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EVALUATION OF ANTIOXIDANT ACTIVITY OF UNRIPE *AEGLE MARMELOS* CORR. FRUITS

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ABSTRACT

Introduction: *Aegle marmelos* Corr. (Rutaceae), commonly known as Bael, is a tree of Indian origin, well known from ancient period and prescribed for various ailments in Ayurveda. Utilization of bael fruit in day-to-day life has a great nutritional, environmental as well as commercial importance. Every part of *Aegle marmelos* including stem, bark, root, leaves, fruit and seeds at all stages of maturity posses medicinal virtues and has been used in Ethno medicine to exploit its medicinal properties. **Objective:** This study was undertaken to examine the antioxidant activity of methanolic extract of *Aegle marmelos* unripe or half ripe fruits. **Material and Methods:** The antioxidant activity was done by using DPPH free radical scavenging assay. The IC50 (The concentration of sample required to scavenge 50% of DPPH free radical) was calculated by plotting graph between % inhibition vs concentration. The ascorbic acid was used as standard antioxidant. **Result and Discussion:** The IC50 value of extract and ascorbic acid was found to be 62.59µg/ml and 2.80µg/ml. The antioxidant activity found in *Aegle marmelos* may be associated with their main phytochemical compounds like flavonoids, phenols and tannins. **Conclusion:** This activity supports that the fruit can be used as natural antioxidant to treat free radical induced cellular damages and can also be used as adjuvant with other drugs to give synergistic effects.

Keywords: Bael, DPPH, **A**scorbic acid, Free radicals, Phenols, Antioxidant

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INTRODUCTION

Plants have long provided mankind with a source of medicinal agents, with natural products once serving as the source of all drugs. [1] Dependence on plants as the source of medicine is prevalent in developing countries where traditional medicine plays a major role in health care.[2, 3] The rural population of a country is more disposed to traditional ways of treatment because of its easy availability and cheaper cost. Herbal therapy, although still an unwritten science, is well established in some cultures and traditions, and has become a way of life in almost 80% of the people in rural areas, especially those in Asia.[4] In order to promote the use of medicinal plants as potential sources of active compounds, it is pertinent to thoroughly explore their composition and activity and thus validate their use.[5] The effectiveness of phytochemicals in the treatment of various diseases may exist in their antioxidant effects.[6] Antioxidants are important in the prevention of human diseases. Antioxidant compounds may function as free radical scavengers, complexing agents for pro-oxidant metals, reducing agents and quenchers of singlet oxygen formation.[7,8,9] Antioxidants are often used in oils and fatty foods to retard their auto-oxidation; therefore, the importance of search for natural antioxidants has greatly increased in the recent years.[10]

In recent years, multiple drug/ chemical resistance in both human and plant pathogenic micro organism has been developed due to indiscriminate use of synthetic drugs. This drives the need to screen medicinal plants for novel bioactive compounds as they are biodegradable, safe and have fewer side effects. [11]

Aegle marmelos (L.) Corr. ex Roxb., locally known as 'bael', is indigenous to India, grows wild throughout the deciduous forests of central and southern parts. It is considered highly religious since it is used extensively to worship Gods specially for worshiping Lord Siva. It is a spiny, small or medium sized tree, having alternate, tri-foliate leaf, white scented flowers and bears berry (amphisaraca) type of fruits.[12,13] All parts of this tree, viz. root, leaf, trunk, fruit and seed are useful in several ailments like diabetes,[14] diarrhoea, cancer, ulcers, etc and is one such herbal source which is rich in bio active compounds having oxygen scavenging activity. The phytochemical screening of different extracts of fruit extract revealed that methanolic extract of fruit pulp contains maximum amount of functional and bioactive compounds such as carotenoids, phenolics, alkaloids, coumarins, flavonoids, terpenoids and other antioxidants which may protect us

against various chronic diseases. In addition, it also contains many vitamins and minerals including vitamin C, vitamin A, thiamine, riboflavin, niacin, calcium, and phosphorus.[15] Hence the present study was designed to evaluate antioxidant activity of *A. marmelos* and to compare the IC50 of *A. marmelos* with IC50 of Standard Antioxidant, Ascorbic acid.

MATERIALS AND METHODS

Plant material

The fruits were collected from the local market of Delhi. The drug was authenticated as *Aegle marmelos* (L.) Correa ex Roxb. by Dr. H. B. Singh (Taxonomist), National Institute of Science Communication and Information Resources, NISCAIR, New Delhi. The reference number is NISCAIR/RHMD/Consult/-2010-11 /15 09 / 107. A voucher specimen is preserved in the RHMD department of NISCAIR, New Delhi.

Chemicals and reagents

DPPH or 1, 1-diphenyl-2-picryl-hydrazyl (Sigma Aldrich), Ascorbic acid (Rankem RFCL Limited), TRIS [2-amino-2 (hydroxy methyl) propane 1-3di-ol] buffer (pH 7.4) (Qualigens Fine Chemicals) and Methanol (Rankem RFCL Limited).

Preparation of plant material

The pulp of half riped fruit of *Aegle marmelos* was chopped into pieces and dried in sun light. The completely dried fruits were powdered and 15 g of dried fruit powder was taken and extraction was carried out in Soxhlet apparatus using methanol as solvent for 4 hrs. The extract was concentrated to syrupy liquid by evaporating the solvent on water bath and stored at 4□C.

Preparation of reagents

Solution of 500µM DPPH was prepared by dissolving 23 mg of DPPH in 100 ml of methanol. TRIS [2-amino-2 (hydroxy methyl) propane 1-3di-ol] buffer (pH 7.4) was prepared by adding 0.605g of TRIS buffer in 30 ml of water and adding 0.33 ml of concentrated hydrochloric acid and diluted to 100 ml with distilled water. TRIS buffer prevents the sudden pH change during the preparation of test dilutions.[16,17]

Preparation of reference standard solution

Dilutions of standard antioxidant Ascorbic acid were prepared in concentrations of 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 2.8, 3.2, 3.6 & 4.0 µg/0.5 ml in methanol.

Preparation of sample solution and dilutions

Stock solution was prepared by dissolving 20mg of methanolic extract of *A. marmelos* in 10 ml methanol. Prepare the initial dilutions from stock solution using volume 0.02 ml (8µg / 5ml), 0.04 ml (16µg / 5ml), 0.06 ml (24µg / 5ml), 0.08 ml (32µg / 5ml), 0.10 ml (40µg / 5ml), 0.12 ml (48µg / 5ml), 0.14 ml (56µg / 5ml), 0.16 ml (64µg / 5ml), 0.18 ml (72µg / 5ml) and 0.20 ml (80µg / 5ml). The volume make up was done by methanol.[18] The final concentrations used for taking the UV absorbance were 8, 16, 24, 32, 40, 48, 56, 64, 72, and 80µg/ml.

Measurement of In Vitro Antioxidant Activity

Antioxidant activity of the methanolic extract of *Aegle marmelos* was determined by using a method based on the reduction of coloured methanolic solution of radical 1, 1-di phenyl-1-2-picryl hydrazyl (DPPH). The radical scavenging activity of tested sample was expressed as inhibition percentage. Ascorbic acid was used as reference standard.

In 5 ml volumetric flasks added 1 ml of DPPH solution, 0.5 ml of TRIS Buffer and 0.5 ml of final dilutions of different concentrations range prepared from *A. marmelos* methanolic extract stock solution and make up the volume to 5 ml with methanol. In same way, control dilutions of DPPH were prepared, replacing 0.5 ml of prepared drug dilutions with methanol. Absorbance of all the dilutions was taken after 30 minutes at λ max 517nm using methanol as blank.

Statistical Analysis

The percentage inhibition was calculated using:

Percent Inhibition = [(Ac - As)/Ac] x 100
Where,
AC is absorbance of control,
As is the absorbance of sample

IC_{50} value (a concentration at 50% inhibition) was determined from the curve between percentage inhibition and concentration. All determinations were done in triplicate and the IC_{50} value was calculated by using the equation of line.[18]

RESULTS AND DISCUSSION

The absorbance of sample (methanolic extract of *A. marmelos*) and standard (Ascorbic acid) were taken in triplicate. It was observed that with the increase in concentration of test and standard, there was a decrease in absorbance values. The respective absorbance and percentage inhibition of free radicals by test and standard dilutions was calculated with the above mentioned formula and presented in Table 1 for standard and Table 2 for test drug. The Methanolic extract of *A. marmelos* tested *in vitro* for antioxidant activity using DPPH free radical scavenging model showed appreciable scavenging activity as evidenced by IC_{50} values. Figure 1 depicted that the IC_{50} value of *A. marmelos* extract was 62.59, whereas standard antioxidant Ascorbic acid showed an IC_{50} of 2.08 µg as seen in Figure 2.

Table 1: Absorbance and percentage inhibition of methanolic solution of Ascorbic Acid (standard antioxidant)

Conc. (µg)	Absorbance	Percentage Inhibition
0.4	1.674 ± 0.012	16.75
0.8	1.542 ± 0.057	23.32
1.2	1.462 ± 0.033	27.29
1.6	1.368 ± 0.022	31.97
2.0	1.255 + 0.036	37.59
2.4	1.173 + 0.035	41.67
2.8	0.997 ± 0.063	50.42
3.2	0.832 ± 0.110	58.62
3.6	0.763 ± 0.102	62.05
4.0	0.692 ± 0.011	65.58

Table 2: Absorbance and percentage inhibition of methanolic extract of *A. marmelos.*

Conc. (µg)	Absorbance	Percentage Inhibition
8	1.825 ± 0.061	22.99
16	1.723 ± 0.102	27.29
24	1.622 ± 0.102	31.56
32	1.499 ± 0.199	36.75
40	1.422 ± 0.127	40.00
48	1.329 ± 0.169	43.92
56	1.275 ± 0.165	46.20
64	1.154 ± 0.194	51.30
72	1.106 ± 0.169	53.33
80	0.99 ± 0.193	58.22

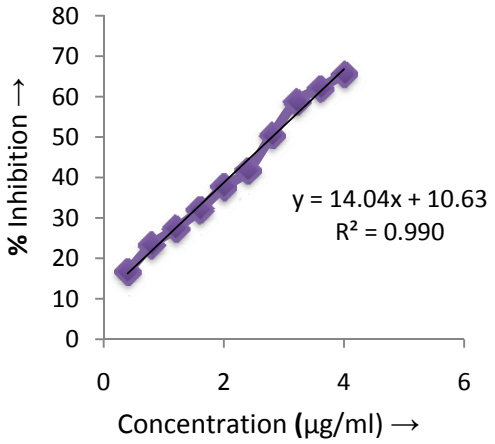

Figure 1: Free radical (DPPH) scavenging activity of methanolic solution of ascorbic acid (standard antioxidant)

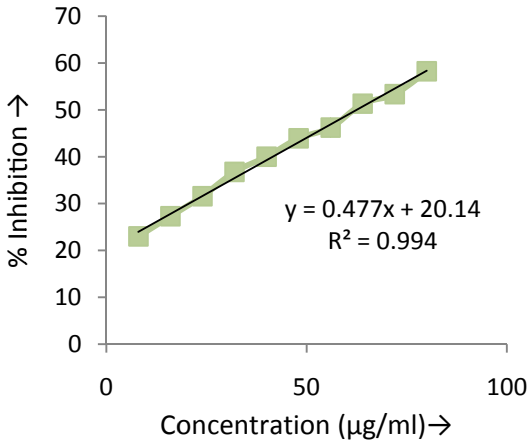

Figure 2: Free radical (DPPH) scavenging activity of methanolic extract of *A. marmelos* in *in-vitro* system.

CONCLUSION

This study is sufficient to conclude that the aqueous extract of *Aegle marmelos* is very active against the free radicals. The free radical scavenging and antioxidant activity found in *Aegle marmelos* may be associated with their main phytochemical compounds like flavonoids, phenols and tannins. This gives the support that the fruit can be used as the medicinal plant to treat free radical damages in cells.

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CONFLICT OF INTEREST

Authors have no conflict of interests.

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