic medicines (Vongtau *et al.,* 2005; Oluyemi *et al.,* 2007). The ROS and other oxidant result in disease and disorders as proved by different evidence. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health (Halliwell *et al.,* 1981). Human body has an inherent antioxidative mechanism and many of the biological functions such as the anti-mutagenic, anti-carcinogenic, and anti-aging responses originate from this property (Gulcin *et al.,* 2012; Gocer *et al.,* 2011). Antioxidants stabilize or deactivate free radicals, often before they attack targets in biological cells (Nunes *et al.,* 2012) Recently interest in naturally occurring antioxidants has considerably increased for use in food, cosmetic and pharmaceutical products, because they possess multifacetedness in their multitude and magnitude of activity and provide enormous scope in correcting imbalance (Djeridane *et al.,* 2006; Wannes *et al.,* 2010).

It is well known that free radical reaction is actively involved in disease pathology resulting in several chronic and acute disease in human such as neurodegeneration, atherosclerosis, immunosuppression, aging and diabetes (Harman *et al.,* 1998). If the balance between inherent antioxidant capacity of the body and ROS is disrupted then medicinal supplements and dietary are provided during attacked by disease. Several researches on vegetables, herbal plants and fruits specified the presence of antioxidants including flavonoids, phenolics, proanthocyanidins and tannins. Antioxidant from medicinal plants offers quite well protection against disease.

The ingestion of natural antioxidants has been inversely associated with morbidity and mortality from degenerative disorders (Gulcin *et al.,* 2012). Liver diseases remain a serious health problem. Free radicals result in the damage of cell by covalent binding as well as lipid peroxidation. This further causes the injury to tissue. Antioxidant agents of natural origin have attracted special interest because of their free radical scavenging abilities (Osawa *et al.,* 1990). The use of medicinal plants with high level of antioxidant constituents has been proposed as an effective therapeutic approach for hepatic damages (Govind *et al.,* 2011).

Reactive oxygen species (ROS) and Reactive Nitrogen Species (RNS) are the products of normal cellular metabolism recognized for playing the either harmful or beneficial effect in living system. Increase in concentration of free radicals or decreased endogenous antioxidant mechanism can lead to oxidative stress which is responsible for the development of many degenerative diseases (Saikat *et al.,* 2014).

GC-MS is an important technique to analyze the plant extract in order to determine the presence of essential herb compound which are often used in pharmaceutical, drug, cosmetic or food industry, environmental and forensic applications (Uma *et al.,* 2009). This technique is the combination of two separate analytical methods to separate and determine the chemical components of a given mixture. Separation is done by Gas Chromatography whereas the components analysis is carried out by mass spectroscopy. Chemical studies have shown that it mainly contains cardenolides, pregnane glycosides and volatile components. Maximum volatile components belong to the class of long chain unsaturated fatty acids. These are the building elements of several valuable compounds and also an essential energy source. Due to these features, the volatile compounds play vital role in the biological system (Mu *et*

al., 2001). In recent years, increasing research has been carried out on fatty acids and the results obtained show that they possess significant sedative and hypnotic effects (Zhang *et al.,* 1995).

The current study was conducted to prepare the methanol extract of *Utleria salicifolia*, *Plectranthus vettiveroides* and *Nothapodytes nimmoniana* leaves. The extracts were assessed for their antioxidant activity. The content of the extracts were determined by Gas Chromatography - Mass Spectrometry analysis.

Materials and Methods

Preparation of plant extract

The plant samples were collected from the following locations, *Utleri salicifolia*, *Curcuma zeodatia*, *Nothapodytes nimmoniana* from the Western Ghats of Kerala. *Plectranthus vettiveroides* from Tamilnadu. *Cayratia pedate* from the Western Ghats bordering Kerala and Tamilnadu and Karnataka states. *Rhaphidophora persuta* from the Western Ghats of Karnataka and *Syzygium travancoricum* from fresh water Myristica swamps of Kerala and Uttar Kannada district of Karnataka.

Utleria salicifolia, *Plectranthus vettiveroides* and *Nothapodytes nimmoniana* leaves were selected to study antioxidant activity and GC-MS analysis. The collected leaves were washed thoroughly with tap water followed by distilled water several times in order to remove the dust and soil particles.

The leaves were then shade dried and used for extraction. 100 gm of all the three plant leaves powder were treated with methanol and extracted using soxhlet apparatus. The extract thus obtained was concentrated by evaporation in rotary vacuum evaporator.

In-vitro Antioxidant Assay

The antioxidant activity of the leaves extracts was carried out following four protocols.

DPPH radical scavenging assay method

2.8 ml of leaves extract (20-100 µg/ ml) was mixed with 200 µL of DPPH (100 µM in methanol) and incubated for 20 min in dark condition. Absorbance was taken at 517 nm. A mixture of DPPH and methanol was used as control. Ascorbic acid was taken as reference standard. Percentage of DPPH inhibition was determined according to Prasad (2015).

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad \text{...Eq 1}$$

Metal ion chelating assay

This assay was carried out by determining the chelating potential of Fe ion present in the extract. 2,2'-bipyridyl competition assay was conducted by mixing 0.25mL(1mM) $FeSO_4$ solution to the equal volume of concentrated extract (200-1000 µg/ml). To this mixture 1mL Tris HCl buffer (pH 7.4) and 0.25mL (0.1%) 2,2'-bipyridyl solution were added along with 0.4mL hydroxylaminehydro chloride and 2.5mL ethanol. Final volume of the solution was adjusted to 5 ml by distilled water. The resulting solution was incubated at room temperature for 10 minutes. The absorbance was taken at 522 nm with EDTA as reference chelating agent. The Fe^{2+} chelating activity of the extract was determined as per the following equation.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad \text{...Eq 2}$$

Superoxide anion radical scavenging assay

NBT reduction method was adopted to assess superoxide anion radical scavenging activity. 0.1 ml concentrated plant extract (200-1000 µg/ml) was mixed with 1mL NBT (in phosphate buffer pH 7.4) and 1mL of NADH solution. 100 µL (60 µM) PMS was added to initiate the reaction and the reaction mixture was incubated for 15 min at 30°C. The absorbance was measured at 560 nm with ascorbic acid as reference standard. The inhibition percentage was calculated by the following equation.

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad \text{...Eq 3}$$

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of the plant extract was determined using 2-deoxy-2- 3+ ribose oxidative degradation in Fe - EDTA- 15 Ascorbate-H O system method. 3.5 ml leaves extract was mixed with 28 mM 2-deoxy-2-ribose, 1.04 mM EDTA and 1 mM ascorbic acid. The resulting solution was incubated for 1 hr at 37°C. The preventive effects of extract on deoxyribose damage, imposed by hydroxyl radicals were determined spectrophotometrically at 532 nm against blank for each concentration. Mannitol was taken as the reference. The inhibition percentage was calculated as:

$$\text{Inhibition (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of test}) \times 100}{\text{Absorbance of control}} \quad \text{...Eq 4}$$

GC-MS analysis of the leaf's extracts

The GC-MS was run with a column oven temperature of 60°c and injection temperature of 250°C with split mode of injection and liner velocity flow control. The pressure applied for GC is 57.4kpa which gives the column flow of 1.00ml/min and linear velocity of 36.5 cm/sec, with a purge flow of 3.0 ml/min and split ratio is 10.0. The ion source temperature was set at 200°C and the interface temperature is 300°C, with 2.00 min of solvent cut time. The Mass Spectra was taken with intervals of 0.50 sec, with a scan range of 40-600 m/z with a scan speed of 1250. The total time taken is 34.00 min and FTD detector is used for detection.

Results and Discussion

Antioxidant activity of *Nothapodytes nimmoniana* extract

Table 1 and Figure 1 exhibited the DPPH radical scavenging capabilities of *Nothapodytes nimmoniana* leaf and ascorbic acid as well. As a standard ascorbic acid showed higher inhibition percentage as compared to leaves extract.

Inhibition percentage enhances with an increase in leaf extract concentration and a maximum 75% inhibition was observed at 100 µg/ml leaf extract concentration. For ascorbic acid inhibition became constant from 60 to 100 µg/ml concentration.

Metal ion chelating activity of leaf extract was compared to EDTA in Table 2 and Figure 2. Inhibition became constant at 35% at leaf extract concentration of 60 to 100% whereas inhibition increases with increase in EDTA content.

Assessment of Superoxide radical scavenging of leaf extract was depicted in Table 3 and Figure 3. Maximum 30% inhibition was observed at 60 µg/ml leaf extract whereas 45% inhibition was obtained at 60 µg/ml ascorbic acid content.

Hydroxyl radical scavenging assessment of leaf extract was exhibited in table 4 and Figure 4. Maximum 25% inhibition was achieved at 60 µg/ml leaf extract. Further increase in extract concentration did not affect the inhibition percentage. 60 µg/ml mannitol showed 45% inhibition which was the maximum.

Antioxidant activity of *Utleria salicifolia* extract

The study carried out on the antioxidant activity of the methanol extract from the leaves of *Utleria salicifolia* using DPPH radical, metal chelating, hydroxyl and super oxide radical scavenging assays was described.

Table 5 and Figure 5 exhibits the DPPH radical scavenging capabilities of *Utleria salicifolia* leaf extract and ascorbic acid as well. As a standard ascorbic acid showed higher inhibition percentage as compared to leaves extract. Inhibition percentage enhances with an increase in leaf extract concentration up to 80 µg/ml and a maximum 45% inhibition was observed at this concentration.

For ascorbic acid inhibition became constant from 60 to 100 µg/ml concentration.

Metal ion chelating activity of leaf extract was compared to EDTA in Table 6 and Figure 6. Inhibition became constant at 50% at leaf extract concentration of 80 to 100% whereas inhibition increases with increase in EDTA content.

Assessment of Superoxide radical scavenging of leaf extract was depicted in Table 7 and Figure 7. Maximum 30% inhibition was observed at 60 µg/ml leaf extract whereas 45% inhibition was obtained at 60 µg/ml ascorbic acid content.

Table.1 Variation of inhibition percentage with respect to the concentration of leaf extract and ascorbic acid

Concentration of leaf extract (µg/ml)	% of inhibitions	Concentration of ascorbic acid (µg/ml)	% of inhibitions
20	40	20	60
40	50	40	80
60	60	60	85
80	70	80	85
100	75	100	85

Table.2 Metal ion chelation activity Assay of methanol extract of *Nothapodytes nimmoniana* leaves and standard EDTA

Concentration of leaf extract (µg/ml)	% of inhibitions	Concentration of EDTA (µg/ml)	% of inhibitions
20	20	20	50
40	30	40	55
60	35	60	60
80	35	80	65
100	35	100	70

Table.3 Superoxide radical scavenging assay of methanol extract of *Nothapodytes nimmoniana* leaves and standard Ascorbic acid

Concentration of leaf extract (µg/ml)	% of inhibitions	Concentration of Ascorbic acid (µg/ml)	% of inhibitions
20	20	20	30
40	25	40	40
60	30	60	45
80	30	80	45
100	30	100	45

Table.4 Hydroxyl radical scavenging assay of methanol extract of *Nothapodytes nimmoniana* leaves and standard Mannitol

Concentration of leaf extract (µg/ml)	% of inhibitions	Concentration of Mannitol (µg/ml)	% of inhibitions
20	10	20	30
40	20	40	40
60	25	60	45
80	25	80	45
100	25	100	45

Table.5 DPPH radical scavenging capabilities of methanol extract of *Utleria salicifolia* leaves and standard ascorbic acid

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Ascorbic acid (µm/ml)	% of inhibitions
20	20	20	60
40	30	40	80
60	40	60	85
80	45	80	85
100	45	100	85

Table.6 Metal ion chelation activity Assay of methanol extract of *Utleria salicifolia* leaves and standard EDTA

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of EDTA (µm/ml)	% of inhibitions
20	25	20	50
40	30	40	55
60	40	60	60
80	50	80	65
100	50	100	70

Table.7 Superoxide radical scavenging assay of methanol extract of *Utleria salicifolia* leaves and standard Ascorbic acid

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Ascorbic acid (µm/ml)	% of inhibitions
20	20	20	30
40	25	40	40
60	30	60	45
80	30	80	45
100	30	100	45

Table.8 Hydroxyl radical scavenging assay of methanol extract of *Utleria salicifolia* leaves and standard Mannitol

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Mannitol (µm/ml)	% of inhibitions
20	25	20	30
40	35	40	40
60	35	60	45
80	35	80	45
100	35	100	45

Table.9 DPPH radical scavenging assay of methanol extract of *Plectranthus vettiveroides* leaves and standard Ascorbic acid

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Ascorbic acid (µm/ml)	% of inhibitions
20	50	20	60
40	60	40	80
60	70	60	85
80	80	80	85
100	85	100	85

Table.10 Metal ion chelation activity Assay of methanol extract of *Plectranthus vettiveroides* leaves and standard EDTA

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of EDTA (µm/ml)	% of inhibitions
20	25	20	50
40	25	40	55
60	25	60	60
80	25	80	65
100	25	100	70

Table.11 Superoxide radical scavenging assay of methanol extract of *Plectranthus vettiveroides* leaves and standard Ascorbic acid

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Ascorbic acid (µm/ml)	% of inhibitions
20	30	20	30
40	40	40	40
60	40	60	45
80	40	80	45
100	40	100	45

Table.12 Hydroxyl radical scavenging assay of methanol extract of *Plectranthus vettiveroides* leaves and standard Mannitol

Concentration of leaf extract (µm/ml)	% of inhibitions	Concentration of Mannitol (µm/ml)	% of inhibitions
20	10	20	30
40	20	40	40
60	20	60	45
80	20	80	45
100	20	100	45

Fig.1 DPPH radical scavenging capabilities of methanol extract of *Nothapodytes nimmoniana* leaves and standard ascorbic acid

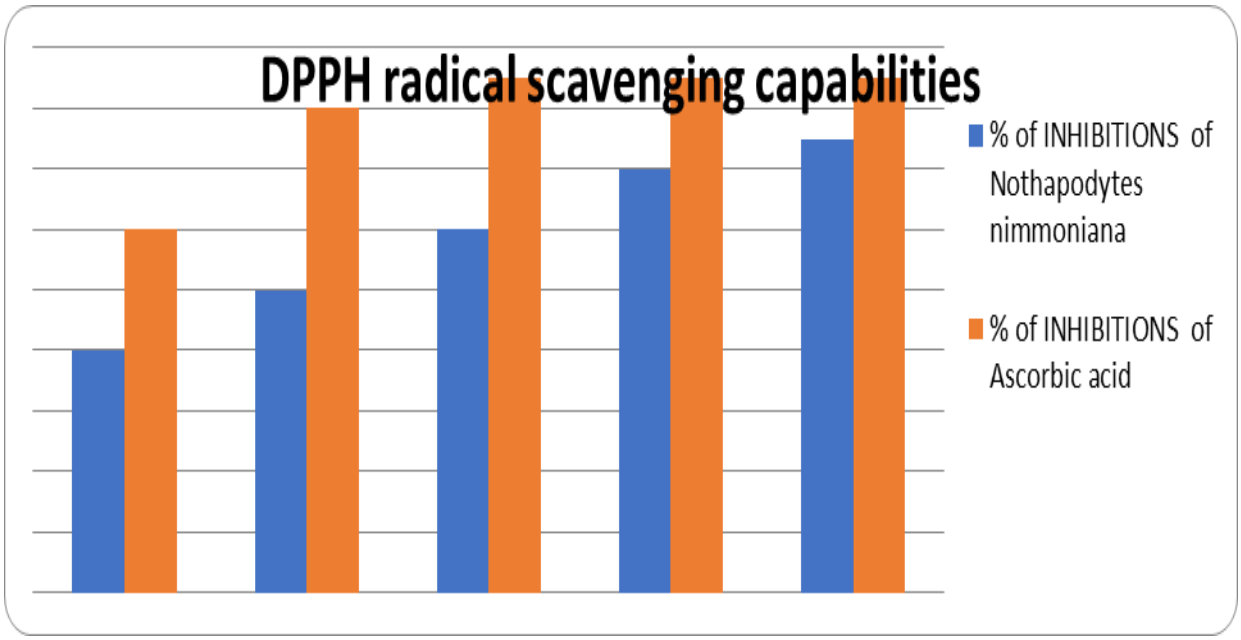


Fig.2 Metal ion chelating capabilities of methanol extract of *Nothapodytes nimmoniana* leaves and EDTA

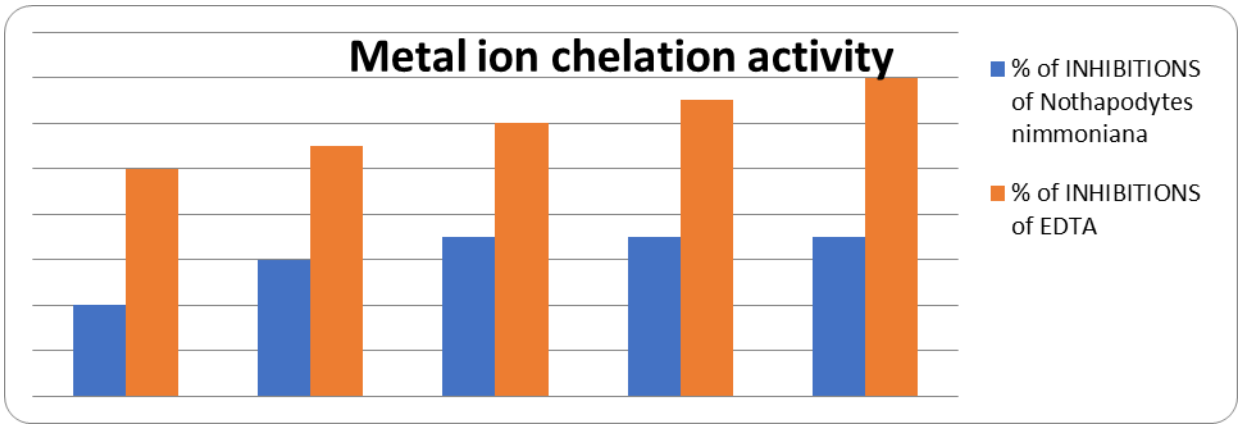


Fig.3 Superoxide ion radical scavenging capabilities of methanol extract of *Nothapodytes nimmoniana* leaves and Ascorbic acid.

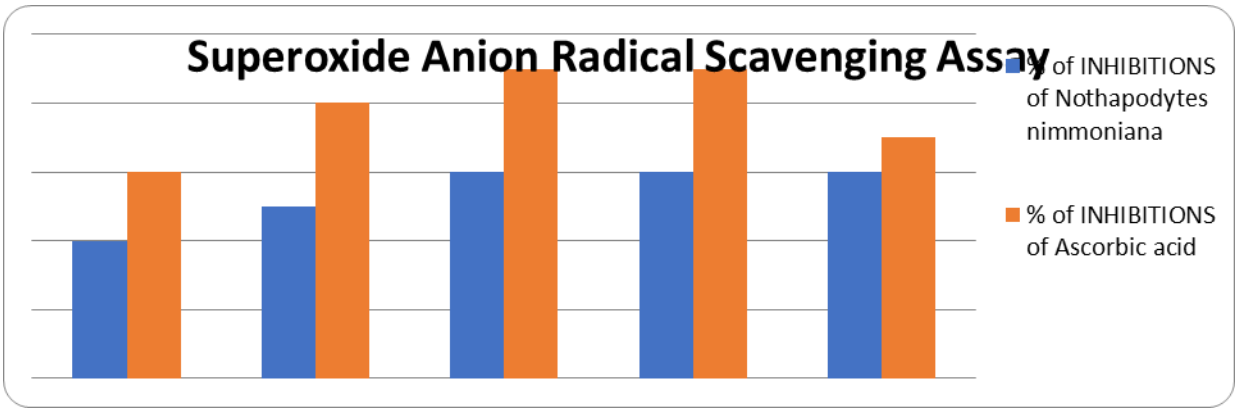


Fig.4 Hydroxyl radical scavenging capabilities of methanol extract of *Nothapodytes nimmoniana* leaves and Mannitol

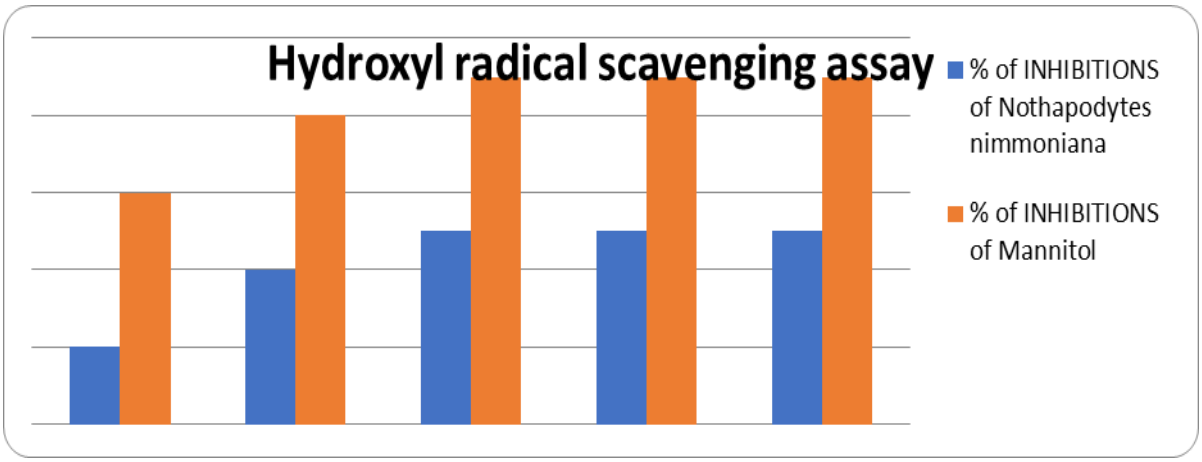


Fig.5 DPPH radical scavenging capabilities of methanol extract of *Utleria salicifolia* leaves and standard ascorbic acid

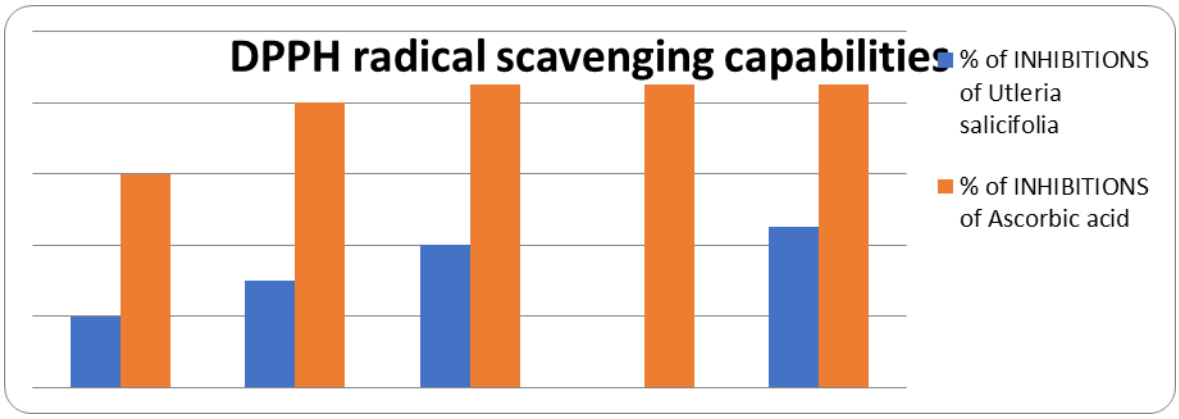


Fig.6 Metal ion chelating capabilities of methanol extract of *Utleria salicifolia* leaves and EDTA

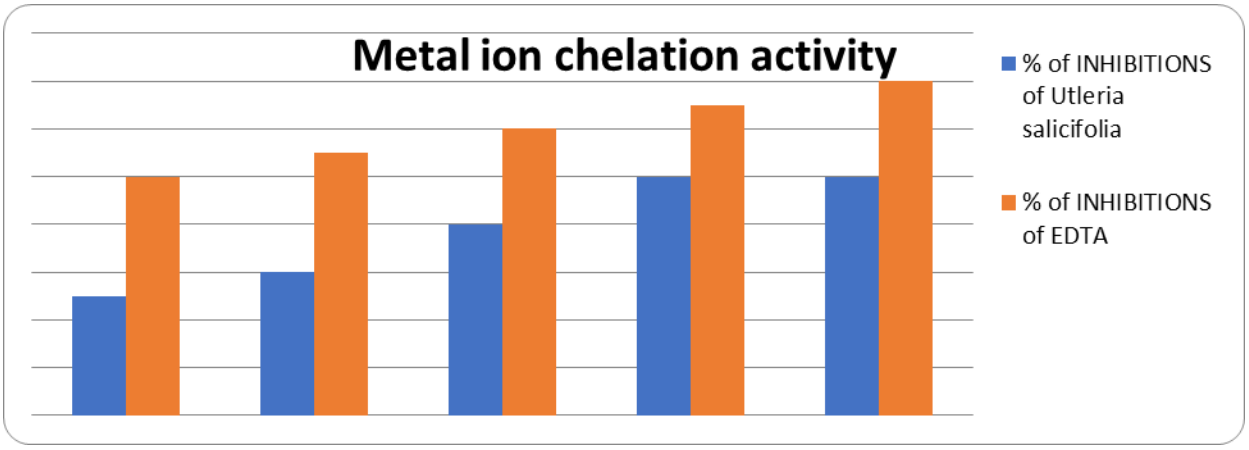


Fig.7 Superoxide radical scavenging capabilities of methanol extract of *Utleria salicifolia* leaves and Ascorbic acid

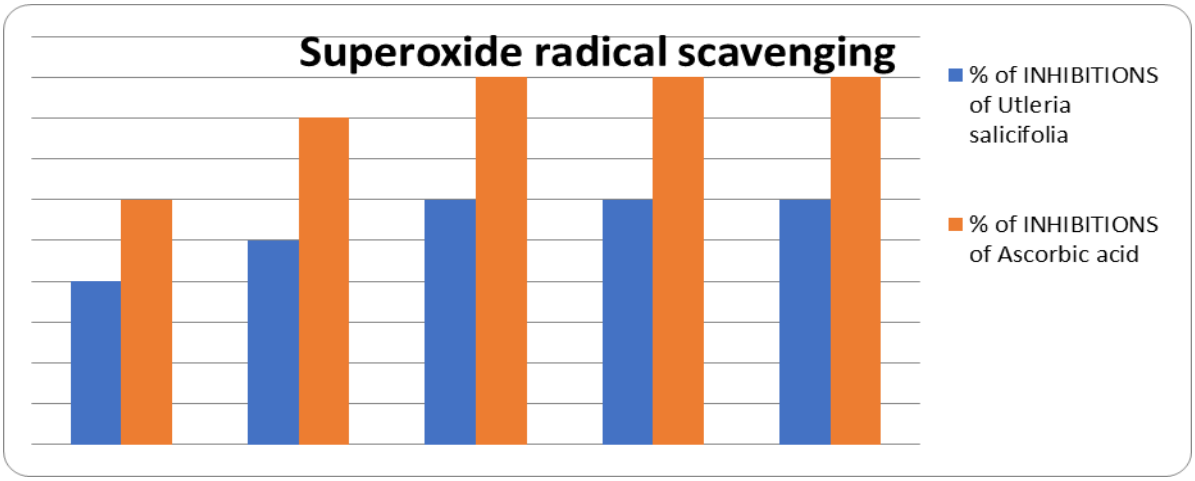


Fig.8 Hydroxyl radical scavenging capabilities of methanol extract of *Utleria salicifolia* leaves and Mannitol

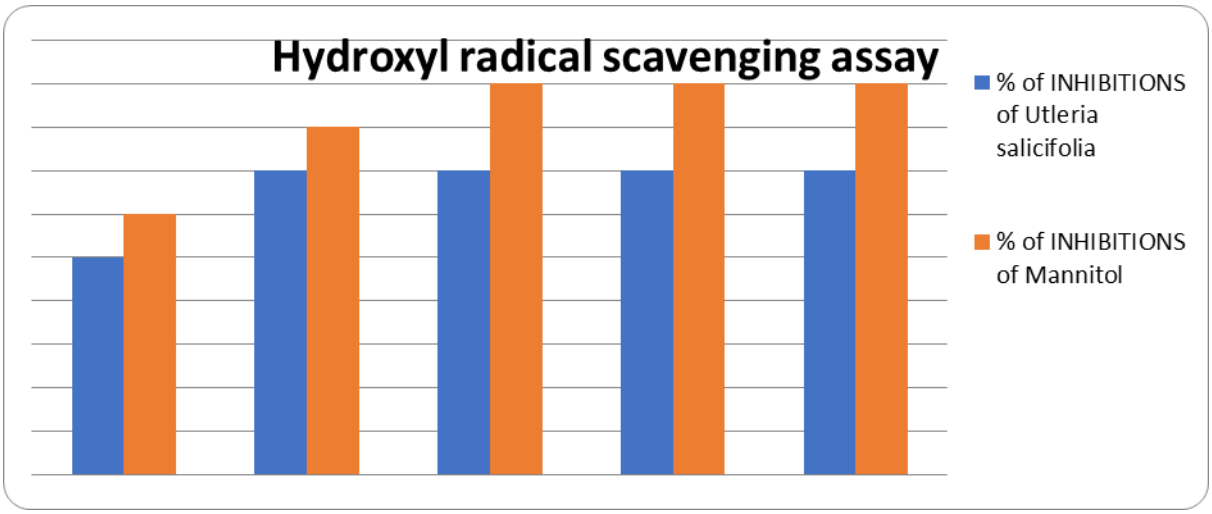
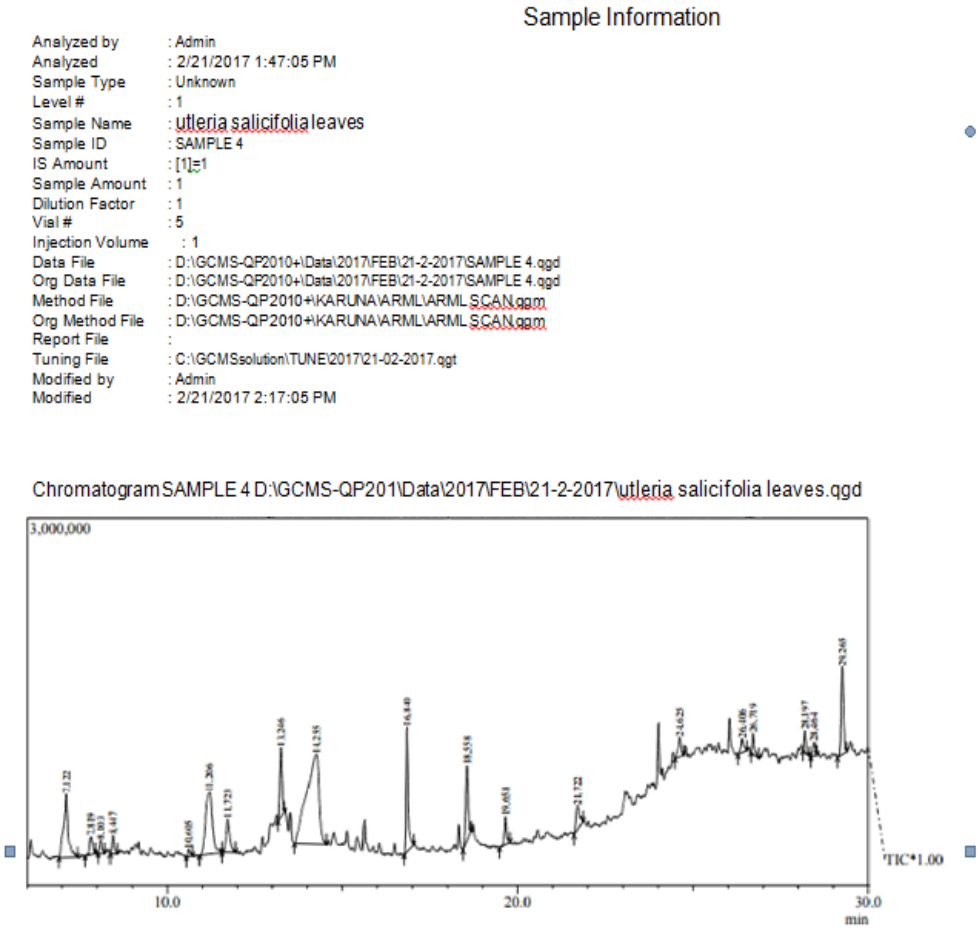


Fig.9 Gas chromatography of *Utleria salicifolia* leaf extract



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	3.566	3.408	3.633	394816	0.66	Propanoic acid, 2-oxo-, methyl ester
2	3.712	3.633	3.992	2584233	4.32	2-Propanone, 1,3-dihydroxy-
3	4.148	4.100	4.225	140028	0.23	2-Cyclopenten-1-one, 2-hydroxy-
4	4.809	4.575	4.892	1056980	1.77	Glycerin
5	4.951	4.892	5.058	409586	0.68	2-Hydroxy-gamma-butyrolactone
6	7.122	6.908	7.442	4805728	8.04	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-
7	7.819	7.658	7.942	797453	1.33	1,2-Benzenediol
8	8.103	8.017	8.217	526556	0.88	Benzofuran, 2,3-dihydro-
9	8.447	8.375	8.575	646758	1.08	1,2,3-Propanetriol, monoacetate
10	10.605	10.542	10.725	259240	0.43	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobut
11	11.206	10.917	11.550	7152499	11.96	Sucrose
12	11.723	11.550	11.950	2028680	3.39	1,6-Anhydro-.beta.-D-glucopyranose (levogluc
13	13.246	13.158	13.342	2850378	4.77	Hexanoic acid, 2-ethylhexyl ester
14	14.255	13.625	14.542	18650932	31.18	3-O-Methyl-d-glucose
15	16.840	16.758	17.042	4418881	7.39	n-Hexadecanoic acid
16	18.558	18.442	18.675	3253287	5.44	8,11,14-Eicosatrienoic acid, (Z,Z,Z)-
17	19.658	19.475	19.775	1011783	1.69	Benzyl .beta.-d-glucoside
18	21.722	21.617	21.858	1406374	2.35	4H-1-Benzopyran-4-one, 2,3-dihydro-5,7-dihy
19	24.625	24.483	24.750	1052172	1.76	1,4-Methanoazulene, 7-bromodecahydro-4,8,8-
20	26.406	26.292	26.542	672554	1.12	3,3,7,11-Tetramethyltricyclo[5.4.0.0(4,11)]und
21	26.719	26.650	26.883	789040	1.32	Vitamin E
22	28.197	28.142	28.325	710328	1.19	Eicosane
23	28.464	28.367	28.542	559641	0.94	Stigmasterol
24	29.265	29.125	29.400	3630378	6.07	.gamma.-Sitosterol

Fig.10 DPPH radical scavenging capabilities of methanol extract of *Plectranthus vettiveroides* leaves and standard Ascorbic acid

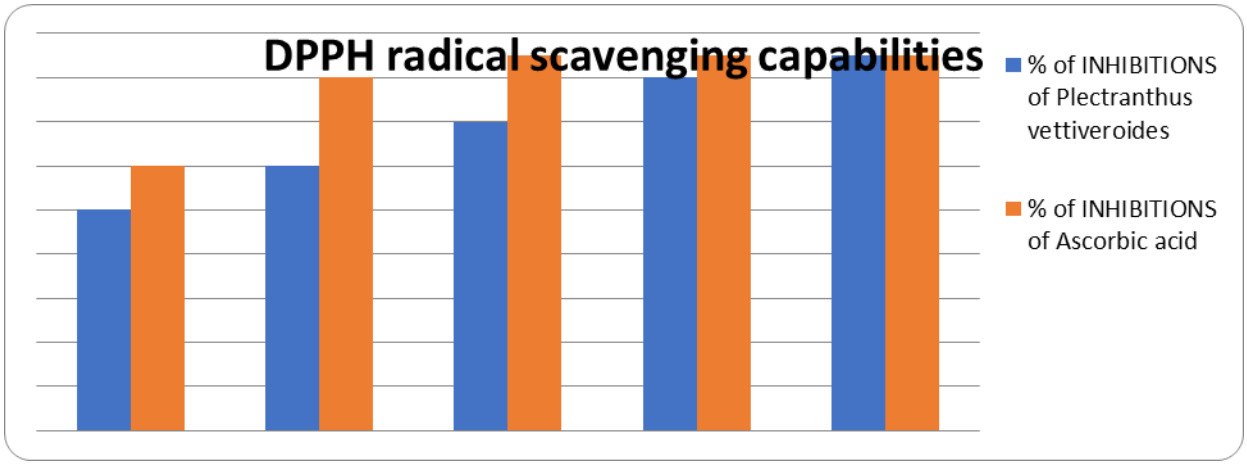


Fig.11 Metal ion chelation capabilities of methanol extract of *Plectranthus vettiveroides* leaves and EDTA

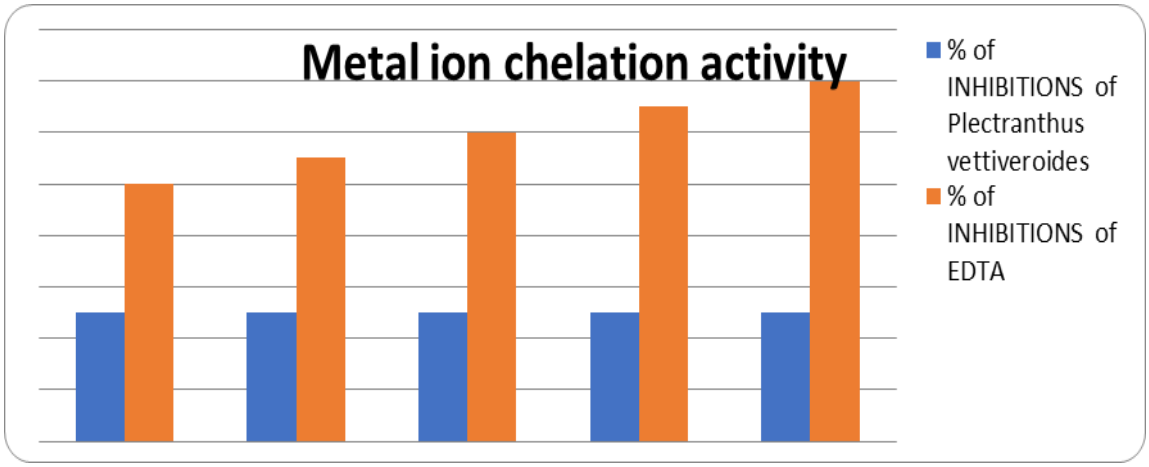


Fig.12 Superoxide radical scavenging capabilities of methanol extract of *Plectranthus vettiveroides* leaves and standard Ascorbic acid

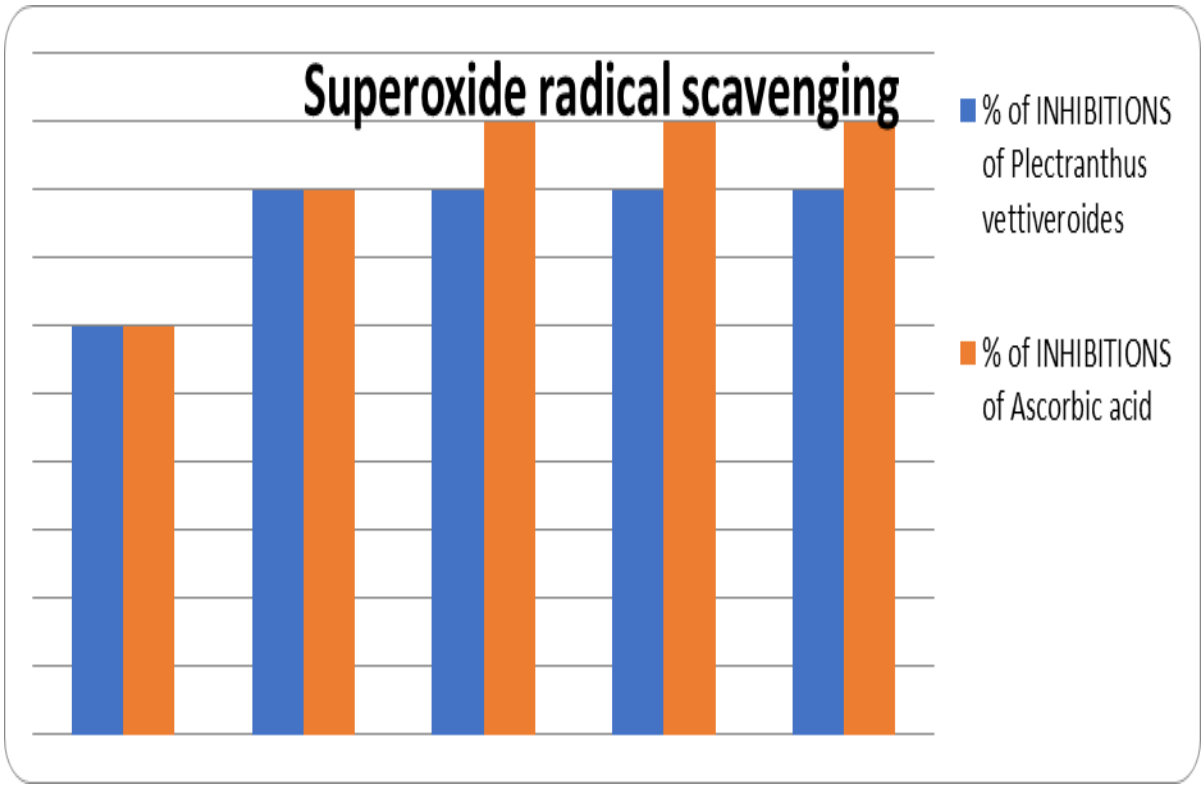


Fig.13 Hydroxyl radical scavenging capabilities of methanol extract of *Plectranthus vettiveroides* leaves and Mannitol

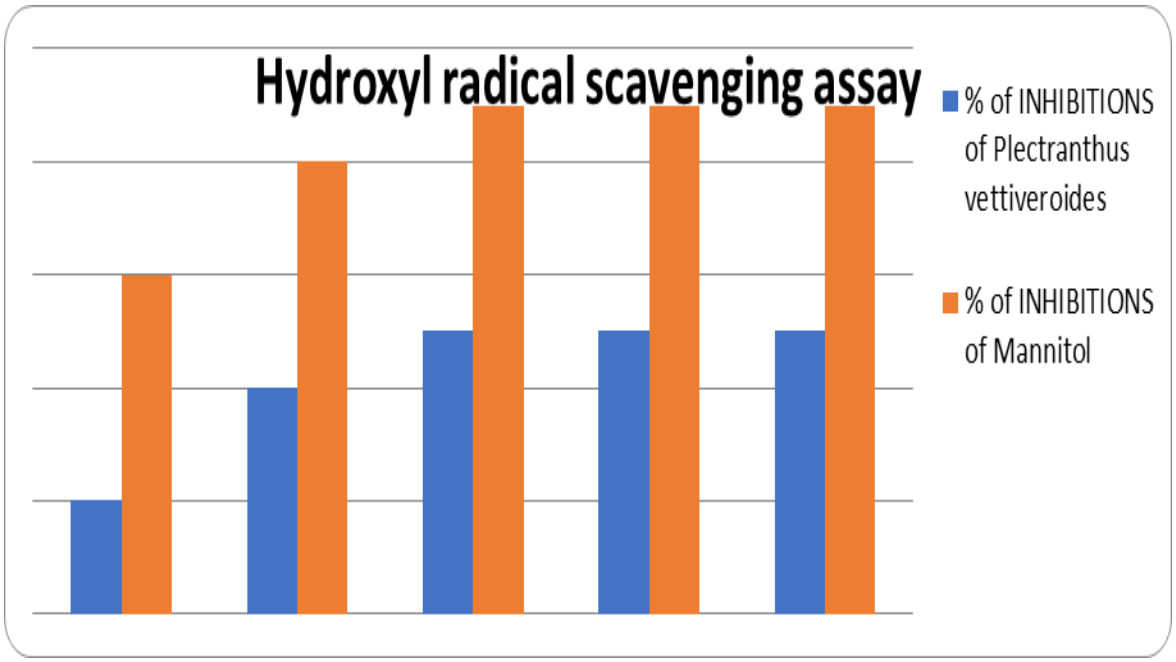
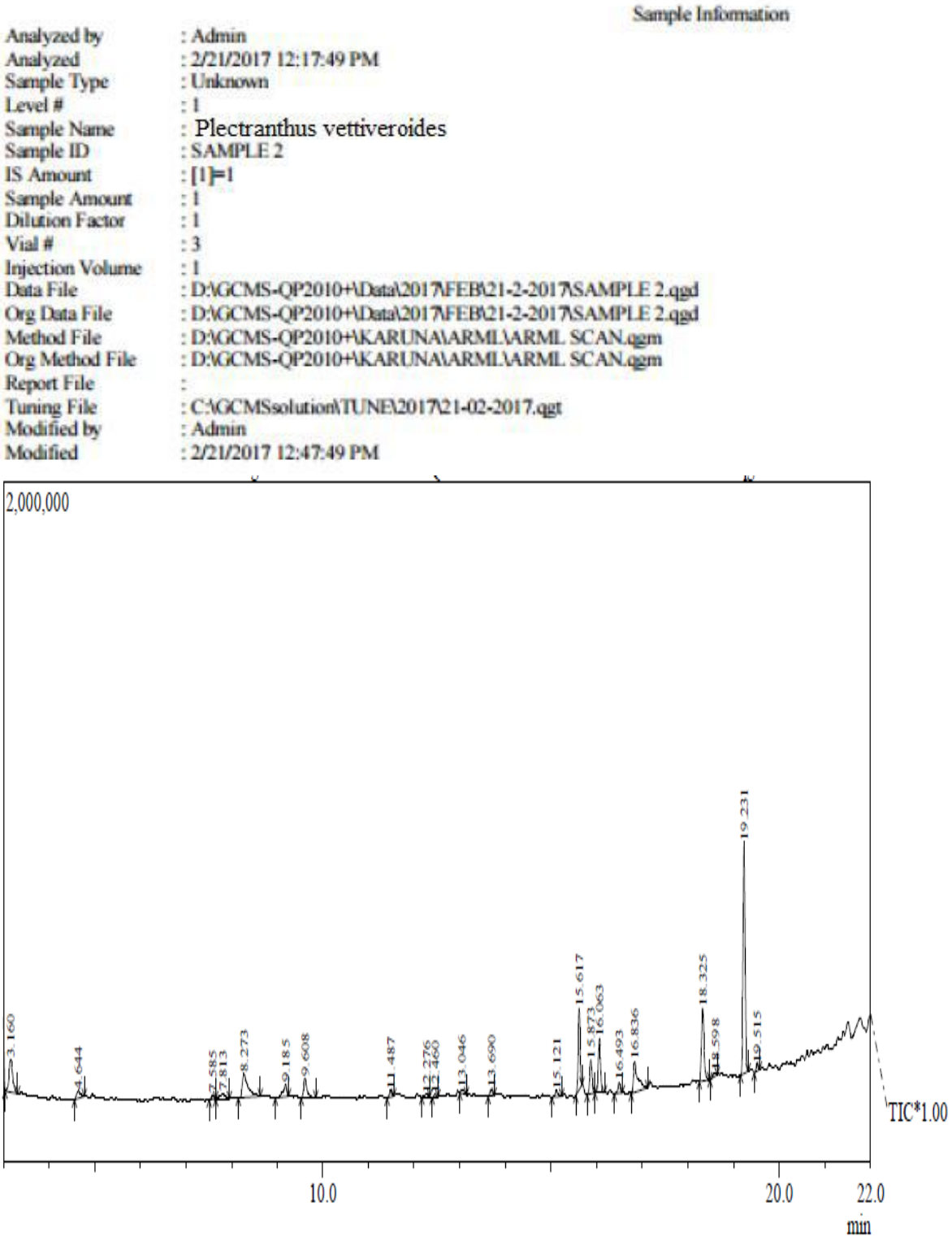


Fig.14 Gas chromatography of *Plectranthus vettiveroides* leaf extract



Peak Report TIC

Peak#	R.Time	I.Time	F.Time	Area	Area%	Name
1	3.160	3.033	3.317	557119	7.09	Furfural
2	4.644	4.558	4.775	128550	1.63	Propanal, 2,3-dihydroxy-
3	7.585	7.508	7.658	50931	0.65	1H-Imidazo[1,2-b]pyrazole, 2,3-dihydro-
4	7.813	7.658	7.950	116582	1.48	2-Octen-1-ol, (E)-
5	8.273	8.150	8.633	613919	7.81	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
6	9.185	8.958	9.250	189305	2.41	Tridecane
7	9.608	9.525	9.842	294639	3.75	Phthalic anhydride
8	11.487	11.408	11.550	65916	0.84	Bicyclo[3.2.1]oct-2-ene, 3-methyl-4-methylene
9	12.276	12.167	12.350	48927	0.62	Bioallethrin
10	12.460	12.400	12.517	22443	0.29	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,
11	13.046	13.000	13.150	69134	0.88	Phthalic acid, di-(1-hexen-5-yl) ester
12	13.690	13.617	13.758	71575	0.91	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)-
13	15.121	15.017	15.233	67520	0.86	7-Oxabicyclo[4.1.0]heptane, 1-methyl-4-(2-me
14	15.617	15.550	15.675	743046	9.45	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
15	15.873	15.792	15.967	331603	4.22	1-Octadecyne
16	16.063	15.967	16.175	554241	7.05	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
17	16.493	16.383	16.558	108227	1.38	Heptacosanoic acid, methyl ester
18	16.836	16.758	17.117	610640	7.77	Tetradecanoic acid
19	18.325	18.250	18.475	860663	10.95	Phytol
20	18.598	18.483	18.642	55113	0.70	2H-Pyran, 2-(2-heptadecynyloxy)tetrahydro-
21	19.231	19.158	19.325	2234470	28.42	1-Octadecyne
22	19.515	19.458	19.567	68515	0.87	1,2-Benzenediol, 3,5-bis(1,1-dimethylethyl)-
				7863078	100.00	

Hydroxyl radical scavenging assessment of leaf extract was exhibited in table 8 and Figure 8. Maximum 35% inhibition was achieved at 40 µg/ml leaf extract. Further increase in extract concentration did not affect the inhibition percentage. 60 µg/ml mannitol showed 45% inhibition which was the maximum.

GC-MS analysis of *Utleria salicifolia* extract

Figure 9 exhibits the chromatogram obtained from GC-MS analysis of *Utleria salicifolia* extract. Presence of essential organic component was observed while the obtained peaks were analyzed.

Antioxidant activity of *Plectranthus vettiveroides* extract

The study carried out on the antioxidant activity of the methanol extract from the leaves of *Plectranthus vettiveroides* using DPPH radical, metal chelating, hydroxyl and super oxide radical scavenging assays is described. Table 9 and Figure 10 exhibited the DPPH radical scavenging capabilities of *Plectranthus vettiveroides* leaf extract and ascorbic acid as well. As a standard ascorbic acid showed higher inhibition percentage as compared to leaves extract. Inhibition percentage enhances with an increase in leaf extract concentration to 100 µg/ml and a maximum 85% inhibition was observed at this concentration. For ascorbic acid inhibition became constant at 85% from 60 to 100 µg/ml concentration.

Metal ion chelating activity of leaf extract was compared to EDTA in Table 10 and Figure 11. There is no change of inhibition with respect to leaf extract concentration whereas inhibition increases with increase in EDTA content.

Assessment of Superoxide radical scavenging of leaf extract is depicted in Table 11 and Figure 12. Maximum 40% inhibition was observed at 40 µg/ml leaf extract whereas a constant 45% inhibition was obtained at 60 µg/ml ascorbic acid content.

Hydroxyl radical scavenging assessment of leaf extract is exhibited in table 12 and Figure 13. Maximum 20% inhibition was achieved at 40 µg/ml leaf extract. Further increase in extract concentration did not affect the inhibition percentage. 60 µg/ml mannitol showed 45% inhibition which was the maximum.

GC-MS analysis of *Plectranthus vettiveroides* extract

Figure 14 exhibits the Chromatogram obtained from the GC-MS analysis of *Plectranthus vettiveroides* leaf extract. Presence of essential organic component was observed while the obtained peaks were analyzed.

It is concluded in the current study better activity of all the three plant species was recorded. This was due to efficient extraction of phytochemicals. The extracts from three different plant leaves showed antioxidant activity which was substantiated by four different assay methods. Further Gas Chromatography analysis verified the presence of important compound in the plant extracts. Further study can be carried out on isolating a specific bioactive compound with commercial value. Being a rich source of antioxidant, these medicinally important plants have values as functional ingredients in food industry.

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