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Comparative studies on phytochemical screening and antioxidant activity of *Zehneria scabra* (Linn. F) Soud and *Zehneria maysorensis* (Wight & Arn.) Leaves

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Abstract

The present study was carried out to evaluate antioxidant activity of petroleum ether, methanol and water extracts of *Zehneria scabra* and *Zehneria maysorensis*. The preliminary phytochemical tests showed the presence of alkaloid, tannin, saponins, phenol, flavonol glycosides, cardiac glycosides, phytosterol, fixed oils and fats, gums and mucilage. A dose dependent antioxidant activity was observed in case of extracts. Phenolics (36.6 g gallic acid equivalents (GAE)/100 g extract), tannin (21.6 g GAE/100 g extract), and flavonoid content (67.5 g rutin equivalents/100g extract) were found to be highest in acetone extract. *Zehneria scabra* and *Zehneria maysorensis* both methanol extract showed maximum antioxidant activity in DPPH (21.6 and 40.87 µg/mL), ABTS cation radical scavenging activity (66923.6 and 71958.3µmol TE/g extract). Hence, Zehneria can be a valuable source for antioxidant and seemed to be applicable in medicine.

Keywords: *Zehneria scabra*, *Zehneria maysorensis*, Phytochemical, Antioxidant.

1. Introduction

Since prehistoric times, humans have used natural products such as plants, animals, microorganisms and marine organisms in medicines to alleviate and treat diseases. According to fossil records, the human use of plants as medicines may be traced back at least 60,000 years [1]. India is well-known for the use of medicinal plants as a folklore medicine from ancient times. At present many compounds have been isolated from plants for treating numerous diseases. Medicinal plant research has succeeded in overwhelming the problems associated with synthetic drugs in maintaining low toxicity and less side effects [2]. Oxidation in living organisms is essential for the generation of energy during catabolism but these metabolic processes result in the continuous production of free radicals and reactive oxygen species (ROS) *in vivo*. Free radicals or more generally ROS are highly reactive species that are generated by cells during respiration and cell-mediated immune functions [3]. Free radicals are also generated through environmental pollutants, cigarette smoke, automobile exhaust, radiation and pesticides [4]. However, synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis [5]. For this reason, interest in the use of natural antioxidants has increased. Plants constitute an important source of active natural products, which differ widely in terms of structure, biological properties and mechanism of actions. Various phytochemical components are known to be responsible for antioxidant, antimicrobial and anti-inflammatory activities of plants [6].

2. Materials and Methods

2.1 Materials

The fresh plant materials of *Zehneria scabra* was collected during the month of September, 2017 from Ooty, The Nilgiris District, Tamil Nadu, India. The *Zehneria maysorensis* was collected during the month of September, 2017 from Mukkali, The Palakad District, Kerala, India. The taxonomic identities of the plant were confirmed by the Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu.

Plate 1: *Zehneria scabra*

Plate 2: *Z. maysorensis*

The plant materials were washed under running tap water to remove the surface pollutants and the leaves were separated mechanically. The leaves were air dried under shade. The dried samples were powdered and used for further studies.

2.2. Extraction of plant material

Fifty grams of dried powder of the samples were mixed with 250 ml of each solvent (Petroleum ether, Methanol and Hot water). The mixture was stirred in a shaker (LABTRON Ls-100) at 3000 rpm at room temperature for 48 hr. The extract was filtered and solvents were evaporated using a rotary evaporator at 50ºC. The extracts were stored at -20ºC until further analysis [7].

2.3. Extract recovery percentage

The amount of crude extract recovered after successive extraction was weighed and the percentage of yield was calculated by the following formula

$$\text{Extract recovery percent} = \frac{\text{Mount of extract recovered (g)}}{\text{Amount of plant sample (g)}} \times 100$$

2.4. Qualitative phytochemical screening

The powder extracts of *Z. scabra* and *Z. maysorensis* were analyzed for the presence of major phytochemicals such as carbohydrates, proteins, amino acids, alkaloids, saponins, phenolic compounds, tannins, flavonoids, glycosides, flavanol glycosides, cardiac glycosides, phytosterols, fixed oils and fats and gums and mucilages according to standard methods such as Hager's test, Frothing test, Borntrager's test, Keller Killiani test, Libermann and Burchard's test, saponification test, etc. [8].

2.5. Quantification Assays of secondary metabolites

2.5.1. Quantification of total phenolics

The total phenolics of the different plant extracts were determined according to the method described by [9]. 100 µL of different plant extracts were taken into a series of test tubes and made up to 1 mL with distilled water. A test tube with 1 mL of distilled water served as blank. Then, 500 µL of Folin – Ciocalteau reagent (1 N) was added to all the test tubes including blank. After 5 minutes, 2.5 mL of sodium carbonate solution (20%) was added to all the test tubes. The test tubes were vortexed well to mix the contents and incubated in dark for 40 minutes. The formation of blue colour in the incubated test tubes indicated the presence of phenolics. Soon after incubation the absorbance was read at 725 nm against the reagent blank. Gallic Acid standard was also prepared and the results were expressed as Gallic Acid Equivalents (GAE). The analyses were performed in triplicates.

2.5.2. Quantification of tannins

The total phenolics contain both tannin and non-tannin phenolics. The amount of total tannins was calculated by subtracting the total non-tannin phenolics from total phenolics[9]. For the determination of total non-tannin phenolics 500 µL of extracts were incubated with 100 mg of polyvinyl polypyrrolidone (PVPP) and 500 µL of distilled water in a 2 mL eppendorf tube for 4 hours at 4º C. After incubation the eppendorf tubes were centrifuged at 4000 rpm for 10 minutes at 4º C. The supernatant contains only the non- tannin phenolics since the tannins would have been precipitated along with PVPP. The supernatant was collected and the non-tannin phenolics were determined by the same method described for the quantification of total phenolics. The analyses were also performed in triplicates and the results were expressed in Gallic Acid Equivalents (GAE). From these two results, the tannin content of the plant samples were calculated as follows,

Tannins = Total phenolics- Non tannin phenolics

2.5.3. Quantification of total flavonoids

The flavonoid contents of all the extracts were quantified according to the method described by [10]. About 500 µL of all the plant extracts were taken in different test tubes and 2 mL of distilled water was added to each test tube. A test tube containing 2.5 mL of distilled water served as blank. Then, 150 µL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 minutes. After incubation, 150 µL of 10%

$AlCl_3$ was added to all the test tubes including the blank. All the test tubes were incubated for 6 minutes at room temperature. Then 2 mL of 4% NaOH was added to all the test tubes which were then made up to 5 mL using distilled water. The contents in all the test tubes were vortexes well and they were allowed to stand for 15 minutes at room temperature. The pink colour developed due to the presence of flavonoids was measured spectrophotometrically at 510 nm. Rutin was used as the standard for the quantification of flavonoids. All the experiments were done in triplicates and the results were expressed in Rutin Equivalents (RE).

2.6. *In vitro* Antioxidant Assays

2.6.1. DPPH scavenging activity

The antioxidant activity of the extract was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH**,** according to the method [11]. Sample extracts at various concentrations were taken and the volume was adjusted to 100 µL with methanol. About 3 mL of a 0.004 % methanolic solution of DPPH was added to the aliquots of both samples and standards (BHT and Rutin) and shaken vigorously. Negative control was prepared by adding 100 µL of methanol in 3 mL of methanolic DPPH solution. The tubes were allowed to stand for 30 minutes at 27°C. The absorbance of the samples and control were measured at 517 nm against the methanol blank. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH concentration.

2.6.2. $ABTS^{\bullet+}$ scavenging activity

The total antioxidant activity of the samples was measured by ABTS radical cationde- colorization assay according to the method [12]. $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark for 12–16 hours at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89 v/v) and equilibrated at 25°C to give an absorbance of 0.700 ± 0.02 at 734 nm. About 1 mL of diluted ABTS solution was added to about 30 µL sample solution and 10 µL of Trolox (final concentration 0–15 µM) in ethanol. A test tube containing 1 mL of diluted ABTS solution and 30 µL of ethanol served as the negative control. All the test tubes were vortexed well and incubated exactly for 30 minutes at room temperature. After incubation, the absorbance of samples and standards (BHT and Rutin) were measured at 734 nm against the ethanol blank. The results were expressed as the concentration of Trolox having equivalent antioxidant activity expressed as µg/ g sample extracts.

2.6. Statistical analyses

All the experiments were done in triplicates and the results were expressed as Mean ± SD.

3. Results and Discussion

3.1. Extract Recovery Percentage

The percentage yield of *Z. scabra* and *Z. maysorensis* leaf extracts from different solvents are presented in Table 1. The maximum yield was obtained from hot water extract of both the samples of the present study followed by methanol and petroleum ether extracts. These results showed that the high polar solvents could dissolve more constituents.

Table 1: Percentage yield of *Z. scabra* and *Z. maysorensis* leaf extracts

S. No.	Solvents	Yield Percentage	
		Z. scabra	*Z. maysorensis*
1	Petroleum ether	0.53	0.92
2	Methanol	1.42	1.52
3	Hot water	**7.26**	**6.02**

3.2 Qualitative Phytochemical Screening

The qualitative phytochemical screening of leaves of *Z. scabra* and *Z. maysorensis* for major primary and secondary phytochemicals are shown in Table 2.The results revealed that the primary metabolites such as carbohydrates and protein are present in both the samples. The results of the qualitative phytochemical screening showed the presence of all the phytochemical constitution such as alkaloid, tannin, saponins, phenol, flavonol glycosides, cardiac glycosides, phytosterol, fixed oils and fats, gums and mucilages in both the samples. Alkaloid, tannin, saponins and phytosterol are found to be in high concentration which was indicated by the high intensity of the colour developed.

Table 2: Phytochemical screening of *Z. scabra* and *Z. maysorensis* leaf

Phytochemicals	Leaf	
	Z. scabra	*Z. maysorensis*
Carbohydrate	+++	+++
Protein	++	++
Alkaloid	+++	+++
Tannin	+++	+++
Saponins	+++	+++
Phenol	++	++
Flavonol glycosides	++	++
Cardiac glycosides	+	++
Phytosterol	+++	+++
Fixed oils and fats	++	++
Gum and mucilage	++	++

(+): Presence of chemical compound (+) < (++) < (+++):
Based on the intensity of characteristic colour

3.3. Quantitative Analysis

3.3.1 Determination of total phenolics and tannin content

The amount of total phenolics in leaf extracts of *Z. scabra* and *Z. maysorensis* were analyzed and the results were shown in Table 3. The total phenolics were found to be higher in methanol extract of both leaf samples (207.27 and 89.09 mg GAE / g extract of *Z. Scabra* and *Z. maysorensis*, respectively). Among the two samples of the study, *Z. scabra* leaf showed the presence of high phenolics contents.

The tannins were found to be higher in both methanol extract of *Z. scabra (*180.76 mg GAE/g extract) and *Z. maysorensis* (77.63 mg GAE/g extract). Among the two samples of the study, *Z. Scabra* leaf showed the presence of high tannin contents.

Table 3: Phenolics and Tannins contents of *Z. scabra* and *Z. maysorensis* leaf extracts

Samples	Extracts	Total Phenolics (GAE g/50 g extract)	Tannins (GAE g/50 g extract)
Z. scabra	Petroleum ether	63.3 ± 11.37	42.38±12.83
	Methanol	**207.2±3.03**	**180.76±3.74**
	Hot water	177.4 ± 6.12	148.64± 8.42
Z. maysorensis	Petroleum ether	72.93±4.87	52.28±1.82
	Methanol	**89.0±6.60**	**77.63 ± 8.33**
	Hot water	62.5±8.34	23.39±11.51

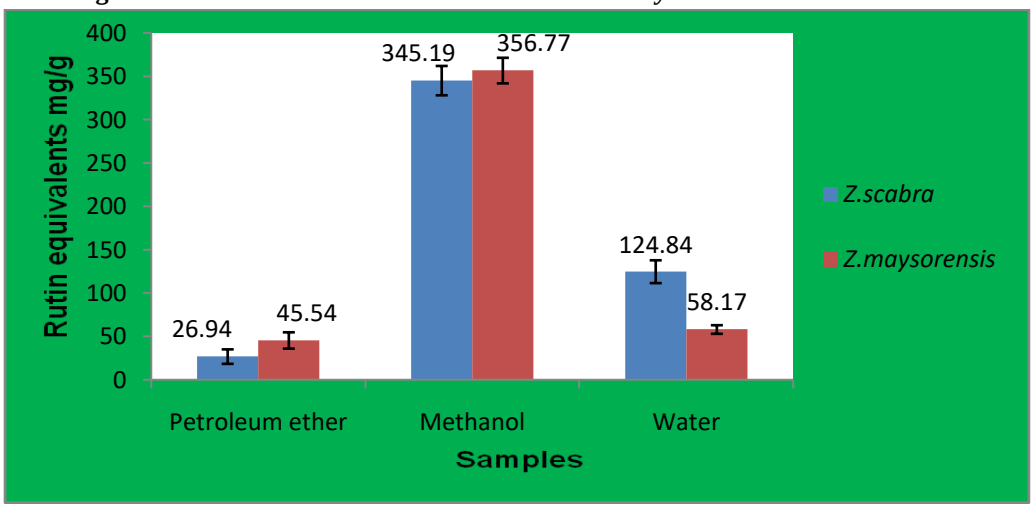
Values are mean of triplicate determinations (n=3) ± standard deviation

In most of the research reports, the total phenolics have been determined to be the main antioxidative compounds [13]. Natural tannins act as powerful antioxidant agents due to the presence of higher number of hydroxyl groups, especially many ortho-di-hydroxy or galloyl groups [14].

3.3.2. Quantification of flavonoids

The flavonoid contents in leaves of *Z. scabra* and *Z. maysorensis* were analyzed and were presented in Fig 1. The methanol extracts were found tocontain appreciable levels of flavonoid in *Z. scabra* (345.1 mg RE/g extract) and *Z. Maysorensis* leaf (356.7 mg RE / g extract).When compared with the other extracts.

Figure: 1 Flavonoid contents of *Z. scabra* and *Z. maysorensis* leaf extracts



Values are mean of triplicate determination (n=3) ± standard deviation

Flavonoids have free radical scavenging and antioxidation properties, which are useful for their pharmacological activities including anti-inflammatory and anticancer properties [15].

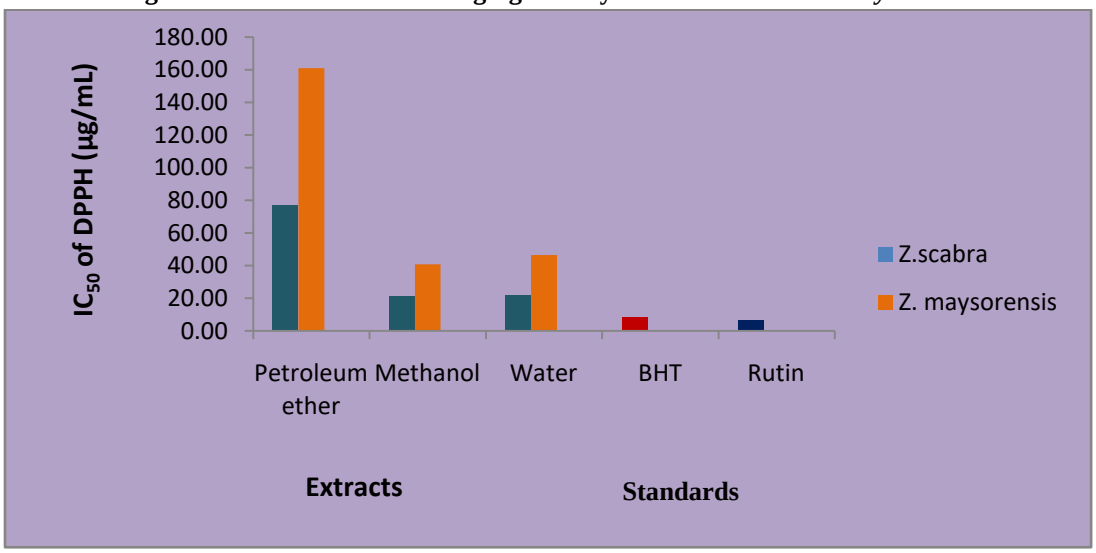
Moreover, the flavonoids are responsible for many pharmacological properties including antibacterial and larvicidal properties. Petroleum ether extract of this plant is also a potent source of natural mosquito larvicidal agent [16]. But, the present study showed the presence of high content of flavonoid in methanolic extract of leaves.

3.4 *In vitro* Antioxidant Assays

3.4.1. DPPH radical scavenging activity

The DPPH radical scavenging activities of *Z. Scabra* and *Z. maysorensis* are shown in Fig 2. In this assay, the commercial antioxidants BHT and rutin were used as standards. Concentration of the sample necessary to decrease initial concentration of DPPH by 50% (IC_{50}) under the experimental condition was determined. Among the extracts analyzed, both the methanol extract of *Z. Scabra* (21.08µg/ml) and *Z. Maysorensis* (40.87 µg/ml) showed better IC_{50} values for DPPH radical scavenging activities compared to other solvent extracts. The IC_{50} value of petroleum ether extracts showed that it has very less free radical scavenging activity. The IC_{50} value of standard natural antioxidant Rutin and synthetic antioxidant BHT was found to be 6.35 and 7.93 µg/ml, respectively.

Figure 2: DPPH radical scavenging activity of *Z. Scabra* and *Z. maysorensis*



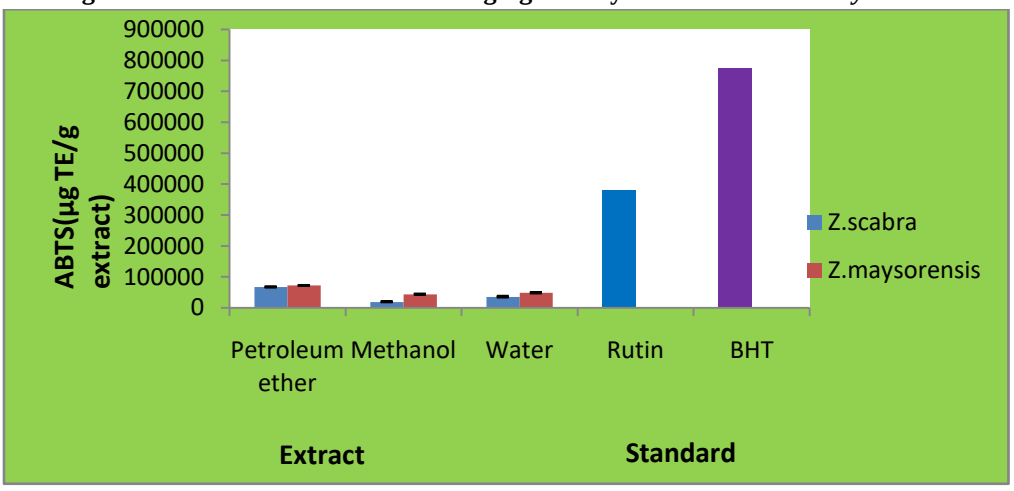
The high molecular weight phenolic compounds like tannins have more ability to scavenge the free radicals. The results of DPPH and tannin contents can be correlated where better results were obtained in methanol extract. The significant activity of methanol extract of leaf may be due to the presence of phenolic compounds in the plant parts [17]. On the other hand, the DPPH radical scavenging efficiency of extracts from *Z. Scabra* and *Z. maysorensis* might have also been partly attributed to Millard reaction products other than phenolic constituents because they also effectively participate as radical scavengers [18].

The higher apparent antioxidant capacity of smaller molecules due to their better access to the DPPH radical site suggests that the extracts may contain smaller compounds than larger compounds [19].

3.4.2. ABTS cation radical scavenging activity

The TEAC (Trolox Equivalents Antioxidant Capacity) was measured using the improved ABTS^{+} radical decolourisation assay; one of the most commonly employed methods for antioxidant capacity, which measures the ability of a compound to scavenge ABTS cation radical. The results were expressed as µg Trolox Equivalents/g of extract. The results of ABTS cation radical scavenging activities of both leaf extracts of *Z. scabra* and *Z. maysorensis* are shown in Fig 3. The petroleum ether extracts of both leaf showed higher radical scavenging activities when compared with methanol and water extracts. The ABTS cation radical scavenging of standard natural antioxidant Rutin and synthetic antioxidant BHT was found to be very high. The total antioxidant capacity of standards and leaf extracts are in the order of BHT >Rutin> petroleum ether > Hot water> methanol extract.

Figure 3: ABTS cation radical scavenging activity *Z. scabra* and *Z. maysorensis*



Moreover, the radical is suitable for evaluating antioxidant capacity of phenolics due to their comparatively lower redox potentials. Many phenolic compounds can thus react with the ABTS cation radical because of this thermodynamic property [20]. The ABTS assay indicated that the extracts of *Z. Scabra* and *Z. Maysoensis* possess strong hydrogen donating ability and could serve as free radical scavengers by acting as primary antioxidants when they are ingested along with nutrients.

4. Conclusion

The present study revealed that the leaf extract of *Z. scabra* and *Z. maysorensis* showed significant antioxidant properties. The extracts are found to have different levels of activity in all tests. It was found that the methanol extract of *Z. scabra* leaf could become a promising source of antioxidant as revealed by various *in vitro* models. The present study demonstrates the usage of the plant as dietary supplements and as therapeutic agent. However, further studies are warranted for much higher levels of compound isolation and for providing a promising herbal medicine.

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