

**ANTIOXIDANT PROPERTY OF DIFFERENT PHENOTYPIC TRAITS OF *AEGLE MARMELLOS*(L.) CORR. –VILVAM****V.N. ARIHARAN^{*1}, K.KALIRAJAN¹ AND P.NAGENDRA PRASAD²**¹PG and Research Department of Chemistry, Sri Paramakalyani College, Alwarkurichi -627412²Department of Biomedical Engineering, Noorul Islam Centre For Higher Education, Kumaracoil - 629180**ABSTRACT**

An increasing demand for natural additives has shifted the attention from synthetic to natural antioxidants. The main objective of this research work to establish the antioxidant property of different Phenotypic traits of *Aegle marmelos* leaf in methanol and water extracts. In the present study three different morphological traits namely Trait I, Trait II and Trait III of *Aegle marmelos* leaf extracts on water and methanol solvents were subjected to antioxidant studies such as DPPH(2,2-diphenyl-1-picrylhydrazyl) and FRAP (Iron Chelating activity). The results were presented and discussed in the communication.

KEYWORDS : *Aegle marmelos*, Vilvam, Bael, Antioxidant, DPPH, FRAP**V.N. ARIHARAN**PG and Research Department of Chemistry,
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INTRODUCTION

Antioxidants interfere with the production of free radicals and are known to defuse free radicals leading to limited risk of oxidative stress and associated disorders¹. Reactive oxygen species (ROS), the by-product of cell metabolism are involved in a variety of diseases and can cause damage to cellular bio-molecules like nucleic acid, proteins, lipids and carbohydrates and consequently may adversely affect immune functions. At cellular and molecular levels they inactivate ROS and inhibit or delay oxidative processes by interrupting the radical chain reaction of lipid peroxidation². Phytochemicals with antioxidant capacity naturally present in food, especially in fruits and vegetables are of great interest due to their beneficial effects on human health as they offer protection against oxidative deterioration³. Antioxidants or ingredients having antioxidant properties are used extensively for improvement of food stability. Natural antioxidant is considered to be safe as it occurs in plants. It occurs mostly in greens, vegetables and fruits. *Aegle marmelos* (Linn.) belongs to family Rutaceae, commonly known as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit and golden apple in English. It is found throughout India and is known from Pre-historic time. It is indigenous to India and is used in folk medicines. The Ayurvedic practitioners use almost all of its parts, but the greatest medicinal value ascribed to its leaves and fruits⁴. Vilvamis a perennial tree, wild in the Sub Himalaya tract, Central and South India. *Aegle marmelos* is a medium sized armed deciduous tree grows up to a height of 9-10 meters with straight, sharp, axillary thorns and yellowish brown shallowly furrowed corky bark. The leaves are trifoliate alternate, leaflets are ovate to lanceolate with pellucid – punctuate aromatic oil glands^{5, 6}. The lateral leaves are subsessile and the terminal one is long petioled. The flowers are greenish white sweet scented present in the axillary panicles. The fruits are Globus woody berry with yellowish ring, seeds numerous embedded in orange brown sweet gummy pulp. The leaves are used as astringent, laxative, febrifuge and expectorant. In order to find out potential sources of natural antioxidants the leaves of *Aegle marmelos* were studied for its DPPH (2,2-diphenyl-1-picrylhydrazyl) and Iron

chelating activity (FRAP). The medicinal properties of these plants had been described¹⁶. *Aegle marmelos* has been used from time immemorial in traditional systems of medicine for curing constipation, diarrhea, dysentery, peptic ulcer and respiratory infections^{7,8}. *A. marmelos* have been used as food and for medicinal purposes for decades⁹. The leaves are useful in ophthalmia, inflammations, catarrh, diabetic and asthmatic complaints^{10, 11}. The leaves are used for the heart and brain disorders. *Aegle marmelos* is a holy tree found in Hindu Siva and Sakthi temples as Sthala virusha. The leaves are used for the Pooja and given as Prasada to the devotees⁴

MATERIALS AND METHODS

Plant material

The authors have identified 5 different traits of different morphological traits of *Aegle marmelos*⁴. In this 3 traits leaves sample were collected and subjected to antioxidant studies. *Aegle marmelos* leaves were collected from Kadayam Vilva Vananthar temple with 3 small Leaves (Trait I) and 7 leaves (Trait II) and Sastha Temple Ariyankavu 3 leaves with big size (Trait III) with different morphology. The leaves were shade dried, powdered and stored in an air-tight container at 25°C for the evaluation.

Preparation of the extract

Ten grams of accurately weighed powdered leaf of *A. marmelos* was extracted with 250ml methanol and water separately in Soxhlet apparatus for 48hrs and the extracts were condensed and used for the studies

DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging assay

DPPH radical scavenging activity of the extract was determined according to the method reported by Blois^{12,13}. An aliquot of different concentrations of samples (2 µg, 10µg, 100µg and 450µg) solution in methanol was mixed with 2.5 ml of 0.5 mM methanolic solution of DPPH. The mixture was shaken vigorously and incubated for 37 minutes in the dark at room temperature. The absorbance was measured at 517 nm using UV

spectrophotometer. Ascorbic acid was used as a positive control. DPPH free radical scavenging ability (%) was calculated by using

the formula. % of inhibition = $\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$.

$$\% \text{ of inhibition} = \left(\text{OD of control} - \text{OD of } \frac{\text{sample}}{\text{absorbance}} \text{ of control} \right) \times 100$$

Iron chelating activity (FRAP)

The method of Benzie and Strain was adopted for the assay^{14,15}. The principle is based on the formation of O-Phenanthroline-Fe²⁺ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200µM) and 2 ml of various concentrations of different samples ranging from 2 to 450µg was incubated at room temperature for 10 minutes and the absorbance of the same was measured at 510 nm.

RESULTS AND DISCUSSION

Aegle marmelos showed a good antioxidant property in water and methanol extracts. The DPPH Scavenging activity of three traits of *Aegle marmelos* in water and methanol extracts was given in table 1 and table 2 respectively. The Iron Chelating Activity of the three traits of *Aegle marmelos* extracts were tabulated in table 3 and 4 respectively. The Graph of DPPH and FRAP activity of three traits of *Aegle marmelos* is presented in graph 1,2,3 and 4.

Table 1
DPPH Scavenging activity of 3 traits of Aegle maemelos in Water extract

	2µg	10 µg	100 µg	450 µg
Trait I	15.33333	69.7619	77.48227	92.8951
Trait II	36.8946	56.1315	68.79978	76.90074
Trait III	0.392157	5.576208	39.95272	83.13413
STANDARD	84.321	88.214	91.55	94.25

Table 2
DPPH Scavenging activity of 3 traits of Aegle maemelos in Methanol extract

	2 µg	10 µg	100 µg	450 µg
Trait I	58.01653	76.73993	86.64564	86.3807
Trait II	43.5692	58.71111	69.03755	70.06648
Trait III	12.41379	59.14614	76.30597	84.11507
STANDARD	84.321	88.214	91.55	94.25

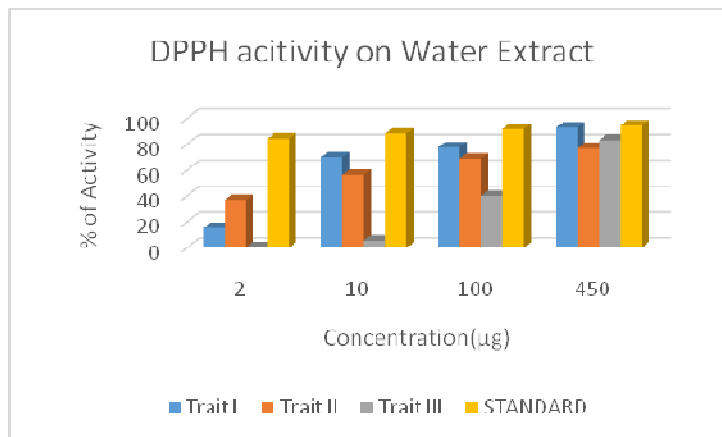
Table 3
FRAP Iron Chealating activity of 3 traits of Aegle maemelos in Water extract

	2 µg	10 µg	100 µg	450 µg
Trait I	21.33333	59.7619	74.48227	86.8951
Trait II	52.48154	71.63751	73.96468	67.56066
Trait III	11.39216	25.57621	69.95272	84.13413
STANDARD	82.321	86.214	91.55	93.25

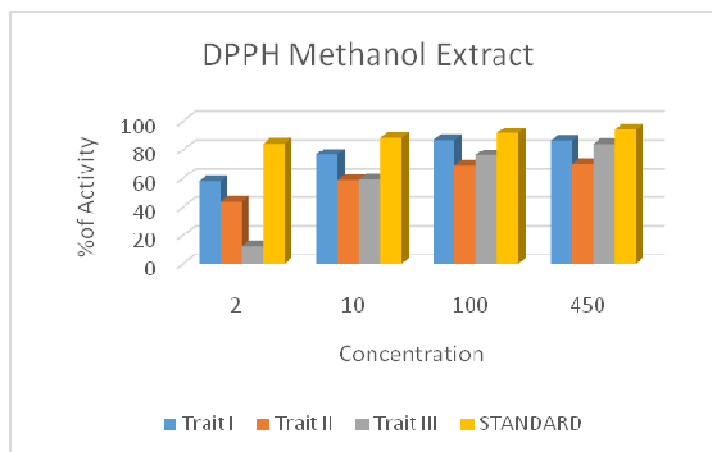
Table 4
FRAP Iron Chealating activity of 3 traits of Aegle maemelos in Methanol extract

	2 µg	10 µg	100 µg	450 µg
Trait I	61.01653	78.73993	84.64564	86.3807
Trait II	37.5692	48.71111	62.03755	71.06648
Trait III	22.41379	63.14614	75.30597	82.11507
STANDARD	82.321	86.214	91.55	93.25

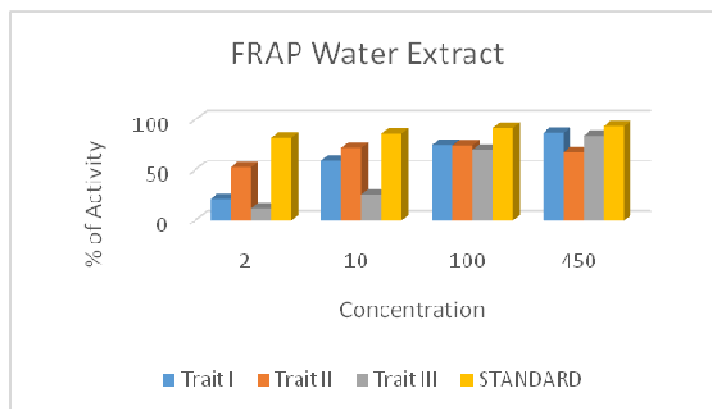
Graph 1
DPPH Scavenging activity of 3 traits of *Aegel maemelos* in Water extract



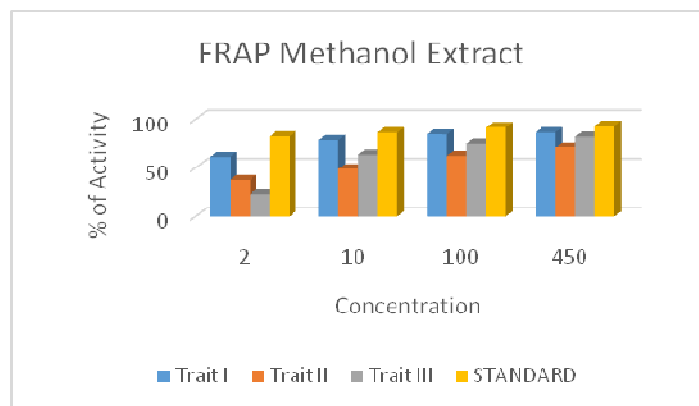
Graph 2
DPPH Scavenging activity of 3 traits of *Aegel maemelos* in Methanol extract



Graph 3
Iron Chelating activity (FRAP) of 3 traits of *Aegel maemelos* in Water extract



Graph 4
Iron Chelating activity (FRAP) of 3 traits of *Aegle marmelos* in Methanol extract



The antioxidant property of the water and methanolic leaf extracts of 3 different traits at four different concentrations were studied. Out of the three different traits studied the traditional small 3 leaves shows maximum DPPH activity and Iron Chelating activity (FRAP) in all concentrations. Among the three Phenotypic traits of *Aegle marmelos* evaluated for antioxidant property the Trait 1 (3 leaves with Small Leaves) which is the traditional one from which the other two traits might have (Trait II and trait III) evolved by nature. As *Aegle marmelos* is rich in antioxidant, it can be used as food additives to delay the oxidative deterioration of foods and as nutraceutical in medicinal formulation against degenerative diseases. The antioxidant compounds are mainly derived from natural sources such as plants. Antioxidant activity of these plants is due to the presence of flavones, isoflavones,

flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins. The *Aegle marmelos* can be used as a natural antioxidant, antipyretic, antibiotic and immuno modulatory drug.

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