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In vitro antioxidant activity and total phenolic content of leaf extracts of *Limonia crenulata* (Roxb.)

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ABSTRACT

This study was conducted to investigate the antioxidant effect of the methanol, ethanol and aqueous extracts of the leaves of Limonia crenulata (Roxb.). The antioxidant activity was evaluated by 1, 1-diphenyl-2-picrylhydrazyl (DPPH) method. The antioxidant activity was compared to standard antioxidants ascorbic acid and selenium. The ethanolic crude extracts showed very promising antioxidant activity compared with methanolic and distilled water extracts. The ethanol extract had the highest total phenolic content followed by methanol and distilled water extract. The findings of the present study suggested that Limonia crenulata (Roxb.) could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

Key Words: *Limonia crenulata* (Roxb.), DPPH method, Antioxidant, Oxidative stress.

INTRODUCTION

Antioxidant means “Against oxidation” antioxidants work to protect lipids from peroxidation by free radicals. Antioxidants are effective because, they are willing to give up their own electrons to free radicals.

When a free radical gains the electron from an antioxidant it no longer needs to attack the cell and the chain reaction of oxidation is broken. After donating an electron an antioxidant becomes a free radical by definition. Antioxidants in this state are not harmful because they have the ability to accommodate the change in electrons without becoming reactive. The human body has an elaborate antioxidant defense system. Antioxidants are manufactured within the body and can also be extracted from the food humans eat such as fruits, vegetables, seeds, nuts, meats, and oil. There are two lines of antioxidant defense within the cell. The first line, found in the fat soluble

cellular membrane consists of vitamin E, β – carotene, and Co – enzyme Q of these, vitamin E is considered the most potent chain breaking antioxidant within the membrane of the cell. Inside the cell water soluble antioxidant scavengers are present [1].

Antioxidants are recognized for their potential in promoting health and lowering the risk for cancer, hypertension and heart disease [2, 3]. The uses of natural antioxidants from plant extracts have experience growing interest due to some human health professionals and consumer's concern about the safety of synthetic antioxidants in foods [4, 5].

The antioxidants may be enzymatic or non – enzymatic, super oxide dismutase, glutathione peroxidases, catalase and peroxidases are some examples which come under enzymatically potential antioxidants. In the non-enzymatic category some of the known and documented antioxidants are vitamin C, vitamin E, vitamin A, carotenoids, uricacid, ubiquinone and synthetic compounds like melatonin, Dihydro eplandrosterone (DHEA) etc. [6]. The antioxidant activity has been described for several triterpenes among other related compounds [7].

Limonia crenulata (Roxb.) is an Indian medicinal plant. All parts of the tree are medicinally useful. Literature in Indian traditional medical systems like Ayureda, Siddha and Unani were prescribed this as an Indian folk medicine which has much potential information on its therapeutic uses. In Ayurveda *Limonia crenulata* (Roxb.) is used as a folk medicine for renitent fever, puerperal fever, lightening of skin, diarrhoea, ulcer, inflammation, skin irritation, dyspepsia, diabetes and many other diseases. Hence in the present investigation to evaluate the in-*vitro* antioxidant activity of ethanol, methanol and aqueous leaf extracts of *Limonia crenulata* (Roxb.) by DPPH method.

MATERIALS AND METHODS

Collection of plant materials

The selected medicinal plant was *Limonia crenulata* (Roxb.) was collected from Keezanatham, Ariyalur (Dt.), Tamilnadu, India. Collected plant leaves were carefully examined and identified with the help of regional Floras [8, 9, 10]. Specimen was further confirmed with reference to Herbarium sheets available in the Rabinat Herbarium, St. Joseph's College, Thiruchirappalli, Tamilnadu, India.

Preparation of powder [11]

The disease free plant leaves were collected and dried under shade. These dried leaves were mechanically powdered, sieved using 80 meshes and stored in an airtight container. These powdered materials were used for further investigation.

Preparation of extracts

Plant extracts were prepared according to the methodology of Indian Pharmacopoeia [12]. The shade dried plants leaves were allowed to pulverization to get coarse powder. The coarse powder material was subjected to Soxhlet extraction separately and successively with methanol and ethanol extracts. The aqueous extraction achieved through the percolation method. These extracts were concentrated to dryness in flash evaporator under reduced pressure and controlled temperature (40-50ºC).

Antioxidant activity of *Limonia crenulata* (Roxb.) by DPPH method

The antioxidant activities of all extracts were evaluated through free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the method proposed by Akowuah *et al*. [13]. Two ml of 0.1mM DPPH methanolic solution was added into 200 µl of sample extracts and 0.8 ml methanol. The mixture was thoroughly mixed and kept in the dark for 1 hr. The control was prepared by mixing 2 ml of DPPH and 1 ml methanol. The absorbance was measure at 517 nm using spectrophotometer. Samples were measured in three replicates. Percentage of DPPH scavenging activity was calculated as % inhibition of DPPH = [Abs control –Abs sample / Abs control] x 100.

Estimation of Total Phenolic content

Total phenolic contents of all plants extracts were determined using Folin-Ciocalteu reagent as described by Singlaton and Rossi [14]. Samples were inserted into different test tube and mixed thoroughly with 5 ml Folin-Ciocalteu reagent (previously pre-dilute 10 times with distilled water). After 5 mins, 4 ml of 7.5% sodium carbonate (Na_2CO_3) was added and allowed to react for 2 hrs at room temperature. The absorbance was measure at 765 nm using spectrophotometer. Samples were measured in three replicates. Standard curve of gallic acid solution (10, 20, 40, 60, 80 and 100 ppm) was prepared using the similar procedure. The results were expressed as mg GAE/100 g extract sample.

RESULTS

Antioxidant activity of *Limonia crenulata* (Roxb.)

Many aromatic plants have been known to support various biological activities such as antimicrobial and antioxidant properties. The radical scavenging effects (percentage of quenched radicals) were determined for *Limonia crenulata* (Roxb.) leaf extracts. The leaf extracts or their constituents when mixed with DPPH decolorized due to hydrogen donating ability. All the tested samples (ethanol, methanol and aqueous extracts) revealed scavenging effects on DPPH (70 to 83%) as shown in Fig. I.

The ethanol extract of *Limonia crenulata* (Roxb.) was found to act as strong free radical scavengers (83.69%) in comparison with commercial antioxidants ascorbic acid and selenium as indicated by DPPH assays (Table I).

Table I. Antioxidant activity of *Limonia crenulata* (Roxb.)

Leaf extracts (µl)	% DPPH of inhibition				
	Leaf extracts			Standard	
	Ethanol	Methanol	Distilled Water	Ascorbic acid	Selenium
200	83.69 ± 0.7	77.89 ± 0.8	70.59 ± 2.7	66±1.0	64±1.0

Values are mean (n=3) ±standard deviation

Total phenolic content

Total phenolic contents of plants extract were tested using the diluted Folin-Ciocalteu reagent. Table II showed total phenolic content of plants extracts. Result clearly showed that ethanol extract had the highest total phenolic content followed by methanol and distilled water extract which mean value of 165.34 mg GAE/100 g extract, 104.7 mgGAE/100 g extract and 103.3 mg GAE/100 g extract, respectively.

Fig. I. Antioxidant activity of *Limonia crenulata* (Roxb.)

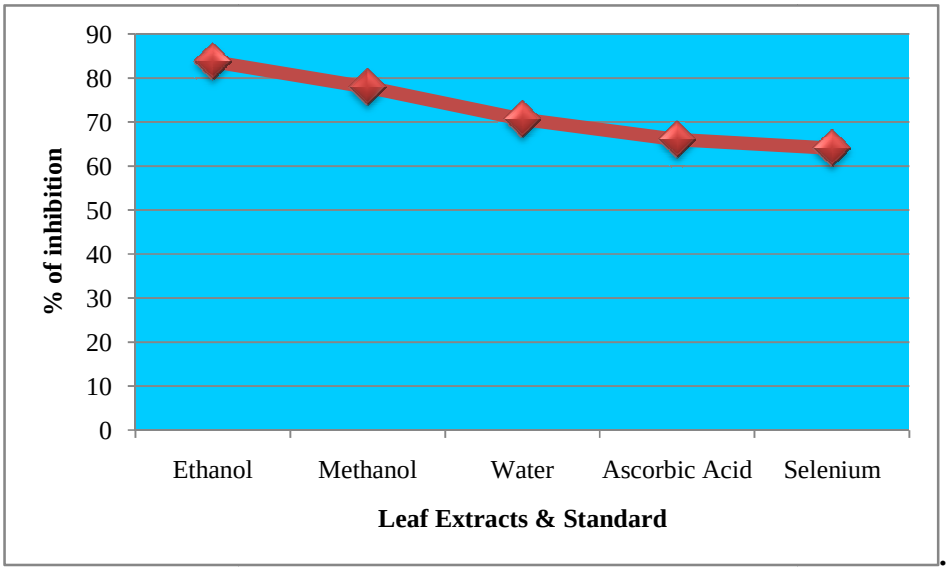

Table II. Mean ± SD of total phenolic content of leaf extracts

S. No	Leaf extracts	Total phenolic (mg GAE/100g extracts)
1	Ethanol	165.3 ± 1.0
2	Methanol	104.7 ± 1.2
3	Distilled Water	103.3 ± 1.1

DISCUSSION

Antioxidants are believed to neutralize the free radicals in lipid chains by contributing a hydrogen atom usually from a phenolic hydroxyl group, which in turn converts phenolic groups into stable free radicals that do not initiate or propagate further oxidation of lipids.

The ethyl acetate extract of *Pereskia grandifolia* (Haw.) (Cactaceae), played a considerable role in antioxidant activity by DPPH method. This is the first report on the antioxidant activities on leaves of *P. grandifolia* [15].

However, hydro ethanolic extracts of *Agle marmelos* and *Trigonella foenum* was found to be more active in DPPH scavenging ability in comparison to the methanol and aqueous extract which contains higher levels of phenols [16]. Recently Attarde *et al*. [17] reported that petroleum ether, chloroform and methanolic extract of leaves of *Limonia acidissima* L. have potential antioxidant ability using DPPH method.

The present study to evaluate the *in vitro* antioxidant activity of ethanol, methanol and distilled water leaf extracts of *Limonia crenulata* (Roxb.) by DPPH method. The DPPH radical scavenging activity of ethanol extracts showed a challenging result (83.69%), methanol and distilled water extracts were found to be 77.89% and 70.59% respectively.

Similar work was done by Sathishkumar *et al.* [18] that the ethanol and acetone extracts of *Polyalthia longifolia* leaves showed significant DPPH radical scavenging activity were found to be 87.92% and 92.84% respectively. The ethanol extract had the highest total phenolic content followed by methanol and water extract.

In this study, it seemed that, the higher total phenolic content of plants extracts resulted in higher antioxidant activity as similarly reported by Cai *et al*. [19]; Shan *et al*., [20] and Wong *et al*. [21].

CONCLUSION

The findings of the present study suggested that *Limonia crenulata* (Roxb.) could be a potential natural source of antioxidants and could have greater importance as therapeutic agent in preventing or slowing oxidative stress related degenerative diseases.

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