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PHYTOCHEMICAL EVALUATION AND DETERMINATION OF ANTIOXIDANT ACTIVITY IN DIFFERENT PARTS OF *AEGLE MARMELOS*

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ABSTRACT: Free radicals are unstable molecules that are formed during normal cell metabolism through chemical changes that take place in a cell. This causes damage to cellular components such as cells, proteins, and DNA, or the cell membrane. Free radicals play a role in a complex interplay of different mechanisms in both normal agings, neurodegenerative, and other diseases. Aqueous and methanolic solvent extracts of pulp (without seed), pericarp, leaf, and seed of *Aegle marmelos* were subjected to phytochemical screening tests, quantitative determination of total phenol content (TPC), total flavonoid content (TFC), total flavonol content (TF) and *in-vitro* antioxidant activities such as DPPH, hydrogen Peroxide (H_2O_2), reducing power assay (RPA), ferric ion reducing antioxidant power (FRAP), ABTS and phosphomolybdenum total antioxidant assay (PM) by using standard procedures for the detection. The aqueous extract of pulp was found to possess maximum TPC (23.88±0.91mg GAE/g), TFC (63±0.25mg QE/g) and TF (13.30±0.53mg QE/g) values for methanolic leaf extract, respectively. The lower EC_{50} values were observed for DPPH assay for aqueous extract of seed is 160.117±1.62 µg/ml while EC_{50} values of pulp aqueous extract for hydrogen peroxide assay was 422.792±0.56 µg/ml. The Reducing power assay value was found to be maximum in pericarp aqueous extract 67.57±1.39 g AA/100g followed by pulp aqueous extract 131± 1.53 mg GAE/g in FRAP, pulp aqueous extract 63.42±2.61 mg AA/g in ABTS and methanolic extract of pericarp 288.33±0.14 mg AA/g in phosphomoly-bdenum assay, respectively. Thus, the main aim of the present study was to investigate phytochemicals quantitatively along with the antioxidant activity of methanolic and aqueous extract of pulp (without seed), pericarp, leaf, and the seed of *Aegle marmelos* by using different *in-vitro* models.

INTRODUCTION: Medicinal plants are, in many ways having their own immense value in the current era. All Plants have the largest history as used for food source, medicine, and for the purpose of daily requirements [1].

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More than 80,000 are utilized for medicinal purposes but 250,000 known plant species on the Earth. India is one of the world's which contains 12 biodiversity centers with the presence of over 45,000 many types of plant species. About 15,000-20,000 plants have a powerful medicinal value. Only 7,000-7,500 type species are consumed in routine by traditional communities for their medicinal values [2].

The medicinal activities of plants have also been evaluated due to their powerful pharmacological activities, pharmaceutical activity, low toxicity, and

economic viability. Many types of plants are manufacture material that is useful to the life support of health in humans and other animals.

In India, drugs of the herbal beginning have been used by *Unani, Siddha*, and *Ayurveda*, systems of medicines ago-earliest times. Among all conventional systems, *Ayurveda* is the most ancient yet still successfully application, mostly in India, China, Germany, Sri Lanka, Bangladesh, Japan, Bhutan, America, and many other countries [3]. It has a sound philosophical and practical basis as well as a holistic approach to cure or combat the diseases to maintain a healthy state of mind and body. The sacred texts in *Ayurveda* have explained several plant species for their use in various ailments. Several classical literatures like *Charak Samhita* and *Sushrut Samhita* had described properties and used of 1,100 and 1,270 species respectively [2]. More than 8,000 herbal remedies have been codified in *Ayurveda* till date.

Aegle marmelos (Linn), golden apple is family Rutaceae is highly reputed Ayurvedic herbal. This plant is commonly known as the Bael in Hindi, Vilvam in Tamil, and Bilva in the Sanskrit fruit tree. It is native to India and is used in traditional medicines. *A. marmelos* is a slow-growing tree of medium size (up to 12-15 m tall) throughout the deciduas forest of India of height up to 1200 meters with flaking, soft, short trunk, thick bark and spreading spiny branches. It is occurring all over India, especially in dry forest on hilly slopes and plains of Eastern Central, sub- Himalayan forest, Bengal and South India.

Every part of the *A. marmelos* plants, such as pulp, pericarp, seed, bark, stem, root, leaf, trunk, fruit, and flower, are used in the traditional system of medicine. The parts of the root are used in dyspepsia (agnimandya), chronic diarrhea with mal absorption (graham roga), and dysentery (pravahika). The dried roots are used in the disorder of the nervous system (vatavadhjy), rheumatism (amavata), oedema (uotha), and vomiting (chardi). The plant is reported to have anti-inflammatory [4], antidiarrheal [5], hypoglycemic [6] and antipyretic [4, 7] properties. The fruit, seeds, leaf and bark of *Aegle marmelos* (bael) are reported to exhibit antioxidant activity, while the leaves have shown anticancer, antidiabetic, and antilipidemic effects in rats [8, 9].

The homeopathic specialist used to each of their parts but the largest medicinal properties ascribed to the pulp of its fruit. Free radical is an unstable molecule that produces oxidative stress during normal cell metabolic processes in the body. It is induced by various factors included such as environmental, physical, air pollution, UV radiation, fried food, drinking alcohol, poor nutrition, sunlight, and some chemicals which cause of various reactive free radicals and succeeding damage to macromolecules like Proteins, Lipids, and DNA so occurs the many types of diseases like as heart disease, cardiovascular disease, diabetes mellitus, Alzheimer disease, Parkinson's disease, atherosclerosis, cancer, inflammation, aging and many other. Therefore, it was to evaluate antioxidant activity of *A. marmelos* fruit pulp and other parts to confirm its folk medicinal claim. A large number of naturally occurring products have been reported to contain a large quantity of antioxidant compounds other than vitamin E, C, and carotenoid [10]. These antioxidants play an active role in neutralizing, delaying, intercepting or preventing oxidative chemical reactions catalyzed by free radical [11]. The antioxidant properties of herbal plants might be due to the presence of phenol compounds such as flavonoids, flavonol [12, 13]. Phenolic diterpene and phenolic acids [14]. Synthetic antioxidants like tertiary butylated hydroxy quinine, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and gallic acid esters have been suspicious to be carcinogenic. Hence, strongly restrict have been placed on their use, and there is a current to exchange them with natural producing antioxidants. Besides, these synthetic antioxidants also show less solubility and moderate antioxidant activity [15]. Hence, searching for natural antioxidants has much been increased in the recent stage work giving details of the experiments. In the recent examination, antioxidant activity of aqueous and methanolic extracts of *Aegle marmelos* (bael) fruit pulp (without seed), pericarp, leaf, and seed were accessed.

MATERIALS AND METHODS:

Collection of Plant Samples: The different parts (Fruit pulp without seed, pericarp, leaf, and seed) *of Aegle marmelos* were collect from Deendayal Research Institute, Chitrakoot (M.P.) INDIA in April 2016, and identification was done by the

Department of Botany, University of Allahabad, India.

Preparation of Powder: The different parts (Fruit pulp without seed, pericarp, leaf, and seed) were collected. They were rinsed with tap water followed by distilled water to remove the dirt on the surface individually. Pulp, pericap, and seed were removed from whole fruit, and pericarp cut into small pieces. They were shade air-dried for 3 days. Dried samples were ground into a fine powder with the help grander mixer and kept in desiccators, then store in an airtight container.

Preparation of Aqueous and Methanolic Extracts for Phytochemical Test: The extraction was carried out, 10g dried powdered plant parts were taken dissolved in 100ml distilled water and methanol (1:10) individually for 72 hours in a round bottom flask at room temperature by placing on a shaker water bath. After 3 days, all sample filter through Whatman filter paper no. 1. The filtrate extract was store durable glass bottle keep at 4 ºC for further phytochemical experimental use.

Preparation of Crude Aqueous and Methanolic Extracts for Antioxidant Test: The extraction was carried out; 10g dried powdered plant parts were taken and dissolved in 100 ml distilled water and methanol (1:10) individually for 72 h in a round bottom flask at room temperature by placing on a shaker water bath. After 3 days, all sample filter through Whatman filter paper no. 1. The filtrate extract put on a hot water bath for concentrating. The obtained crude extracts were kept in desiccators. Crude extract was store airtight glass bottle keep in 4 ºC for further experimental use.

Chemicals and Standard: All chemicals used were of 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid, butylated hydroxyl toluene (BHT), trichloroacetic acid (TCA), ferric chloride, ascorbic acid, 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), ABTS ± [(2,20-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt], quercetindehydrate, anhydrous sodium carbonate (Na_2CO_3), aluminum trichloride, potassium acetate, sodium acetate, ferric chloride hexahydrate ($FeCl_3.6H_2O$, folin–ciocalteu's reagent, dragendorff's reagent, mercuric chloride, potassium iodide, iodine were purchased from Sigma-Aldrich was from sisco research laboratories (India). Ethanol, methanol, hydro-chloric acid (HCl), sulfuric acid (H_2SO_4), H_2O_2, chloroform, ammonia, glacial acetic acid, sodium hydroxide (NaOH) was purchased from Merck and potassium peroxodisulfate from Fluka. All solvents used for Methanol (purity 99.00%), were obtained from Merck, India. All other chemicals and solvents used were of standard analytical grade.

Phytochemical Qualitative Analysis: For preliminary qualitative phytochemical analysis, the freshly prepared liquid aqueous and methanolic extracts of different parts of *Aegle marmelos* were studied for their phytoconstituents such as alkaloids, tannins, flavonoids, saponins *etc*. by using standard different phytochemical tests.

Test for Alkaloids:

Mayer's Test (Potassium Mercuric Iodide Reagent): Add a few drops of Mayer's reagents to 1 ml of the acidic, aqueous extract of the drug. The white or pale yellow color is formed. The mayer's reagent was prepared as follows: 1.36 g of mercuric chloride was dissolved in 60 ml of distilled water. Both the solutions were mixed and diluted to 100 ml with distilled water [16].

Wagners Reagent: Take 1ml of aqueous extract in test tubes and add 2-3 drops of Wagner's reagent it gives orange-red precipitation(1.27g of iodine and 2g of potassium iodine dissolve in 100ml d/w) [17].

Test for Carbohydrate:

Anthrone's Test: To 2 ml of anthrone's test solution, add 0.5 ml of aqueous extract of drug. A green or blue color indicates the presence of carbohydrates [18].

Fehling's Test (for Reducing Sugar): To 2 ml of aqueous extract of drug, add 1 ml of mix. Of equal parts of Fehling's solution, A and Fehling's solution B and boil the content of the test tube for few minutes. A red or brick red precipitate was formed [16]. The Fehling's solution was prepared as follows:

Solution A: Copper sulphate – 34.64 gm, sulphuric acid – 0.5 ml, Distilled water 500 ml.

Solution B: Sodium potassium tartarate-176 gm, Sodium hydroxide-77 gm, distilled water- 500 ml. Then the two solutions mixed in equal volumes immediately before use.

Test for Proteins:

Biuret Test: The test solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of violet/pink color. (10 g of sodium hydroxide dissolved in 100ml d/w) (0.1g of copper sulphate dissolve in 100ml d/w) [19, 20].

Xantho Protein Test: A little residue was taken with 2 ml of water, and 0.5 ml of concentrated nitric acid was added to it. The development of yellow color indicates the presence of protein[16].

Test for Terpenoids: Take 5ml of aqueous extract, add 2ml chloroform followed by the addition of 3ml conc. sulfuric acid, observe the reddish-brown interface for the presence of terpenoids [17].

Test for Triterpenes:

Salkowski's Test: A few drops of Conc. Sulphuric acid was added to the filtrate by the wall of the test tube. The appearance of the golden yellow color indicated the presence of triterpenes [20, 21].

Test for Flavonoids:

Shinoda Test: In the test tube containing 0.5 ml of alcoholic extract of drug and drops of concentrated HCl followed by 0.5g of magnesium metal. The appearance of pink, crimson or magenta color within minutes or two indicates the presence of flavonoids [16].

Sodium Hydroxide (NaOH): To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow color was produced in the plant extract, which becomes colorless on addition of a few drops of dilute acid, indicating the presence of flavonoids [18, 22].

Test for Glycosides:

Keller Killiani Test: The test solution was treated with a few drops of glacial acetic acid and Ferric chloride solutions and mixed. Concentrated sulphuric acid was added and observed for the formation of two layers. The lower reddish-brown layer and an upper acetic acid layer, which turns bluish-green, would indicate a positive test for glycosides [19, 20].

Test for Phenols: (1 mL of extract + 5 mL distilled water + few drops of neutral ferric chloride) appearances of dark green color indicated the presence of phenol (10g ferric chloride dissolve in 100ml d/w.) [20, 21].

Test for Poly Phenols: Take 2ml ethanolic extract of plant sample and add 1ml folin-ciocalteu reagent and 9ml d/w. between 1-8 min. and add sodium carbonate solution (8ml) vortex to mix then kept test tube in the dark take O.D at 760nm (10.598g sodium carbonate dissolve in 1000ml d/w) [17].

Test for Tannin:

5% Ferric Chloride Solution: The test extract was taken in water, warmed, and filtered. 5 ml of filtrate was allowed to react with 1ml of 5% ferric chloride solution. If dark green or deep blue color is obtained, tannin is present (5g of ferric chloride dissolve in 100ml d/w) [20, 21].

Quantitative Analysis:

Determination of Total Phenolic Content (TPC): The concentration of phenolics in different parts of *Aegle marmelos* samples was estimated using a modified spectrophotometric folin-ciocalteu method [23]. Briefly, an aliquot 1 mL of extract (1mg/1ml conc.) was mixed with 1 mL of folin-ciocalteu phenol reagent. After 3 min, 1 mL of 10% Na_2CO_3 solution was added to the mixture and adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm using a T 60 UV/VIS spectrophotometer (PG Instruments Ltd, London, UK). Gallic acid was used to calculate a standard curve (20, 40, 60, 80 and 100 μg/mL; $r^2 = 0.998$). A blank was prepared in the same manner, where 1 mL of distilled water was used instead of the sample or standard. The concentration of phenolic compounds was measured in triplicate. The results were reported as the mean ± standard deviation and expressed as mg of gallic acid equivalents (GAEs) per g extracts. All analyses were carried out in triplicate. The percentage of total phenolic content (TPC) was calculated from the calibration curve of gallic acid plotted, and total phenolic content was expressed as milligram gallic acid equivalent per gram extract (mg GAE/g extract).

Total phenolic content was calculated using the formula:

$$TPC = C \times V/M$$

Where, 'c' is the concentration of gallic acid in mg/ml; 'V' is the volume of plant extract in ml; and 'm' is the weight of pure plant extract in g.

Determination of Total Flavonoid Content (TFC): Total flavonoid content (TFC) was determined using the aluminum colorimetric method with some modifications using quercetin as a standard [24, 25]. A calibration curve of quercetin was prepared in the different concentration range of 0-200 µg/ml (10, 30, 50, 100, 150, and 200µg/ml). Briefly, an aliquot 0.5 ml of extract and 0.5 ml standard were placed in different test tubes, and to each 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, 1.5 ml of 80% methanol, and 2.8 ml of distilled water were added and mixed. A blank was prepared in the same manner where 0.5 ml of distilled water was used instead of the standard or sample, and the amount of aluminum chloride was also replaced by distilled water. All tubes were incubated at room temperature for 30 min. The absorbance was taken at 415 nm. The concentration of flavonoids was expressed as mg quercetin equivalent (QE) per g of extract. All analyses were carried out in triplicate. The percentage of total flavonoids content (TFC) was calculated from the calibration curve of quercetin plotted, and total flavonoids content was expressed as milligram quercetin equivalent per gram extract (mg GAE/g extract).

Total flavonoids content was calculated using the formula:

$$TPC = C \times V/M$$

Where 'c' is the concentration of quercetin in mg/ml; 'V' is the volume of plant extract in ml; and 'm' is the weight of pure plant extract in g.

Total Flavonol Contents (TF): Total flavonol content was analyzed using the aluminum chloride colorimetric method) with some modifications [26, 27]. In this method, quercetin was used to make a standard calibration curve in the different concentration range of 0–100 µg/ml (10, 20, 40, 60, 80, and 100µg/ml). In different test tubes, each 1ml extract and 1ml standard solutions were placed, and then 1ml of 2% aluminum chloride, 3ml of 5% sodium acetate, was added and mixed well. The mixture was then centrifuged at 3000 rpm for 20 min to get a clear solution. A blank was prepared in the same manner where 1ml of distilled water was used instead of the standard or sample, and the amount of aluminum chloride was also replaced by distilled water. The absorbance of standard and sample was taken at 440 nm. Results were expressed as mg quercetin equivalent (QE) per gram of extract. All analyses were carried out in triplicate. The percentage of total flavonol content (TF) was calculated from the calibration curve of quercetin plotted, and total flavonol content was expressed as milligram quercetin equivalent per gram extract (mg GAE/g extract).

Total flavonol content was calculated using the formula:

$$TPC = C \times V/M$$

Where, 'c' is the concentration of quercetin in mg/ml; 'V' is the volume of plant extract in ml; and 'm' is the weight of pure plant extract in g.

Free Radical Scavenging Antioxidant Activity: Free Radical scavenging activity was analyzed by using the DPPH method [28] with some modification 0.5 ml different solvent extracts of pulp, pericarp, leaf, and seed of *Aegle marmelos* with different concentration, *i.e.*, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 mg/ml (1ml/1mg stock solution). Add 1.5 ml of 0.3 mM methanolic solution of DPPH and incubate for 20 min in the dark. Absorbance was measured at 517 nm against blank samples (DPPH solution only), and L-ascorbic acid (1.0 mg/ml) used as the standard. The % inhibition of both standard and samples was calculated for each concentration, and graphs were plotted (% inhibition against concentration). From these graphs, EC_{50} values were calculated for standard and extracts. Since the lower EC_{50} value means higher antioxidant activity. EC_{50} means effective concentration required for 50% inhibition. All analyses were carried out in triplicate. The capability to scavenge the DPPH radical was calculated using the following Equation-:

$$\text{DPPH radical scavenging activity (\%)} = Ab- As \times 100 /Ab$$

Where, Ab = Absorbance of Blank (control- only DPPH solution), As = Absorbance of Test sample

Hydrogen Peroxide Activity: The ability of the different extracts to scavenging of hydrogen peroxide was determined according to method [29]. A solution of hydrogen peroxide (40 mM) was

prepared in phosphate buffer (pH= 7.4). Plant extract (0.1 - 1.0 mg/ml) added to 0.6 ml of 40 mM solution of H_2O_2. (Butylated hydroxytoluene) BHT of 1.0 mg/ml concentration used as standard. Absorbance was taken at 230 nm against the blank solution (Phosphate buffer without H_2O_2). The % inhibition of both standard and samples was calculated for each concentration, and graphs were plotted (% inhibition against concentration). From these graphs, EC_{50} values were calculated for standard and extracts. Since the lower EC_{50} value means higher antioxidant activity. EC_{50} means effective concentration required for 50% inhibition. All analyses were carried out in triplicate.

H_2O_2 scavenging activity was measured by the following equation:

$$H_2O_2 \text{ Scavenging activity (\%)} = Ao\text{-}A1 \times 100/Ao$$

Where Ao = Absorbance of Control (blank), A1 = Absorbance of Sample

Reducing Power Assay: Substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form Potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form a ferric, ferrous complex that has an absorption maximum at 700nm. This experiment, some modified, was carried out a Spectrophotometric method [30] used for the measurement of reducing power. 1ml of plant extract solution was mixed with 2.5ml phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferricyanide [K_3Fe (CN_6)] (10g/Liter), then the mixture was incubated at 50Cº for 20 minutes. To this, 2.5mL of trichloroacetic acid (100g/Liter) was added and centrifuged at 3000rpm for 10 min. Finally, 2.5ml of the supernatant solution was mixed with 2.5ml of distilled water, and 0.5ml $Fecl_3$ (1g/Liter) was added and allowed to stand for 10 min. The absorbance was measured at 700nm in a UV-visible spectrophotometer (Systronics UV-Visible Spectrophotometer 119, India). Phosphate buffer was used as a blank solution. Ascorbic acid (10, 20, 40, 60, and 80 µg/ml) was used as the standard for construction of the calibration curve, and the reducing power was reported as ascorbic acid equivalent per 100 gm of dry sample [31]. The absorbance of the final reaction mixture of three parallel experiments was expressed as a mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

Ferric Reducing Antioxidant Power (FRAP): Ferric reducing antioxidant power (FRAP) was determined according to the procedure described[32]. Briefly, the FRAP reagent contained 2.5 mL of a 10 mmol/L TPTZ (2,4,6- tripyridyl-s-triazine) solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3$ $6H_2O$ and 25 mL of 0.3 mol/L acetate buffer, pH 3.6 and was prepared freshly and warmed at 37 °C. Aliquots of 1 ml sample filtrate were mixed with 0.2 mL distilled water and 1.8 mL FRAP reagent, and the absorbance of the reaction mixture at 593 nm was measured spectrophotometrically after incubation at 37 °C for 10 min.

Gallic acid (10, 30, 70, 100, 200, 400, and 600 µg/ml) was used as the standard. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of mg of standard used per gram of dry weight extract. The absorbance of the final reaction mixture of three parallel experiments was expressed as a mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

ABTS Radical Scavenging Assay: In this method, the radical scavenging capacity was measured by using ABTS + solution radical cation. The assay was performed according to the method described by Thaipong *et al.*, [33] and Gan *et al.* [34] Standard solutions (150 µl) and sample extract (150 µl) were placed in different test tubes, then ABTS working solution (2850 µl) was added to each test tube. These tubes were kept in the dark for 30 min. After that their absorbance was taken at 734 nm. Ascorbic acid of the standard was prepared in the range of 15, 30, 75, 90, and 125 µg/ml. The % inhibition of both standard and samples were calculated, and the concentration of ABTS content in the extract was reported as mg of an ascorbic acid equivalent (AA)/g extract. The absorbance of the final reaction mixture of triplicate experiments was expressed as a mean ± standard deviation. Decreased absorbance of the reaction mixture indicates stronger antioxidant activity.

Phosphomolybdenum Antioxidant Assay: The total antioxidant activity of the extract was evaluated by the phosphomolybdenum assay

method [35,] which is based on the reduction of Mo (VI) to Mo(V) by the extract and subsequent formation of a green phosphate- Mo(V) complex in acidic condition. A 0.3 ml extract (2mg/ml) was combined with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate), and the reaction mixture was incubated at 95 °C for 90 min. Then the absorbance of the solution was measured at 695 nm using a UV-visible spectrophotometer against black after cooling to room temperature. The antioxidant activity was expressed as the number of gram equivalents of ascorbic acid. The absorbance of the final reaction mixture of triplicate experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger antioxidant activity.

RESULTS AND DISCUSSION: The qualitative phytochemical analysis of aqueous and methanolic extracts of pulp (without seed), pericarp, leaf, and seed samples of *Aegle marmelos* revealed the presence of some primary and secondary metabolites such as alkaloids, steroids, and cardiac glycosides as shown in **Table 1**. Tannins were detected in all extracts but not in both extracts of seed and terpenoid were not detected in methanolic extract leaf of *Aegle marmelos*. The preliminary phytochemicals detected were known to comprise medicinal and health-promoting properties.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF AQUEOUS AND METHANOLIC EXTRACT IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of experiments	Observation	Pericarp		Pulp		Leaf		Seed	
			A	M	A	M	A	M	A	M
1	Alkaloids									
	Mayer's test	Pale yellow color appears	+	+	+	+	+	+	+	+
	Wagner's test	Yellow color appear	+	+	+	+	+	+	+	+
2	Carbohydrate									
	Anthrone's test	Reddish-brown color appears	+	+	+	+	+	+	+	+
	Fehling's test	Red color ppt. is formed	+	+	+	+	+	+	+	+
3	Proteins									
	Bieuret's test	Red color appears	+	+	+	+	+	+	+	+
	Xanthoproteic test	Mustard oil color appear	+	+	+	+	+	+	+	+
4	Terpenoid test	Reddish colour formed	+	+	+	+	+	-	+	+
5	Triterpenoid test									
	Salkowski's Test	Violet color ring formed	+	+	+	+	+	+	+	+
6	Flavonoid test									
	Shinoda's test	No change in light brown color	+	+	+	+	+	+	+	+
	Sodium hydroxide test	The light brown color appears	+	+	+	+	+	+	+	+
7	Glycoside test									
	Keller killiani test	Two layers occurs	+	+	+	+	+	+	+	+
8	Phenols test	Dark greenish color	+	+	+	+	+	+	+	+
9	Poly-Phenols test	Green color appear	+	+	+	+	+	+	+	+
10	Tannin test									
	5% Ferric chloride test	Brown or dark green colour appear	+	+	+	+	+	+	-	-

A- Aqueous extract of *Aegle marmelos* , M- Methanolic extract of *Aegle marmelos*, (+) – Presence and (-) - Absent

Total Phenolic Content: The total phenolic content of aqueous and methanolic extracts of pulp, pericarp, leaf, and the seed of *Aegle marmelos* was estimated using standard Gallic acid equivalent of phenols. The different concentration of gallic acid (20-100 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007, and the results were shown in **Fig. 1**. The total phenolic contents for all sample was obtained for 100 µg/ml of extract from the calibration curve of gallic acid, and the obtained results were shown in **Table 2**. The Total phenolic content for all samples was calculated using a standard calibration curve (y=0.009x - 0.140, R^2 =0.998) against absorbance 725 nm. The total phenol in aqueous and methanolic extract of pulp was (23.88±0.91 and 23.11±0.54); pericarp (23.55± 0.36 and 22.33±0.76); leaf (20.33±1.61 and 19± 0.41); seed (21.11±2.61 and 18.88±2.74) mg gallic acid equivalent (GAE)/g of extract, respectively. The results **Table 2** revealed that the aqueous and methanol extract of *Aegle marmelos* pulp, pericarp, leaf, and the seed has almost similar values but an aqueous extract of pulp being slightly maximum in total phenolic content (23.88 mg GAE/g of extract) while methanol extract of seeds shows the minimum value (18.88mg GAE/g of

extract). All experimental results are expressed as means ± SD, and all measurements reading were replicated three times (n=3).

TABLE 2: TOTAL PHENOLIC CONTENTS OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Total phenol content (mg of GAE/g of extract)
1	Gallic acid	Standard	104.88 ± 0.22
2	Pulp	Aqueous extract	23.88 ± 0.91
		Methanolic extract	23.11 ± 0.54
3	Pericarp	Aqueous extract	23.55 ± 0.36
		Methanolic extract	22.33 ± 0.76
4	Leaf	Aqueous extract	20.33 ± 1.61
		Methanolic extract	19.00 ± 0.41
5	Seed	Aqueous extract	21.11 ± 2.61
		Methanolic extract	18.88 ± 2.74

The average value of three calculations are presented as means ± S.D. (standard deviation)

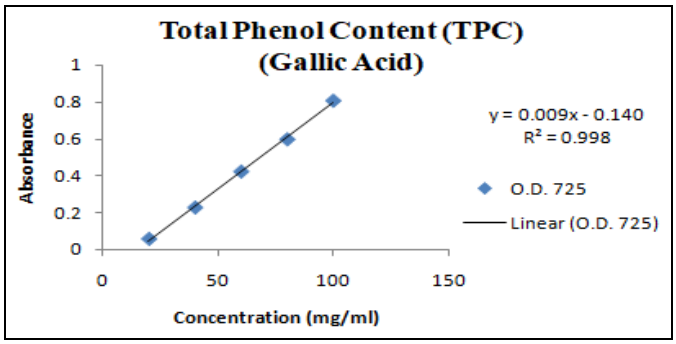

FIG. 1: STANDARD GALLIC ACID CALIBRATION CURVE FOR TOTAL PHENOLIC CONTENT OF *AEGLE MARMELOS* (GAE=GALLIC ACID EQUIVALENT)

Total Flavonoid Content: The total flavonoid content of aqueous and methanol extracts of pulp, pericarp, leaf, and seed of *Aegle marmelos* was estimated using standard quercetin equivalent of flavonoid. The different concentration of quercetin (10-200 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007, and the results were given in **Fig. 2**. The total flavonoid contents for all sample was obtained for 200 µg/ml of extract from the calibration curve of quercetin, and the results obtained are given in **Table 3**. The Total flavonoid content for all the samples was calculated using standard calibration curve (y=0.005x + 0.211, R^2 =0.996) against absorbance 415 nm and was found to be aqueous and methanol extract of pulp (49±0.61 and 47±0.44); pericarp (47±0.66 and 45.4±0.71); leaf (52.6±0.11 and 63 ± 0.25) seed (44.6±0.31 and 47.8±0.47) mg quercetin equivalent (QE)/g of extract respectively. The results **Table 3** revealed that the aqueous and methanolic extract of *Aegle marmelos* pulp, pericarp, leaf, and the seed has almost similar values but methanol extract of the leaf being slightly maximum in total flavonoid content, *i.e.*, 63mg QE/g of the extract while aqueous extract of seed shows minimum value 44.6 mg QE/g of extract. All experimental results are expressed as means ± SD, and all measurements reading were replicated three times (n=3).

TABLE 3: TOTAL FLAVONOID CONTENTS OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Total phenol content (mg of QE/g of extract)
1	Gallic acid	Standard	186.2± 0.42
2	Pulp	Aqueous extract	49 ± 0.61
		Methanolic extract	47 ± 0.44
3	Pericarp	Aqueous extract	47 ± 0.66
		Methanolic extract	45.4 ± 0.71
4	Leaf	Aqueous extract	52.6 ± 0.11
		Methanolic extract	63 ± 0.25
5	Seed	Aqueous extract	44.6 ± 0.31
		Methanolic extract	47.8 ± 0.47

The average value of three calculations are presented as means ± S.D. (standard deviation)

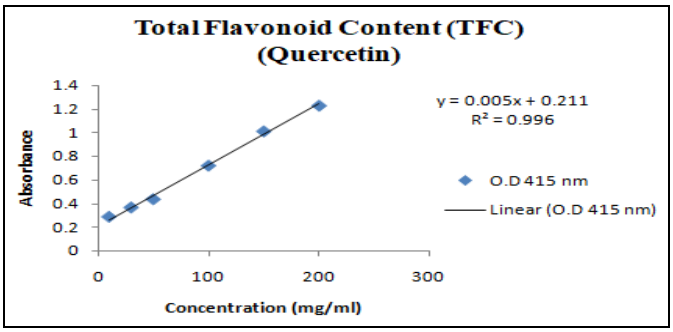

FIG. 2: STANDARD QUERCETIN CALIBRATION CURVE FOR TOTAL FLAVONOID CONTENT OF *AEGLE MARMELOS* (QE= QUERCETIN EQUIVALENT)

Total Flavonol Content: The total flavonol content of aqueous and methanol extracts of pulp, pericarp, leaf, and seed of *Aegle marmelos* was estimated using standard quercetin equivalent of flavonol. The different concentration of quercetin (10-100 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007, and the results were given in **Fig. 3**. The total flavonol content for all samples was obtained for 100 µg/ml of extract from calibration curve of quercetin and the results obtained are given in **Table 4**. The Total flavonol content for all samples were calculated using standard calibration curve (y=0.013x + 0.037, R^2 =0.996) against absorbance 440 nm and was found to be aqueous and methanol extract of pulp (11.69 ± 0.21 and 5.53 ± 0.34); pericarp (13.30 ± 0.53 and 5.15 ± 0.51); leaf (7.46 ± 0.37 and 9.00 ± 0.61);

seed (8.61 ± 0.42 and 13.15 ± 1.51) mg quercetin equivalent (QE) / g of extract respectively. The results **Table 3** revealed that the aqueous and methanol extract of *Aegle marmelos* pulp, pericarp, leaf and seed has almost similar values, but pericarp aqueous extract being slightly high in total flavonol content *i.e.*, 13.30 mg QE/g of the extract while pericarp methanol extract having minimum value5.15 mg QE/g of extract. All experimental results were expressed as means ± SD. All measurements reading was replicated three times (n=3). Phenols and polyphenolic compounds, such as flavonoids, are widely found in food products derived from plant sources and they have been shown to possess significant antioxidant activities [36].

TABLE 4: TOTAL FLAVONOL CONTENTS OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Total flavonol content (mg of QE/g of extract)
1	Gallic acid	Standard	108.69 ± 0.41
2	Pulp	Aqueous extract	11.69 ± 0.21
		Methanolic extract	5.53 ± 0.34
3	Pericarp	Aqueous extract	13.30 ± 0.53
		Methanolic extract	5.15 ± 0.51
4	Leaf	Aqueous extract	7.46 ± 0.37
		Methanolic extract	9.00 ± 0.61
5	Seed	Aqueous extract	8.61 ± 0.42
		Methanolic extract	13.15 ± 1.51

The average value of three calculations are presented as means ± S.D. (standard deviation)

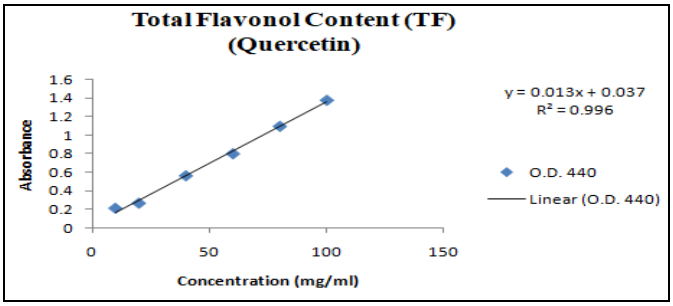

FIG. 3: STANDARD QUERCETIN CALIBRATION CURVE FOR TOTAL FLAVONOL CONTENT OF *AEGLE MARMELOS* (QE= QUERCETIN EQUIVALENT)

DPPH Free Radical Scavenging Assay: The DPPH radical scavenging activity of aqueous and methanol extracts of pulp, pericarp, leaf and seed of *Aegle marmelos* was evaluated and compared with ascorbic acid and the results were given in **Table 5**. The % inhibition at various concentration (100-1000 µg/ml) of all extracts as well as standard ascorbic acid (10 -100 µg/ml) were calculated and plotted in **Fig. 4** using Microsoft Office Excel 2007. The EC_{50} values were calculated from linear regression analysis of SPSS software and were found to be 13.92 ± 0.23µg/ml for standard (ascorbic acid) whereas seed aqueous extract was having highest EC_{50} value 160.117 ± 1.62 µg/ml followed by 404.47 ± 3.77 µg/ml and 557.48 ± 1.87µg/ml for pulp aqueous and methanol extract respectively. Since EC_{50} means effective concentration required for 50% inhibition and the lower EC_{50} value means higher antioxidant activity. Seed Aqueous > Pulp Aqueous > Pulp Methanol > Pericarp Aqueous > Pericarp Methnol> Leaf Methanol > Leaf Aqueous > Seed Methanol. The results show that at 20 µg/ml concentration of ascorbic acid shows maximum inhibition of DPPH *i.e.*, 52%, while at the same concentration all extracts show very low inhibition of DPPH. The results for the DPPH assay of percentage inhibition for standards and all samples (aqueous and methanol extract pulp, pericarp, leaf and seed) are shown in **Fig. 4**.

TABLE 5: EC_{50} VALUE OF DPPH FREE RADICAL SCAVENGING ACTIVITIES OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	DPPH Radical Scavenging EC_{50} (µg/ml)
1	Gallic acid	Standard	13.928 ± 0.23
2	Pulp	Aqueous extract	404.474 ± 3.77
		Methanolic extract	557.48 ± 1.87
3	Pericarp	Aqueous extract	622.145 ± 1.85
		Methanolic extract	737.041 ± 1.68
4	Leaf	Aqueous extract	917.292± 1.04
		Methanolic extract	896.862 ± 0.57
5	Seed	Aqueous extract	160.117± 1.62
		Methanolic extract	1,016.37 ± 6.17

The average value of three calculations are presented as means ± S.D. (standard deviation)

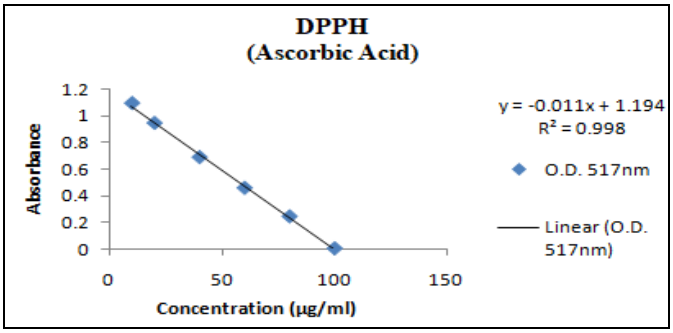

FIG. 4: STANDARD ASCORBIC ACID CALIBRATION CURVE FOR DPPH RADICAL SCAVENGING ACTIVITY OF *AEGLE MARMELOS*

When comparing the EC_{50} values of all extracts of *Aegle marmelos* plants, it was observed that aqueous seed extract possesses lower values and

had the greater potential of antioxidant activity than other parts of *Aegle marmelos* medicinal plant **Fig. 5**.

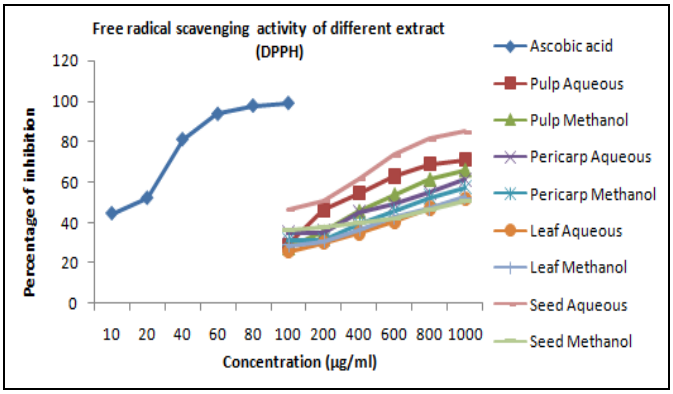

FIG. 5: FREE RADICAL SCAVENGING ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

Hydrogen Peroxide (H_2O_2) Scavenging Activity: The hydrogen peroxide scavenging activity of aqueous and methanol extracts of pulp, pericarp, leaf and seed of *Aegle marmelos* was evaluated and compared with BHT (Butylated hydroxytoluene) and the results were shown in **Table 6**.

TABLE 6: EC_{50} VALUE OF HYDROGEN PEROXIDE (H_2O_2) SCAVENGING ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	DPPH Radical Scavenging EC_{50} (µg/ml)
1	Butylated Hydroxytoluene (BHT)	Standard	265.491 ± 0.45
2	Pulp	Aqueous extract	422.792 ± 0.56
		Methanolic extract	831.434 ± 1.14
3	Pericarp	Aqueous extract	960.853 ± 0.26
		Methanolic extract	590.511 ± 0.58
4	Leaf	Aqueous extract	745.986± 1.13
		Methanolic extract	558.653 ± 0.25
5	Seed	Aqueous extract	759.617± 0.13
		Methanolic extract	429.324± 1.41

The average value of three calculations are presented as means ± S.D. (standard deviation)

The percentage inhibition (% inhibition) at various concentrations (100-1000 µg/ml) of all samples as well as standard ascorbic acid (100-1000µg/ml) were calculated and plotted in **Fig. 6** using Microsoft Office Excel 2007. The aqueous pulp extract showed more percent inhibition than methanol extract of *Aegle marmelos* respectively, against the standard butylated hydroxytoluene (BHT). The EC_{50} values were calculated from linear regression SPSS software and were found to be 265.491±0.45 µg/ml for standard BHT while the lower EC_{50} value was reported for pulp aqueous extract 422.792±0.56 µg/ml and seed methanol extract 429.324±1.41 µg/ml. Since EC_{50} means effective concentration required for 50% inhibition and the lower EC_{50} value means higher antioxidant activity.

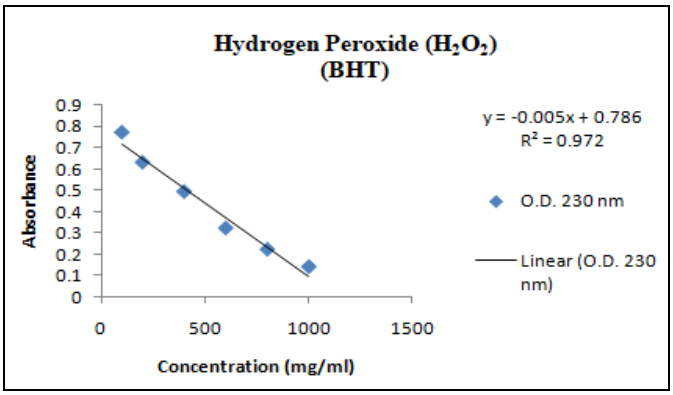

FIG. 6: STANDARD BUTYLATED HYDROXYTOLUENE (BHT) CALIBRATION CURVE FOR HYDROGEN PEROXIDE SCAVENGING ACTIVITY OF *AEGLE MARMELOS*

Reducing Power Assay (RPA): The reducing abilities of aqueous and methanol extracts of pulp, pericarp, leaf and seed of *Aegle marmelos* was evaluated and compared with ascorbic acid and the results were given in **Table 7**. The mean absorbance at various concentrations (10 - 800 µg/ml) of all extracts as well as standard ascorbic acid (10 - 80 µg/ml) were calculated and plotted using Microsoft Office Excel 2007as shown in **Fig. 7**.

The reductive capabilities were found to increase with increasing of concentration of all extracts as well as standard ascorbic acid. The absorbance value was found to be increased with increase in concentration of the extract. The increased absorbance of the reaction mixture indicated increased reducing power and vitamin C was used as a positive control.

The highest reducing power was found for pulp aqueous extract (74.85 ± 2.04 gm of ascorbic acid / 100 gm of dried extract) followed by 67.57 ± 1.39 and 67.42 ± 1.19 gm of ascorbic acid / 100 gm of dried extract for pericarp aqueous extract and seed aqueous extract respectively. The lowest value was reported in pericarp methanol extract (47.57 ± 0.82 gm of ascorbic acid / 100 gm of dried extract) as shown in **Table 7**.

TABLE 7: REDUCING POWER ASSAY (RPA) OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Reducing Power Assay (gm of AA / 100 gm of dried extract)
1	Ascorbic acid	Standard	132.00± 0.22
2	Pulp	Aqueous extract	74.85 ± 2.04
		Methanolic extract	60.57 ± 1.53
3	Pericarp	Aqueous extract	67.57 ± 1.39
		Methanolic extract	47.57 ± 0.82
4	Leaf	Aqueous extract	62 ± 2.56
		Methanolic extract	59.14 ± 0.39
5	Seed	Aqueous extract	67.42 ± 1.19
		Methanolic extract	50.42 ± 1.48

The average value of three calculations are presented as means ± S.D. (standard deviation)

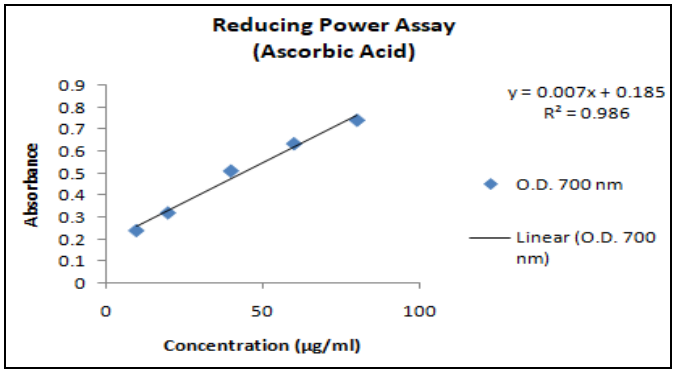

FIG. 7: STANDARD ASCORBIC ACID CALIBRATION CURVE FOR REDUCING POWER ASSAY (RPA) OF *AEGLE MARMELOS*

Ferrous Reducing Antioxidant Power (FRAP): Ferric reducing antioxidant power (FRAP) assay depends on the reduction of ferric ion into ferrous ion. The ferric reducing assay of the all extract suggested that it has the potential to reduce the ferric form. The mean absorbance at various concentrations (10 - 600 µg/ml) of all extracts as well as standard gallic acid (10 - 80 µg/ml) were calculated and plotted using Microsoft Office Excel 2007 as shown in **Fig. 8**. The highest reducing power was found for pulp aqueous extract (74.85 ± 2.04 gm of ascorbic acid / 100 gm of dried extract) followed by 67.57 ± 1.39 and 67.42 ± 1.19 gm of ascorbic acid / 100 gm of dried extract for pericarp aqueous extract and seed aqueous extract respectively. The standard calibration curve (y=0.005x + 0.922, R^2 =0.997) against absorbance 593 nm shows highest ferric reducing antioxidant power for aqueous and methanol extract of pulp (131± 1.53 and 22.2 ± 0.61); pericarp (87.2 ± 0.21 and 109.4 ± 0.25); leaf (5.8 ± 0.43 and 62.6± 0.78); seed (62.6 ± 0.56 and 126.6 ± 0.74) mg gallic acid equivalent (GEA) / g of extract respectively. The results **Table 8**, **Fig. 8** of FRAP revealed that the aqueous pulp extract was having a maximum value of 131 mg GEA / g of the extract while aqueous leaf extract having a minimum value has 5.8 mg GEA/g of extract. All experimental results are expressed as means ± SD, and all measurements reading were replicated three times (n=3).

TABLE 7: FERRIC REDUCING ANTIOXIDANT PROPERTY (FRAP) OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Reducing Power Assay (gm of AA / 100 gm of dried extract)
1	Gallic acid	Standard	495.2± 0.23
2	Pulp	Aqueous extract	131± 1.53
		Methanolic extract	22.2 ± 0.61
3	Pericarp	Aqueous extract	87.2 ± 0.21
		Methanolic extract	109.4 ± 0.25
4	Leaf	Aqueous extract	5.8 ± 0.43
		Methanolic extract	62.6± 0.78
5	Seed	Aqueous extract	62.6 ± 0.56
		Methanolic extract	126.6 ± 0.74

The average value of three calculations are presented as means ± S.D. (standard deviation)

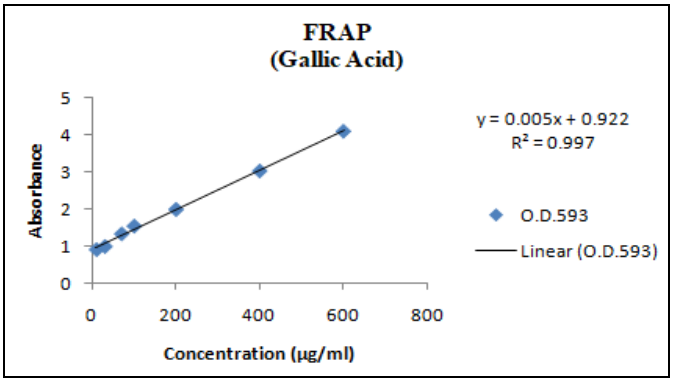

FIG. 8: STANDARD GALLIC ACID CALIBRATION CURVE FOR FERRIC REDUCING ANTIOXIDANT PROPERTY (FRAP) OF *AEGLE MARMELOS*

2, 2'- Azino- Bis- 3- Ethylbenzothiazoline-6-Sulfonic Acid (ABTS): This assay helps to assess the scavenging activity of the extract against the radical ABTS, which is expressed as ascorbic acid equivalent antioxidant capacity (AAAC). The different concentration of ascorbic acid (15- 125 µg/ml) calibration curve was plotted using Microsoft Office Excel 2007, and the results were given in **Fig. 9**. The results for aqueous and methanolic samples were obtained for 15-125µg/ml of extract from the calibration curve of ascorbic acid as given in **Table 9**. The standard curve of ascorbic acid showed (y= 0.007x + 0.987,

R^2 =0.990) against absorbance 734 nm. The results indicate an increase in AAAC value with an increase in concentration. The AAAC value for aqueous and methanol extract of *Aegle marmelos* pulp, pericarp, leaf and seed has almost similar values but aqueous pulp extract having a maximum value 63.42 ± 2.61mg AA/g of the extract while pericarp methanol extract was having minimum value 21.42 ± 2.01 mg AA/g of extract. All experimental results were expressed as means ± SD and all the measurements reading were replicated three times (n=3).

TABLE 9: ABTS ANTIOXIDANT PROPERTY OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	ABTS (mg AA/ g)
1	Ascorbic acid	Standard	140.85± 0.10
2	Pulp	Aqueous extract	63.42 ± 2.61
		Methanolic extract	38 ± 1.80
3	Pericarp	Aqueous extract	62 ± 2.26
		Methanolic extract	21.42 ± 2.01
4	Leaf	Aqueous extract	29.14 ± 1.45
		Methanolic extract	23 ± 1.45
5	Seed	Aqueous extract	40.57 ± 2.32
		Methanolic extract	38.85 ± 2.11

The average value of three calculations are presented as means ± S.D. (standard deviation)

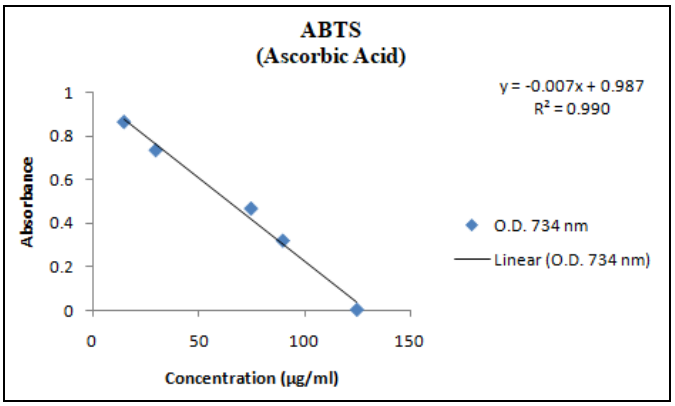

FIG. 9: STANDARD ASCORBIC ACID CALIBRATION CURVE FOR ABTS ANTIOXIDANT PROPERTY OF *AEGLE MARMELOS*

Phosphomolybdenum Assay (PM): The phosphomolybdenum of antioxidant activity of the aqueous and methanol extracts of pulp, pericarp, leaf and seed of *Aegle marmelos* was estimated using standard ascorbic acid equivalent of extract. The different concentration of ascorbic acid (30-1000µg/ml) calibration curve was plotted using Microsoft Office Excel 2007 and the results were given in **Fig. 10** and **Table 10**. The phospho-molybdenum of antioxidant activity for all samples were calculated using standard calibration curve (y=0.003x - 0.393, R^2 =0.997) against absorbance 695 nm The results for aqueous and methanol extract of pulp was (268.66 ± 0.36 and 277.33 ± 0.15); pericarp (286 ± 0.58 and 288.33 ± 0.14); leaf (263 ± 0.42 and 260 ± 0.23); seed (226.66 ± 0.25 and 206.33 ± 0.14) mg ascorbic acid equivalent (AA) / g of extract respectively. The results **Table 10** revealed that the aqueous and methanol extract of *Aegle marmelos* pulp, pericarp, leaf, and seed has almost similar values but pericarp methanol extract being slightly maximum in Phospho-molybdenum assay, *i.e.*, 288.33 mgAA/g of the extract while seed methanol extract having minimum value 206.33 mg AA/g of extract. All experimental results are expressed as mean ± SD and all measurements reading were replicated three times (n=3).

TABLE 10: PHOSPHOMOLYBDENUM ASSAY (PM) ANTIOXIDANT PROPERTY OF AQUEOUS AND METHANOLIC EXTRACTS IN DIFFERENT PARTS OF *AEGLE MARMELOS*

S. no.	Name of sample	Extracts	Phosphomolybdenum Assay (PM) (mg AA /g)
1	Ascorbic acid	Standard	383.33 ± 0.25
2	Pulp	Aqueous extract	268.66 ± 0.36
		Methanolic extract	277.33 ± 0.15
3	Pericarp	Aqueous extract	286.00± 0.58
		Methanolic extract	288.33± 0.14
4	Leaf	Aqueous extract	263.00± 0.42
		Methanolic extract	260.00± 0.23
5	Seed	Aqueous extract	226.66± 0.25
		Methanolic extract	206.33± 0.14

The average value of three calculations are presented as means ± S.D. (standard deviation)

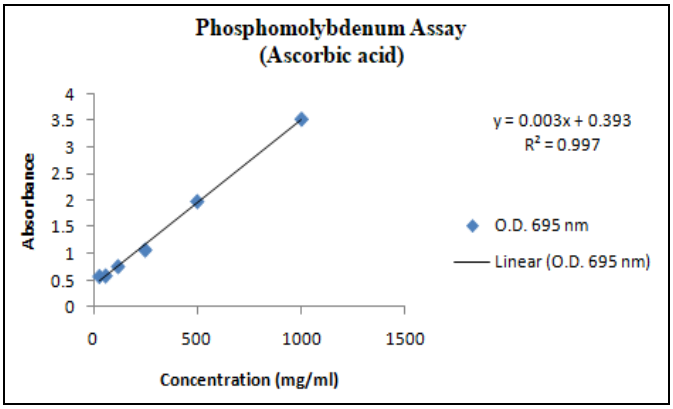

FIG. 10: STANDARD ASCORBIC ACID CALIBRATION CURVE FOR PHOSPHOMOLYBDENUM ANTIOXIDANT PROPERTY OF *AEGLE MARMELOS*

CONCLUSION: *Aegle marmelos* is ethnically used in different types of diseases in humans and animals. The study demonstrated the antioxidant

potentials of different solvent extracts of *Aegle marmelos*. The results obtained in the present study have shown that the presence of some phyto-chemicals such as flavonoids, phenol, carbohydrates, alkaloids, steroids, tannins, and cardiac glycosides in aqueous and methanolic extracts pulp, pericarp, leaf, and seed of *Aegle marmelos*. According to results, the extracts having greater phenolic content can effectively scavenge free radicals under *in-vitro* conditions. Generally, phenolic compounds can trap free radicals and neutralize them, thus preventing our cells from the aging process and various diseases. Further, high phenolic content in plants generally shows some anticancer activities and other disease-preventing properties. This study also revealed that the aqueous extracts of seed and pulp of *Aegle marmelos* have greater DPPH radical scavenging capacity than other aqueous and methanolic extracts in different parts of *Aegle marmelos*. Thus, results from the present study reported that medicinal properties in *Aegle marmelos* plant was due to the presence of some phenolic compounds and other phytochemicals which are a dense source of antioxidant. This is an ongoing study, and further work is being carried to investigate its biological activities.

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