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Screening of the endangered medicinal plant extracts for antioxidant activity

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Abstract
The present study was undertaken to find the antioxidant value of endangered medicinal plants in Gingee hills, Villupuram, Tamilnadu. Antioxidants have been reported to prevent oxidative damage caused by free radical and can be used in curing various human diseases. The endangered medicinal plants *Zehneria scabra* (tuber), *Ormocarpum sennoides* (leaf) and *Bauhinia tomentosa* (leaf) exhibits potent antioxidant activities. The study indicates that the presence of phenolic compounds and flavonoids in the medicinal plants can act as scavengers in preventing oxidative damages caused by the free radicals.

Keywords: antioxidant, oxidative damages, free radicals, Gingee hills

1. Introduction
Traditional knowledge of medicinal plants has always guided the search for new cures. In spite of the advent of modern high throughput drug discovery and screening techniques, traditional knowledge systems have given clues to the discovery of valuable drugs [1]. Traditional medicinal plants such as *Ormocarpum sennoides* (Willd) DC. (Papilionoideae), *Zehneria scabra* (L.f.) Sond. (Cucurbitaceae), *Bauhinia tomentosa* L. (Caesalpinioideae) are found to be over exploited for their medicinal values by the local inhabitants in Gingee hills, Tamilnadu, India. They are now on the verge of extinction from the hills. Antioxidant screening of such plants will establish their therapeutic values and pave way to drug development with a scientific foundation before they disappear.
An antioxidant is a chemical that reduces the rate of particular oxidation reactions in a specific context [2]. The potentially reactive derivatives of oxygen, attributed as reactive oxygen species (ROS) such as hydrogen peroxide, hypochloric acid and proxy nitrite are continuously generated in the human body. The generated ROS and/or inadequate antioxidant defense can easily affect and persuade oxidative damage to various biomolecules including proteins, lipids, lipoproteins and DNA [3] causing several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis and neurodegenerative diseases and also in the ageing process. As antioxidants have been reported to prevent oxidative damage by free radical (generated when cells use oxygen during energy production such as super oxide, hydroxyl radicals and nitric oxide), it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers (also called as "free radical scavengers") [4, 5].
Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders [6, 7]. The substances which act as antioxidants are phenolics, a major secondary metabolite required for the growth and reproduction of plants and are produced as a response for defending injured plants against stress. Likewise, flavonoids are a common group of polyphenolic compounds rich in plant leaves, stem, and bark. The carbonyl group present in phenols, flavonoids protect human beings from any type of attack. Vitamin C and Vitamin E were the first recognized antioxidants, but other substances that have powerful antioxidant properties have also been recognized such as: selenium, carotenoids (beta-carotene, lutein, lycopene, sulforaphane, zeaxanthin, and astaxanthin), bioflavonoids (anthocyanins, proanthocyanidins, quercetin, and apigenin), coenzymes, soy isoflavones (genistein and daidzein), and many less well-known compounds found in fruits and vegetables [8]. The utilities of the investigated medicinal plants are given below (Table 1).

2. Materials and Methods

2.1 Chemicals and Reagents

Chemicals such as sulphuric acid, sodium di-phosphate, ammonium molybdate, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), ethanol, Folin Ciocalteau reagent, sodium carbonate, tannic acid, Sodium nitroprusside, phosphate buffer, Griess reagent, ascorbic acid were procured from Ponmani Chemicals Suppliers, Tiruchirappalli, Tamilnadu.

2.2 Plant Materials

The leaves of *Ormocarpum sennoides* and *Bauhinia tomentosa* and tuber of *Zehneria scabra* were collected from Gingee hills, Villupuram, Tamilnadu during January, 2014. Taxonomic identification of these plants was carried out by John Britto, Director, The Rapinant Herbarium, St. Joseph's College, Tiruchirappalli. A voucher specimen of each experimental plant was deposited at The Rapinant Herbarium.

2.3 Plant Extraction

The samples were shade dried at room temperature and further ground into powder. About 10 gm of each plant powder was extracted in 70 ml of ethanol, aqueous and chloroform by maceration (48 hrs). The solvents were concentrated at temperature below 40 °C and the resulting extracts were used for screening antioxidant properties.

2.4 Phytochemical Screening

Phytochemical screening was performed using standard procedures described by Mukherjee [9]. The physical characteristics of the plant extracts at the time of the study were recorded (Table 2).

2.4 Antioxidant assays

The antioxidant activity of plant materials were assayed by employing the following methods:

(i) Total antioxidant activity

The total antioxidant activity was eluted by using the method described by Prieto *et al.*, [10]. Plant extracts were taken in test tubes having five different concentration solutions such as 10 µl, 20 µl, 30 µl, 40 µl, 50 µl and dissolved in reagent solution (0.6 M Sulphuric Acid, 28 mM Sodium Phosphate, 4 mM Ammonium molybdate) and the resulting mixture was incubated at 950 °C for 90 minutes. After the mixture was cooled to room temperature, the absorbance of the each solution was measured by using UV-Visible spectrophotometer at 695 nm against blank. A calibration curve was constructed, using ethanol (100-500 µg/ml) as standard and total antioxidant activity of the extract (µg/ml) expressed as ethanol equivalents.

(ii). DPPH Radical Scavenging Activity [11]

DPPH (1-1 - diphenyl 2-picryl hydrazyl) is a commercially available stable free radical, which is purple in colour. The antioxidant molecules present in the herbal extracts, when incubated, react with DPPH and convert it into di-phenyl hydrazine, which is yellow in colour. The degree of discoloration of purple to yellow was measured at 517 nm [12, 13], which is a measure of scavenging potential of plant extracts. 10 µl of plant extract was added to 100 µl of DPPH solution (0.2 mM DPPH in methanol) in a microtitre plate. The reaction mixture was incubated at 25 °C for 5 minutes, after that the absorbance was measured at 517 nm. The DPPH with corresponding solvents (without plant material) serves as the control. The methanol with respective plant extracts serves as blank. The DPPH radical scavenging activity of the plant extract was calculated as the percentage inhibition. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity. DPPH radical-scavenging activity (%) was calculated according to the following equation:

$$(\%) = 100 \times (A0-A_1)/A0$$

Where A0 is the absorbance of the control, A1 is the absorbance of the extract/standard, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for % 50 inhibition was determined. The lower inhibition value indicates high antioxidant capacity.

(iii). Nitric oxide scavenging assay

Nitric oxide scavenging activity was measured spectrophotometrically [14]. Extract, prepared in ethanol, was added to different test-tubes in varying concentrations (10 µl, 20 µl, 30 µl, 40 µl, 50 µl). Sodium nitroprusside (5 mM) in phosphate buffer was added to each test tube to make volume up to 1.5 ml. Solutions were incubated at 25 ºC for 30 minutes. Thereafter, 1.5 ml of Griess reagent (1% sulphanilamide, 0.1% naphthylethylenediamine dichloride and 3% phosphoric acid) was added to each test tube. The absorbance was measured, immediately, at 546 nm and percentage of scavenging activity was measured with reference to ascorbic acid as standard.

(iv). Tannins estimation

Tannins were estimated spectrophotometrically [15], with minimal modifications. 0.5 ml of suitably diluted extract was taken in a test tube and volume was made up to 2.5 ml with distilled water. 0.25 ml of 1:19 diluted Folin Ciocalteau reagent and 0.5 ml of 20% sodium carbonate solution were added. The solution was kept for 30 minutes at room temperature. Subsequently, absorbance was measured at 775 nm and concentration was estimated with respect to tannic acid as standard.

3. Results and Discussion

Pakkamalai and Thandavasamuthiram hills of Gingee Range have a rich diversity of medicinal plants. The inhabitants and herbal practitioners in the study area are aware of the uses of many medicinal plants. The selected experimental plants as shown in Table 1 are some of the commonly exploited plants for medicinal purposes by local people. The usages of such plants are mentioned in the same table. The extracts were examined for their physical characterization like colour, odor and consistency. The color of the aqueous extracts of the experimental samples was yellowish brown and while ethanolic extracts showed the colour of yellow and dark yellow. The consistency level of all the extracts were semi-solids and the odors were characteristics in two samples and sample tuber was odorless. Presence of odor showed the presence of desired phytochemicals. The result of the above study is compiled in Table 2. Different chemical tests were performed to determine the nature of the chemical constituents.

Table 1: Various medicinal uses of the experimental plants

Name of the plant	Part used	Uses
Ormocarpum sennoides	leaf	Leaf powder is taken along with honey or milk to strengthen bone and when its paste is tied on the fracture it heals the bone.
Bauhinia tomentosa	leaf	Leaf is taken with honey to stop vomiting; it is cooked and eaten as green vegetable to increase appetite.
Zehneria scabra	tuber	Tuber is consumed for the snakebites; the tuberous herbaceous perennial plant is grown at home to keep away snakes; it is also used for diabetes.

Table 2: Physical characteristics of the extracts

Name of the Extracts	Name of plant	Part used	Consistency	Colour	Odor
Ethanolic extract Aqueous extract	*Ormocarpum sennoides*	leaf	Semi-solid Semi-solid	dark green greenish brown	characteristic characteristic
Ethanolic extract Aqueous extract	*Bauhinia tomentosa*	leaf	Semi-solid Semi-solid	dark green greenish brown	characteristic characteristic
Ethanolic extract Aqueous extract	*Zehneria scabra*	tuber	Semi-solid Semi-solid	yellow Yellowish brown	no odor no odor

Table 3: Chemical tests in the aqueous, ethanolic and chloroform extracts

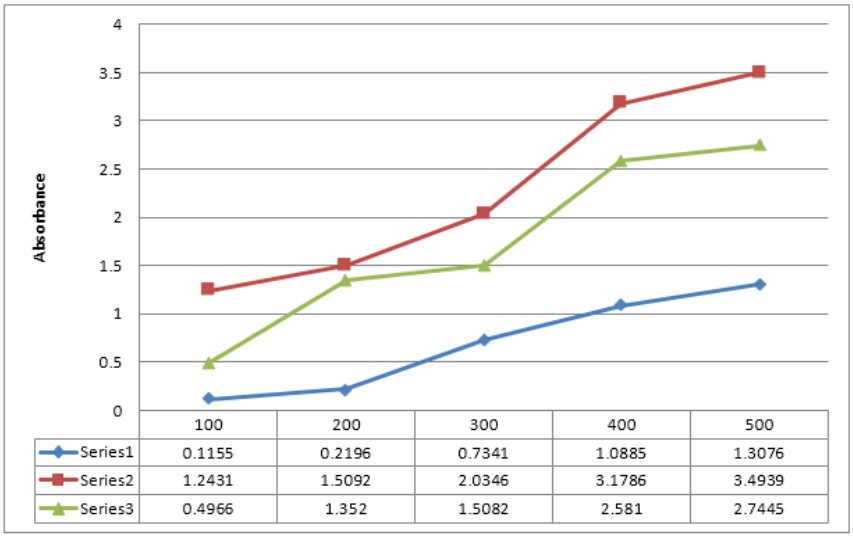
Name of the plants	Part used	Phytoconstituents	Aqueous extract	Ethanolic extract	Chloroform extract
Ormocarpum sennoides	leaf	Phenol	+	+	-
		Steroids	-	-	+
		Tannins	+	+	+
		Flavonoids	+	-	-
		Alkaloids	+	+	+
		Saponins	+	+	-
		Glycosides	-	-	-
		Proteins	+	+	+
		Amino acids	+	+	+
		Phenol	+	+	+
		Steroids	+	-	-
		Tannins	+	+	+
		Flavonoids	+	+	+
Bauhinia tomentosa	leaf	Alkaloids	+	+	-
		Saponins	+	-	-
		Glycosides	+	-	+
		Proteins	+	+	+
		Amino acids	+	-	+
Zehneria scabra	tuber	Phenol	+	+	+
		Steroids	+	+	-
		Tannins	-	-	+
		Flavonoids	-	-	+
		Alkaloids	-	-	-
		Saponins	-	-	
		Glycosides	+	+	-
		Proteins	+	+	+
		Amino acids	+	+	+

(+) = Present, **(-)** = Absent

The triphytochemical screening (aqueous, ethanolic and chloroform) of the extracts of *Ormocarpum sennoides* and *Bauhinia tomentosa* revealed the presence of antioxidant compounds such as phenol, flavonoids and tannins. *Zehneria scabra* (tuber) showed the presence of relatively very less amount of the antioxidant compounds. Only phenolic compound is found in the plants while other antioxidant compounds like flavonoids and tannins are absent. Therefore,

screening of 3 medicinal plant extracts for antioxidant studies was useful to identify the therapeutic values for antioxidant activity.

3.1 Determination of total antioxidant activity .



	100	200	300	400	500
Series1	0.1155	0.2196	0.7341	1.0885	1.3076
Series2	1.2431	1.5092	2.0346	3.1786	3.4939
Series3	0.4966	1.352	1.5082	2.581	2.7445

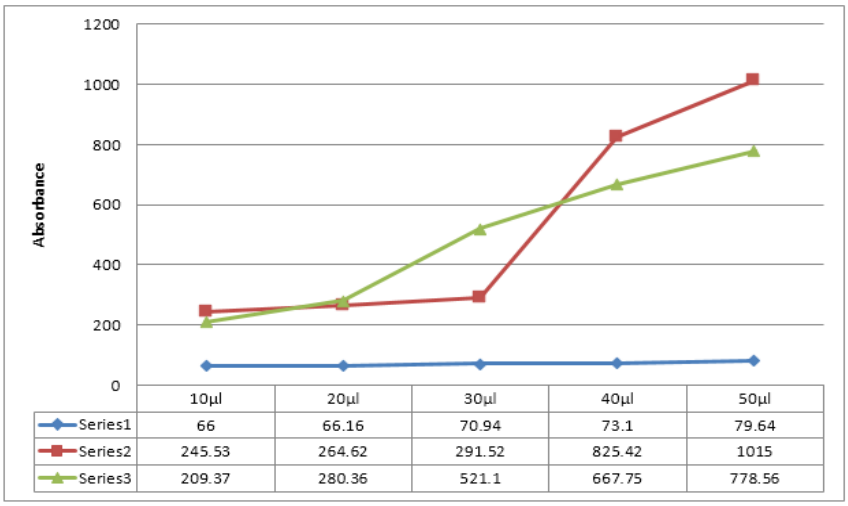
Chart 1: Total Antioxidant Activity

Series 1: *Zehneria scabra* (tuber); **Series 2:** *Ormocarpum sennoides* (leaf);
Series 3: *Bauhinia tomentosa* (leaf)

The total antioxidant assay was successfully used to determine the total antioxidant activity of standard ethanol. Chart 1 gives the entire antioxidant activities from the selected medicinal plants. *Zehneria scabra* (tuber) exhibits very minimum antioxidant activity as compared to *Ormocarpum sennoides* (leaf) and *Bauhinia tomentosa* (leaf). It is noted that *Ormocarpum sennoides* has the highest antioxidant activity.

The range of antioxidant activity varies from 0.1155 µl to 1.3076 µl in *Zehneria scabra* which is found to have the lowest antioxidant activity; 1.2431 µl to 3.4939 µl in *Ormocarpum sennoides* which is found to have the highest antioxidant activity.

3.2 Determination of DPPH Radical Scavenging Activity



	10µl	20µl	30µl	40µl	50µl
Series1	66	66.16	70.94	73.1	79.64
Series2	245.53	264.62	291.52	825.42	1015
Series3	209.37	280.36	521.1	667.75	778.56

Chart 2: DPPH Radical Scavenging Activity

Series 1: *Zehneria scabra* (tuber); Series 2: *Ormocarpum sennoides* (leaf);
Series 3: *Bauhinia tomentosa* (leaf)

Chart 2 shows the scavenging effects on DPPH radical assay. There is an increase in concentration of the samples against the standard blank. The lower inhibition against the standard concentration indicates the presence of high antioxidant activity from the selected medicinal plants. *Zehneria scabra* (tuber) indicates the inhibition range from 66.00 µl to79.64 µl; *Ormocarpum sennoides* presents the range from 245.53 µl to 1015 µl and *Bauhinia tomentosa* gives the range of 209.27 µl to 778.56 µl. All the experimental medicinal plants exhibit a certain amount of antioxidant activity.

3.3 Determination of Nitric oxide Scavenging Activity

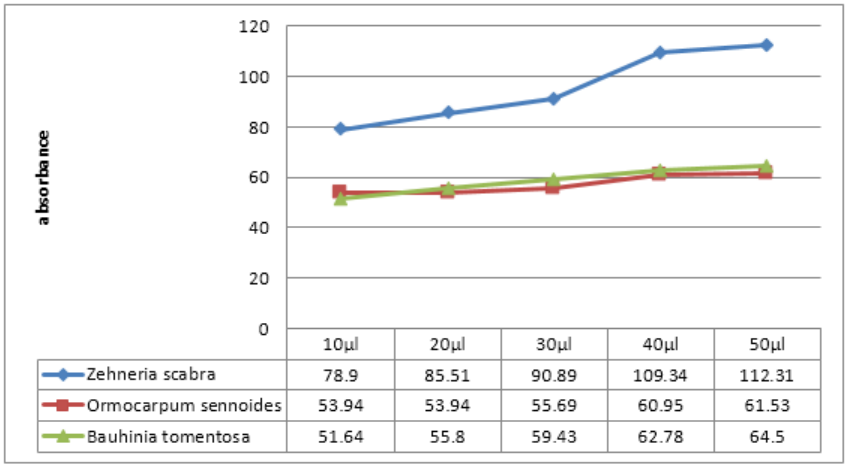


Chart 3: Nitric oxide Scavenging Activity

Extracts of *Zehneria scabra* (tuber), *Ormocarpum sennoides* (leaf) and *Bauhinia tomentosa* revealed the significant presence of antioxidative agents like flavonoids and tannins. Nitric Oxide (NO) scavenging assay is based on the scavenging ability of the extracts as well as ascorbic acid, which is used as standard. The scavenging of number was found to increase in a dose dependent manner. Maximum inhibition of number was observed in the extracts of *Zehneria scabra* with 112.31 mg/ml. The lowest inhibition was found in *Bauhinia tomentosa* with 51.54 mg/ml (Chart 3).

3.4 Estimation of total Tannins

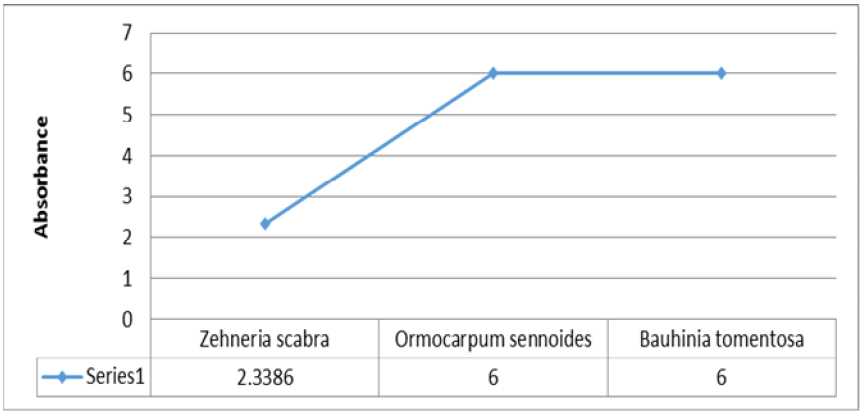


Chart 4: Estimation of total Tannin

Estimation of total tannin is based on oxidation of molecules which contain phenolic hydroxyl groups. For dry leaf extract, total tannins were found to be 2.33, 6 and 6 µgs/mg of samples from Gingee hills, Villupuram, Tamilnadu respectively (Chart 4).

4. Conclusion

In conclusion, the results of the present study suggest that *Ormocarpum sennoides* (leaf) and *Bauhinia tomentosa* (leaf) exhibit good antioxidant activities for total antioxidant assay and DPPH scavenging assay and while *Zehneria scabra* gives a moderate antioxidant activity. The data can enrich the free radical scavenging activities of the study plant materials.

5. Conflict of interest statement

We declare that we have no conflict of interest.

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