Original Research Article

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

N. Sumangala¹*, M. Jayaramu² and M.P. Prasad³

¹Microbiology, Tumkur University, Karnataka, India
²Department of Studies and Research in Environmental Sciences, Tumkur University, Karnataka, India
³Sangene Biotech, Bengaluru, Karnataka, India

*Corresponding author

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.

Keywords
Antioxidant, DPPH, Metal ion, Hydroxyl radical, Superoxide anion radical

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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, Utleria 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).

\[
\text{Inhibition (\%)} = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100 \quad \text{Eq 1}
\]

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 0.4mL of hydroxylaminehydrochloride 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.

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

\[
\text{Inhibition (\%) } = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]

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:

\[
\text{Inhibition (\%) } = \left( \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \right) \times 100
\]

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 *Notapodytes 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

![DPPH radical scavenging capabilities graph](image)

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

![Metal ion chelation activity graph](image)

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

![Superoxide radical scavenging graph](image)
Fig. 8 Hydroxyl radical scavenging capabilities of methanol extract of *Utleria salicifolia* leaves and Mannitol

![Graph showing hydroxyl radical scavenging assay](image)

% of INHIBITIONS of *Utleria salicifolia*

% of INHIBITIONS of Mannitol

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

![Gas chromatogram](image)
**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
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 *Uteria salicifolia* extract**

Figure 9 exhibits the chromatogram obtained from GC-MS analysis of *Uteria 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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