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J. Nat. Prod. Plant Resour., 2013, 3 (4):24-28
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Evaluation of phenolic content and antioxidant activity of *Aegle marmelos* (L.) *Corr. Serr.*

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ABSTRACT

Aegle marmelos (L.) Corr.Serr. (Rutaceae) contains many important phytoconstituents in the various parts of it and they are also responsible for some important biological activity like Aegle marmelos. The antioxidant capacity of Aegle marmelos leaves were assayed for their scavenging abilities against superoxide anion radicals, hydroxyl radical, nitric oxide radicals , hydrogen peroxide, metal chelation and reducing power. All the extracts inhibited all above said free radicals in a dose dependent manner. The high content of phenolic compounds indicated that these compounds contribute to the antioxidant activity. The Aegle Marmelos can be regarded as promising candidates for natural plant sources of antioxidants with high value. The finding indicated promising antioxidant activity of crude extracts of the above plants and needs further exploration for their effective use in both modern and traditional system of medicines.

Key words: *Aegle marmelos* (L.) Corr.Serr. antioxidant activity, solvents

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INTRODUCTION

Medicinal plants constitute the major constituents of most indigenous medicines and a large number of Western medical preparations contain one or more ingredients of plant origin. Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants (Halliwell, 1995).

Antioxidants interfere with the oxidative processes by scavenging free radicals, chealating free catalytic metals and by acting as Electron donors (Gulcin *et al.,* 2005). The natural antioxidant mechanisms may be insufficient in variety of conditions and hance dietary intake of antioxidant in of antioxidant compounds are important (Terao *et al.,* 1994). The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse relationship between dietary intake of antioxidant rich foods and incidence of human diseases (Yildrim *et al.,* 2001). Plant based antioxidants are preferred to the synthetic ones because of their multiple mechanisms of actions and non-toxic nature. These facts have inspired widespread screening of plants for possible medicinal and antioxidant properties. The isolation and characterization of diverse

phytochemicals and the utilization to antioxidants of natural origin to prevent the diseases (Akinmoladun *et al.,* 2007).

Free radicals and reactive oxygen metabolites can react with proteins, nucleic acids and lipids, causing changes in genetic material and inactivation of enzymes (Valko *et al.*, 2007).Therefore the human health depends on the efficiency of antioxidant mechanisms. Because of detrimental influence of peroxides and oxygen radicals on organisms there is growing interest in natural antioxidants, especially in polyphenols (Borowska*et al*., 2003). Lower level of those compounds was observed in samples collected from smokers, elders and patients with some disorders, as well as among people subjected to health threatening agents (Michota-Katulska *et al.,* 2000). Quite efficient scavengers of free radicals are also some vitamins, such as ascorbic acid tocophenols, carotenoids and retinol (Sroka *et al.,* 2005). Antioxidants are present in fruits, vegetables, cereals, leguminous plants, juices, wine, tea and many herbs. Herbaceous plants, besides improving taste of dishes, are the rich source of antioxidants which are more active than those from fruits and vegetables. Recently, the correlation between antioxidant activity and level of polyphenols, betalains or carotenoids in plants was proved (Mikolajczyk and Czapski, 2006). In past decades the growing consumption of synthetic medicines and therapeutic preparations, both prescribed by doctors and available without prescription has been observed simultaneously to the increased amounts and frequency of their use, the number of documented adverse or even toxic effects raised and caused the consumer confidence fall despite the board information campaigns, supported by results of pharmacologic researchers as well as aggressive advertising, the interest in synthetic medicines offered by chemical industry is still decreasing. All above mentioned causes that natural therapeutics and plant preparations are gaining popularity at the moment about 35% medicines are of plant origin and they are recognized as equal to their chemical equivalents very convincing argument for natural preparations is their low price and ability of long-term use with quite good therapeutic efficiency and with no side effects (Bremness, 1991; Schulz and Uberhuber, 1986).

MATERIALS AND METHODS

Plant collection

Aegle marmelos (L.) Corr.Serr. belonging to family Fabaceae has been selected for the study. The plant materials were collected from Thanjavur District, Tamil Nadu, India.

Sterilization of plant materials

The disease free and fresh plant were selected for this investigation. About 2g of fresh and healthy leaves were taken for each solvent extraction. These are washed with tap and distilled water for three times. Then, surface sterilized with 0.1% mercuric chloride or alcohol for few seconds. Then it was washed with distilled water.

Preparation of extracts

About two grams of sterilized plant leaves were kept in the 10ml organic solvents such as ethanol, ethyl acetate and distilled water. Then these are ground with the help of mortar and pestle. The ground plant material was subjected to centrifugation, for 10-15 minutes (at 10000 rpm). Again, it was filtered through whatmann's No.1 filter paper. The supernatant was collected and stored for further antioxidant screening purposes.

Antioxidant activity

Reagents

- DPPH solution
- Ethanol
- Ethyl acetate

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect of the stable DPPH (1, 1-diphenyl -2-picryl-hydrazyl) free radical activity. Ethanol, ethyl acetate and distilled water was added to 0.1mM of DPPH solution with different concentrations of (5, 10 and 15mg/ml) plant extract was freshly prepared and kept in the dark at 4^{o}C. The mixture was left to stand for 5 minutes and absorbance was measured spectrophotometrically at 517 nm. Ethanol, ethyl acetate and distilled water were used to get the absorbance zero. A blank sample containing the distilled water and DPPH was also prepared. All determinations were performed. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

Percentage of inhibition of DPPH activity = 1-(A1/A2) x 100

Where;

A1 = was the absorbance of the test sample

A2 = was the absorbance of the control

The control and standard were subjected to same procedures as the sample, except that the control, only the solvent was added to the standard, sample was replaced with same amount of ascorbic acid, and selenium.

Determination of total phenolic contents

Total phenolic contents in the ethanol, ethyl acetate and distilled water were determined using folin-ciocalteu's method, total polyphenol content was estimated using Folin-ciocalteu's assay developed by veliglu, mazza, Gao, and Oomab with slight modifications.

Reagents

- Folin – ciocalteu's phenol reagent
- 20% sodium carbonate
- Gallic acid

Procedure

One ml of the extract was added to 10ml distilled water and 2ml of Folin – ciocalteu's phenol reagent. The mixture was allowed to stand at room temperature for 5 min and then 2ml of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. The mixture was homogenized and allowed to stand at room temperature for 30 min. gallic acid used as a standard for calibration curve.

RESULTS AND DISCUSSION

The antioxidant activity of different concentration (5, 10 and 15mg/ml) ethanol, ethyl acetate and distilled water of *Aegle marmelos* (L.) Corr.Serr. was determined by DPPH method. The scavenging effects on DPPH radical were determined. Measuring the decay in absorbance at 517nm due to the DPPH radical reduction, indicating the antioxidant activity of the *Aegle marmelos* in a short time. The amounts of total phenolic content were also determined by Folin-ciocalteu's method.

***Antioxidant activity of Aegle marmelos* (L.) Corr.Serr.**

The modal of scavenging the DPPH radical is widely used method to evaluate the free radical scavenging ability of different solvent extracts (Ethanol, Ethyl acetate and Distilled water) and standard such as selenium and ascorbic acid on the DPPH radical which increase with increasing concentration from 5-15mg/ml.

Zahin *et al.*, (2009) reported that the DPPH free radical scavenging ability, the methanolic extract of *H. indicus* and *P. zeylanica* al 100µg/ml showed strong radical scavenging activity with percentage decrease of 77.0% and 73.41% where as *A. calamus* and *H. antidysentrica* showed relatively poor free radical scavenging activity of 20.88% and 6.32% respectively. The order of scavenging activity was maximum in *H. indicus* followed by *P. zeylanica, A. calamus* and *H. antidysenterics*. The values are also comparable with commercial antioxidant L-ascorbic acid (90.0%) and butylated hydroxytoluene (83%) at the same concentration. This suggested that *H. indicus* and *P. zeylanica* contain compounds such as polyphenolics that can donate electron/hydrogen easily. In the present study the ethanol extract of *Aegle marmelos* is higher antioxidant activity values (46.08%, 50.56% and 54.32%) were observed at a different concentration of 5, 10 and 15mg/ml respectively. The scavenging effects on the ethyl acetate extract from *Aegle marmelos* were 40.18% in 5mg/ml, 43.56% in 10mg/ml and 47.24% in 15mg/ml respectively. The scavenging activity was found to be lower in distilled extract 28.51% at 5mg/ml, 30.18% at 10mg/ml and 36.75% at 15mg/ml. Among the three solvents ethanol having higher antioxidant (50.51%) activity than the other two solvents (Table 1).

Ahmed *et al.*, (2011) studied the qualitative DPPH test demonstrated that the water and MeOH extracts of the prangos species display low antioxidant activities. However, in the DPPH test, yellow zones on a purple background were prominent for the MeOH extracts of the fruits of *P. uechtritzii, P. heyniae* and *P. ferulacea.* The lowest inhibition zones were shown on the whole MeOH extracts of *P. meliocarpoides Var. meliocarpoides*. The water extracts of fruits of the *P. ferulacea* and *P. uechtritzii* gave faint yellow zones when compared with their roots and herbs. The highest DPPH scavenging effects, expressed as yellow zones of the root water extracts, in decreasing

order were determined as *P. heyniae* > *P. uechtritzii* > *P. ferulacea* > *P. meliocarpoids* Var. meliocarpoides. In our study the highest activity was observed in ethanol, ethyl acetate and distilled water extracts. The minimum activity was observed on ethyl acetate extract. *Aegle marmelos* leaves extract were compared to standard antioxidant selenium and ascorbic acid. Selenium and ascorbic acid to produced antioxidant than *Aegle marmelos.*

Akinmoladum *et al.,* (2007) suggested the scavenging activity of phenolic group is due to its hydrozyl group. The antioxidant activity has been reported to be concomitant with the development of reducing power. Herbal preparation revealed synergistic effects both in DPPH scavenging and reducing power in comparison with the individual plant extracts selected for the study. The crude extracts of plants are pharmacologically more active than their isolated active principles due to the synergistic effects of various components present in the whole extract. In the present study the amount of total phenolic contents of ethanol, ethyl acetate and distilled extract of *Aegle marmelos* was found 1.921, 1.745 and 1.510mg/g. the total phenolic compounds are major compounds responsible for antioxidant activity. The phenolic compounds may contribute directly to the anti oxidative action. Among the three solvents derivation of phenolic compounds was observed by the ethanol solvent (1.921mg/g) the lowest total phenolic compounds were observed by distilled water extract (1.510mg/g). The antioxidant properties of *Aegle marmelos* possibly attributed to the presence of phenolic compounds (Table 2).

Table 1: Antioxidant activity of *Aegle marmelos* (L.) Corr.Serr.

Concentration of leaves extract (mg/ml)	% of inhibition			Selenium %	Ascorbic acid %
	Ethanol	Ethyl acetate	Distilled water		
5	46.08	40.18	28.51	62.7	65.2
10	50.56	43.56	30.18	73.7	77.8
15	54.32	47.24	36.75	81.5	85.6

Table 2: Total phenolic content in *Aegle marmelos* (L.) Corr.Serr.

Extraction of solvents	Total phenolic contents (mg/g)
Ethanol	1.921
Ethyl acetate	1.745
Distilled water	1.510

CONCLUSION

Antioxidant properties of several herbaceous plants depending of the solvent used for extraction. The herbs selected for analysis are traditional plant with well known therapeutic activity. However, they were poorly characterized with respect of antioxidant properties or polyphenols content. Information on the most efficient way of antioxidant compounds extraction can be applied during production of herbal preparation as well as for. Supplementation of foods tuffin natural antioxidant (functional food).

Acknowledgement
The authors are grateful to the Managing Director, Sri Gowri Biotech Research Academy, Thanjavur (Dt), Tamilnadu for their permission to utilized the laboratory facility.

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