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Quantitative analysis of phenols, flavonoids in different parts of *Aegle marmelos* (Bael) along with the evaluation of Antioxidant potential using different extracts

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Abstract
The present study was undertaken for phytochemical analysis and evaluation of DPPH free radical scavenging activity of different extracts (acetone, ethanol and aqueous) of leaves, stem-bark, roots and fruits of bael. The results revealed that the extract yield of ethanol extracts of different parts of bael was highest (ranged from 9.18 to 37.42 g/100g) followed by aqueous extracts (ranged from 2.65 to 35.40 g/100g) and acetone extracts (ranged from 1.03 to 7.86 g/100g). Total phenolics content in aqueous extract was highest (ranged from 9.05 to 49.00 mg GAE/g) followed by ethanol extract (ranged from 3.21 to 13.32 mg GAE/g) and acetone extract (ranged from 0.62 to 3.75 mg GAE/g). Total flavonoids contents in aqueous extracts was highest (ranged from 2.14 to 11.89 mg CE/g) followed by ethanol extract (ranged from 1.49 to 4.37 mg CE/g) and acetone extract (ranged from 1.14 to 4.31 mg CE/g). DPPH free radical scavenging activity of bael extracts varied widely and it increased with increase of concentration levels. All the parts of bael were found to be rich source of antioxidants and exhibited better DPPH free radical scavenging activity in aqueous extracts in comparison to ethanol and acetone extracts.

Keywords: Bael, phenolics, flavonoids, DPPH, free radical scavenging activity, extracts

1. Introduction
India is a bonanza of aromatic and medicinal plants. It has been estimated that 80% of the population of developing countries still depends on traditional medicines, mostly plant drugs for their primary health care requirements and ensure patient safety by upgrading the skills and knowledge of conventional medicine providers (WHO, 2008) [1]. Plants are the richest source of drugs of traditional medicines, modern medicines, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for artificial drugs (Hammer *et al.*, 1999) [2]. People trust more on natural product obtained from shrubs, herbs and trees as herbal medicines are safe and effective, at low cost with least or no side effects. So, in present scenario, medicinal and aromatic plants occupy a central economic position because of the continuous and increased demands of their products at local, national and international markets. Further, there has been ever increasing demand for more and more drug from plant resources especially from developing country. The rural population of India is more disposed to traditional ways of treatment because of easy accessibility and cheaper cost. Thus, there is prominent interest in the screening of plants and other natural product extracts in modern drug discovery programmes, since structurally novel chemotypes with potent and selective biological activity may be obtained (Cragg *et al*., 1997) [3].
Aegle marmelos is a dryland plant belonging to family Rutaceae and commonly known as Bengal quince, Golden/Stone apple in English, Bel geri in Hindi, Bel Kham in Urdu (Parichha 2004; Sharma *et al*., 2007) [4, 5]. This plant is native to India and also grown in hills and plains of Sri Lanka, Pakistan, Bangladesh, Burma, and Thailand as well as in most of the South Asian countries. In India it is profusely found in Himalayan tract, Bengal, Central and South India. It is the slow growing average sized tree that grows up to 15 m tall with short trunk, thick and exfoliated bark, sometimes spiny branches, the lower ones drooping (Dhankhar *et al*., 2011) [6]. The deciduous alternate leaves which can be single, two or three, are composed of 3 to 5 oval, pointed leaflets measuring upto 4 to 10 cm long, 2 to 5 cm wide and the terminal one with a long petiole (Patel *et al*., 2012) [7]. The peel of the fruit is made up of stony shell. Depending upon the ripening, color of the fruit varies from green to brown. The eatable pulp appears like a boiled pumpkin (yellow or orange) which tastes sweet having pleasant flavour and is fragrant.

The flowers are 1.5 to 2 cm, pale green or yellowish, sweetly scented, bisexual, short, drooping unbranched clusters at the end of twigs and leaf axils. There is surrounding of oily transparent mucilage around the seeds (Suvimol and Pranee, 2008) [8].

Phytochemistry (a branch of natural product chemistry) deals with phytochemicals derived from plants. Phytochemicals are bioactive compounds which are found in plants that work with nutrients and dietary fibre to protect against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavour colour (Craig 1999; Agbafor and Nwachukwu, 2011) [9, 10]. Phytochemists now have been able to isolate, identify and characterize about 70,000 chemical substances present in plants. A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc.). They act as defense chemicals. Their absence does not found to cause any bad effects to plants. Some other secondary metabolites like carotenoids, tocopherols, ascorbates, phenols and flavonoids present in plants are strong natural antioxidants and have an important role in health care system.

Plant's secondary metabolites have been of great interest to man for a long time due to their pharmacological relevance (Arora *et al.*, 2003) [11]. Flavonoids and phenolics are the most important groups of secondary metabolites and bioactive compounds in plants (Kim *et al*., 2003) [12]. Phenolics compound confer unique taste, flavour, and health promoting properties found in vegetables and fruits (Tomas-Barberan and Espin, 2001) [13]. They are crucial for plants growth and reproduction, and are produced as a response to environmental factors (light, chilling, pollution etc.) and to defend injured plants (Valentine *et al*., 2003) [14]. Flavonoids, the most common group of polyphenolic compounds, are found ubiquitously in plants. Common flavonoid groups include aurones, xanthones, and condensed tannins. Most of flavonoids are present in our daily life (Manach *et al*., 2004; Dahan and Altman, 2004) [15, 16]. Till date, about 6000 flavonoids compounds have been isolated and identified, and many are common in higher plants (Tolonen *et al*., 2002; Austin and Noel, 2003) [17, 18].

The only source of natural antioxidants is plants (Walton and Brown 1999) [19]. Antioxidants are substances having the ability to neutralize free radicals (Sies, 1996) [20] and are vital substances which owe the ability to protect the body from damage caused by free radical induced oxidative stress. Antioxidants inhibit the oxidative processes by various mechanisms such as scavenging free radicals, acting as electron donors and by chelating free catalytic metals (Gulcin *et al.*, 2005) [21]. The natural antioxidant mechanisms lack in variety of conditions and hence, dietary intake of antioxidant in the form of antioxidant compounds is important (Terao *et al.*, 1994) [22]. The therapeutic effects of certain medicinal plants are typically attributed to their antioxidant phytochemicals. It has been suggested that there is an inverse correlation between dietary intake of antioxidant rich foods and prevalence of human diseases (Yildrim *et al.*, 2001) [23]. Plant based antioxidants are preferred over artificial ones because of their multiple mechanisms of actions and non-toxic nature. These facts have encouraged widespread screening of plants for medicinal and antioxidant properties. The separation and characterization of different phytochemicals and their utilization as antioxidants of natural origin is preferred to prevent diseases (Akinmoladun *et al.*, 2007) [24]. Most of the antioxidant compounds in a typical diet are obtained from plant sources and belong to various classes of compounds with ample variety of physical and chemical properties.

Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention, disruption or by scavenging reactive metabolites and converting them to less reactive molecules or by increasing the resistance of sensitive biological target to ROS attack (Sen, 1995) [25]. Free radicals, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are related with many pathological conditions like atherosclerosis, arthritis, reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman 1996) [26, 27]. As synthetic antioxidants (like butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters) have been suspected to be carcinogenic therefore, they either need to be replaced with naturally occurring antioxidants or strong restrictions have been placed on their use. Moreover, synthetic antioxidants also show moderate antioxidant activity and low solubility (Barlow, 1990; Branen, 1975) [28, 29]. Hence, in the recent scenario search for natural antioxidant has greatly increased. So far, many crude extracts and pure natural compounds having potent antioxidant potential have been reported (Schuler, 1990; Chu, 2000; Mantle *et al*., 2000) [30, 31, 32]. Nowadays, the focus has been shifted on edible plants especially spices and herbs for safe and effective naturally occurring antioxidants (Miliauskas *et al*., 2004) [33].

2. Experimental section

Bael (*Aegle marmelos* L.) different parts namely stem-bark, leaves, roots and fruits were collected from Chaudhary Charan Singh Haryana Agricultural University, Hisar. All the parts were shade dried followed by oven drying After oven drying, leaves were grounded as such whereas stem-bark and roots were cut into small pieces of 2-3 inches and were grounded and fruit shell was removed, seeds were separated and pulp was grounded. For estimation of total phenolics, total flavonoids and evaluation of antioxidant activity various extracts *viz*. acetone, ethanolic and aqueous extracts were prepared by using soxhlet apparatus. For preparation of extracts, ten gram of powdered samples of different parts of bael were placed in a filter paper (Whatman No. 1) thimble in a classical soxhlet apparatus fitted with a 250 mL round bottom flask. The respective solvents (acetone, ethanol and distilled water) were added up to one and a half siphons that is approximately 150 mL. After the completion of first extraction step of 5h, residue in thimble was again extracted twice with suitable amount of solvents. Filtrates of each solvent from three extraction steps were pooled and their volumes were noted. These extracts were filtered and used for estimation of extract yield, total phenolics, total flavonoids and evaluation of antioxidant activity by DPPH free radical scavenging method.

The commercially available chemicals from Merck, SRL (SISCO Research Laboratories), Qualigens and Sigma-Aldrich, were used for various experimental procedures.

2.1 Estimation of total phenolics content: Total phenolics content of extracts were determined by the Folin-Ciocalteu method (Singleton and Rossi, 1965) [34]. Aliquots of 0.2 mL of extracts were mixed with 1 mL of 1mol/L Folin-Ciocalteu

reagent. After that, 2.0 mL of 20% (w/v) sodium carbonate was added. The solutions were mixed and volume was made upto 10.0 mL with distilled water. The absorbance was measured at 730 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g.

2.2 Estimation of total flavonoids content: Total flavonoids content of extracts was estimated according to the colorimetric assay. In 1 mL of extract, 4 mL of double distilled water and 0.3 mL of 5% (w/v) $NaNO_2$ were added. After 5 min, 0.3 mL of 10% (w/v) $AlCl_3$ was added. Immediately, 2 mL of 1 M NaOH was added and the volume was made up to 10.0 mL with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-VIS double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using catechin as standard. Results were expressed as mg CE/g on dry weight basis.

2.3 DPPH free radical scavenging activity: The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picryl- hydrazyl (DPPH) free radical scavenging activity method. Acetone, Ethanolic and aqueous extracts of different parts of bael were dried up completely and the weight of dry mass was noted. The dry mass of acetone and ethanolic extracts was redissolved in appropriate amount of methanol to make the stock solution (5000 µg/mL). Since, the dry mass of water extract was not soluble in pure methanol, hence, it was redissolved in 50% (v/v) methanol:water to make the stock solution. From stock solution, different concentrations (100 to 5000 µg/mL) were made by appropriate dilutions with respective solvents (*i.e.* methanol for ethanol extracts and with methanol: water for water extracts). For evaluation of antioxidant activity, in 0.2 mL of extracts (various concentrations), 3 mL of 2,2'-diphenyl- 1-picrylhydrazyl radical (DPPH; 0.1 mM in 100% methanol) was added and mixed thoroughly for 5 min. For antioxidant activity in water extracts (various concentrations), DPPH stock solution was prepared in 50% (v/v) methanol: water and remaining procedure was same. A control was also made containing 0.2 mL of each solvent instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-visible double beam spectrophotometer Model 2203 (Systronics Co.) against a blank containing respective solvent. Three replications were carried out for each sample. A graph was drawn by plotting percent DPPH free radical scavenging activity (y-axis) against extract concentration (x- axis). Then using the Microsoft Excel Software, a quadratic regression equation ($y = ax^2 + bx + c$) was obtained. By putting y = 50% in the equation $y = ax^2 + bx + c$; it was converted to the form $ax^2 + bx + c = 0$. IC_{50} was calculated from the equation $ax^2 + bx + c = 0$ by using the formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

Where, $x = IC_{50}$ (µg/mL)

2.4 Calculation

The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$\%\ DPPH^*_{sc} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

3. Results and Discussion

3.1 Extract yield: Extract yield of different extracts (acetone, ethanol and aqueous) of different parts (leaves, stem-bark, roots and fruit) of bael varied widely and is given in Table 1. Extract yield of ethanol extracts of different parts of bael was highest (ranged from 9.18 to 37.42 g/100g) followed by aqueous extracts (ranged from 2.65 to 35.40 g/100g) and acetone extracts (ranged from 1.03 to 7.86 g/100g) (Table 1). Amongst bael parts, fruit had highest mean value of extract yield (26.89 g/100g) followed by leaves (13.11 g/100g), roots (6.46 g/100g) and stem-bark (4.28 g/100g). Purena *et al.* (2018) [35] reported 6.0% extract yield in aqueous solvent and 30.5% in hydro-ethanolic solvent of bael leaves.

Table 1: Extract yield (g/100g) of acetone, ethanol and aqueous extracts of leaves, stem-bark, roots and fruit of Bael

Sr. No.	Part Extracts	Extract yield (g/100g)			
		Acetone	Ethanol	Aqueous	Mean
1.	Leaves	3.22 ± 0.05	21.21 ± 0.10	14.90 ± 0.06	13.11
2.	Stem-bark	1.03 ± 0.03	9.18 ± 0.03	2.65 ± 0.01	4.28
3.	Roots	1.85 ± 0.02	11.71 ± 0.05	5.81 ± 0.05	6.46
4.	Fruits	7.86 ± 0.06	37.42 ± 0.06	35.40 ± 0.11	26.89
Range		1.03 – 7.86	9.18 – 37.42	5.81 – 35.40	
Mean		3.49	19.88	14.69	

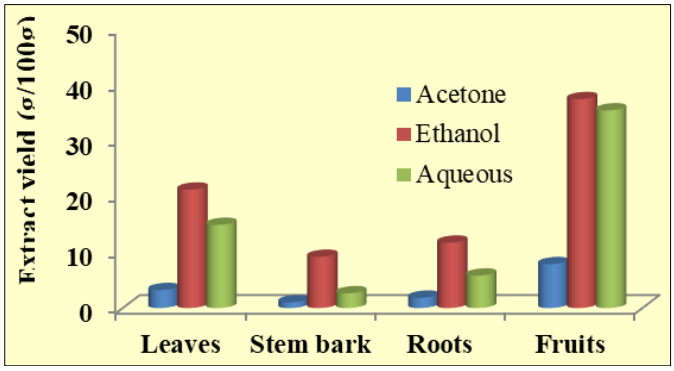

Fig 1: Extract yield (g/100g) of acetone, ethanol and aqueous extracts of different parts of Bael

3.2 Total phenolics content: Total phenolics contents of promising extracts (acetone, ethanol and aqueous) of different parts (leaves, stem-bark, roots and fruit) of bael were estimated. Total phenolics content in aqueous extract was highest (ranged from 9.05 to 49.00 mg GAE/g) followed by ethanol extract (ranged from 3.21 to 13.32 mg GAE/g) and acetone extract (ranged from 0.62 to 3.75 mg GAE/g) (Table 2). Among different parts, fruit had highest mean value of total phenolics (22.02 mg GAE/g) followed by leaves (11.08 mg GAE/g), stem-bark (6.65 mg GAE/g) and roots (4.29 mg GAE/g). According to the studies performed by other research workers, Rajan *et al.* (2011) [36] reported that total phenolics in ethanolic and aqueous extract of bael fruit pulp was 158.66 mg/g and 147.66 mg/g, respectively. Behera *et al.* (2014) [37] reported that total phenolics in ethanolic and aqueous extract of bael fruit pulp was 15.588 µg/mg and 10.509 µg/mg, respectively. Purena *et al.* (2018) reported that total phenolics in aqueous extract of bael leaves was 53.37 µg GAE/ mg and in hydro-ethanolic extract of bael leaves was 75.13 µg GAE/

mg. Shrivastava and Shrivastava (2018) [38] reported that total phenolics in aqueous extract of bael leaves were 1.138 mg/100g. Tagad *et al.* (2018) [39] reported that total phenolics in aqueous extract of bael fruit pulp was 76.28 mg TAE/g fw and in ethanol extract was 80.59 mg TAE/g. Vardhini *et al.* (2018) [40] reported that total phenolics in aqueous extract of bael fruit was 343.00 µg/mg. Hence, review of reported literature revealed the values of total phenolics either in similar range or in slightly lower/higher range.

Table 2: Total phenolics (mg GAE/g) in acetone, ethanol and aqueous extracts of leaves, stem-bark, roots and fruit of bael

Sr. No.	Part Extracts	Total phenolics (mg GAE/g)			
		Acetone	Ethanol	Aqueous	Mean
1.	Leaves	2.64 ± 0.05	8.92 ± 0.09	21.68 ± 0.34	11.08
2.	Stem bark	1.11 ± 0.06	4.66 ± 0.04	14.18 ± 0.18	6.65
3.	Roots	0.62 ± 0.02	3.21 ± 0.01	9.05 ± 0.24	4.29
4.	Fruits	3.75 ± 0.04	13.32 ± 0.11	49.00 ± 0.35	22.02
Range		0.62 – 3.75	3.21 – 13.32	9.05 – 49.00	
Mean		2.03	7.53	23.48	

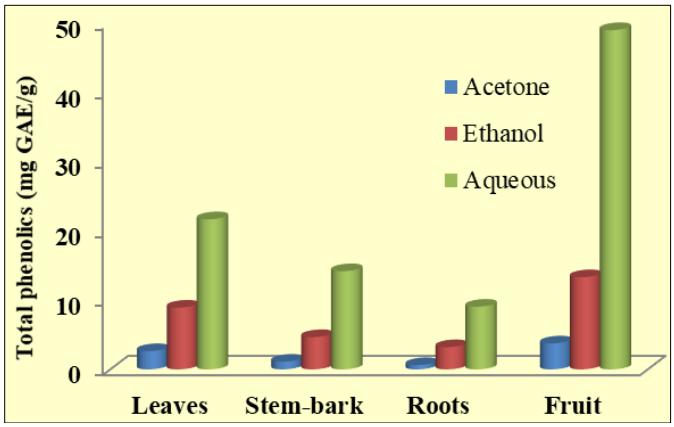

Fig 2: Total phenolics (mg GAE/g) in acetone, ethanol and aqueous extracts of different parts of Bael

3.3 Total flavonoids content: Total flavonoids content of promising extracts (acetone, ethanol and aqueous) of different parts (leaves, stem-bark, roots and fruit) of bael were estimated. Total flavonoids contents in aqueous extracts was highest (ranged from 2.14 to 11.89 mg CE/g) followed by ethanol extract (ranged from 1.49 to 4.37 mg CE/g) and acetone extract (ranged from 1.14 to 4.31 mg CE/g) (Table 3). Amongst different parts, fruit has highest mean value of total flavonoids (6.86 mg CE/g) followed by leaves (4.30 mg CE/g), stem-bark (2.14 mg CE/g) and roots (1.59 mg CE/g). Among the studies performed on total flavonoids but other research workers, Rajan *et al.* 2011 reported that total flavonoids in ethanolic and aqueous extract of bael fruit pulp was 166.3 mg/g and 129.00 mg/g, respectively. Behera *et al.* (2014) reported that total flavonoids in ethanolic and aqueous extract of bael fruit pulp was 32.305 µg/mg and 6.388 µg/mg, respectively. Purena *et al.* (2018) reported that total flavonoids 13.27 µg QE/mg in aqueous extract of bael leaves and 25.27 µg QE/ mg in hydro-ethanolic extract of bael leaves. Tagad *et al.* (2018) reported that total flavonoids in aqueous extract of bael fruit was 15.20 mg RE/g fw and in ethanol extract of bael fruit was 19.48 (mg RE/g fw). Shrivastava and Shrivastava (2018) reported that total flavonoids content in ethanol extract of *A. marmelos* leaves was 3.371 mg/100g and 2.445 mg/100g in aqueous extract Vardhini *et al.* (2018) reported that total flavonoids content in aqueous extract of *A. marmelos* fruit pulp was 21.92 µg/mg. Hence, review of reported literature revealed the values of total flavonoids either in similar range or in slightly lower/higher range.

Table 3: Total flavonoids (mg CE/g) in acetone, ethanol and aqueous extracts of leaves, stem-bark, roots and fruit of bael

Sr. No.	Part Extracts	Total Flavonoids (mg CE/g)			
		Acetone	Ethanol	Aqueous	Mean
1.	Leaves	1.60 ± 0.03	2.13 ± 0.01	9.17 ± 0.03	4.30
2.	Stem-bark	1.27 ± 0.01	1.68 ± 0.02	3.47 ± 0.02	2.14
3.	Roots	1.14 ± 0.02	1.49 ± 0.03	2.14 ± 0.01	1.59
4.	Fruit	4.31 ±0.02	4.37 ± 0.03	11.89 ± 0.01	6.86
Range		1.14 – 4.31	1.49 – 4.37	2.14 – 11.89	
Mean		2.08	2.42	6.67	

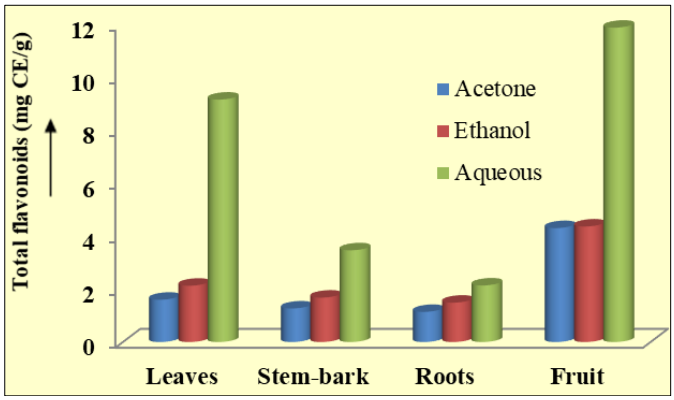

Fig 3: Total flavonoids (mg CE/g) in acetone, ethanol and aqueous extracts of different parts of Bael

3.4 DPPH free radical scavenging activity of acetone extracts of different parts of bael

DPPH is a stable free radical (purple coloured) and it gets transformed to non radical form (yellow coloured) by abstracting one electron and hence, it is widely used as a measure for the electron donation capacity of antioxidants under assay conditions. In present studies, amongst different parts of bael, DPPH free radical scavenging activity (%) of acetone extract of different parts of bael increased with increase of concentration levels (Table 4). Amongst different parts of bael DPPH free radical scavenging activity of fruit was highest ranging from 6.8 to 81.0%, followed by leaves (4.1 to 80.2%), stem-bark (3.7 to 79.5%) and roots (3.2 to 73.5%) at 100 to 5000 µg/mL concentration levels.

The IC_{50} value (µg/mL) of fruit (1436.6) was lowest in comparison to leaves (1588.2), stem-bark (1665.7) and roots (1751.2) thereby showing that fruit exhibited higher activity in comparison to leaves, stem-bark and roots. Choudhary *et al*, (2017) [41] reported that IC_{50} value (µg/ml) of acetone extract of bael leaves collected from local area of Jammu was 229.8.

Table 4: DPPH free radical scavenging activity (%) of acetone extracts of leaves stem-bark, roots and fruit of bael

Part ↓ Conc. (µg/mL)→	DPPH Free Radical Scavenging Activity (%)						
	5000	2500	1000	500	250	100	IC_{50} (µg/ml)
Leaves	80.2 ± 1.01	64.2 ± 0.61	39.2 ± 0.38	25.0 ± 0.50	16.2 ± 0.53	4.1 ± 0.58	1588.2
Stem-bark	79.5 ± 1.20	63.1 ± 0.40	37.1 ± 0.37	24.3 ± 0.70	13.2 ± 0.49	3.7 ± 0.46	1665.7

Roots	73.5 ± 1.28	60.5 ± 1.02	35.2 ± 1.09	23.2 ± 0.64	11.9 ± 0.72	3.2 ± 0.63	1751.2
Fruit	81.0 ± 1.34	67.0 ± 1.08	42.0 ± 0.90	27.9 ± 0.86	18.1 ± 0.97	6.8 ± 1.05	1436.6
Range	73.5 – 81.0	60.5 – 67.0	35.2 – 42.0	23.2 – 27.9	11.9 – 18.1	3.2 – 6.8	1436.6-1751.2

3.5 DPPH free radical scavenging activity of ethanolic extracts of leaves, stem-bark, roots and fruit of bael

In present studies, (Table 5) amongst different parts of bael, DPPH free radical scavenging activity (%) of ethanol extract of fruit was highest ranging from 7.1 to 83.1%, followed by leaves (5.4 to 81.0%), stem-bark (4.9 to 80.2%) and roots (4.0 to 74.3%) at 100 to 5000 µg/mL concentration levels. The IC_{50} value (µg/mL) of fruit (1310.7) was lowest in comparison to leaves (1527.3), stem-bark (1621.3) and roots (1713.1) thereby showing that fruit exhibited higher activity in comparison to leaves, stem-bark and roots. Dheeba *et al.* (2010) [42] reported 35.15 to 56.39% DPPH activity (from 20 to 100 µg/ml concentrations) of ethanol extracts of bark of bael of Tamil Nadu region. Kumar *et al.* (2016) [43] reported that IC_{50} value (µg/ml) of 50% ethanolic solvent of bael leaves of New Delhi region was 160.47. Raja and Khan (2017) [44] reported 86.10% DPPH free radical scavenging activity (at 800 µg/ml concentration) of ethanol extract of *A. marmelos* leaves of Allahabad region.

Table 5: DPPH free radical scavenging activity (%) of ethanol extracts of leaves, stem-bark, roots and fruit of bael

Part Conc. (µg/mL)	DPPH Free Radical Scavenging Activity (%)						
	5000	2500	1000	500	250	100	IC_{50} (µg/ml)
Leaves	81.0 ± 1.00	65.6 ± 1.05	40.1 ± 1.04	26.1 ± 1.00	17.5 ± 0.45	5.4 ± 0.53	1527.3
Stem-bark	80.2 ± 1.03	64.1 ± 1.02	38.0 ± 1.26	25.1 ± 0.80	14.8 ± 0.91	4.9 ± 0.87	1621.3
Roots	74.3 ± 1.26	61.0 ± 1.50	36.8 ± 0.80	24.1 ± 0.96	13.9 ±1.13	4.0 ± 0.87	1713.1
Fruit	83.1 ± 1.43	71.3 ± 1.40	46.1 ± 1.02	29.3 ± 1.50	19.8 ± 1.56	7.1 ± 0.85	1310.7
Range	74.3 – 83.1	61.0 – 71.3	36.8 – 46.1	24.1 – 29.3	13.9 – 19.8	4.0 – 8.7	1310.7-1713.1

3.6 Evaluation of DPPH free radical scavenging activity of aqueous extracts of leaves, stem-bark, roots and fruit of bael

In present studies, (Table 6) amongst different parts of bael, DPPH free radical scavenging activity (%) of aqueous extract of fruit was highest ranging from 8.5 to 86.6%, followed by leaves (6.2 to 82.9%), stem-bark (5.2 to 81.7%) and roots (4.6 to 75.4%) at 100 to 5000 µg/mL concentration levels. The IC_{50} value (µg/mL) of fruit (1126.8) was lowest in comparison to leaves (1386.4), stem-bark (1441.2) and roots (1632.1) thereby showing that fruit exhibited higher activity in comparison to leaves, stem-bark and roots. Dheeba *et al.* (2010) reported 33.36 to 70.30% DPPH activity (from 20 to 100 µg/ml concentrations) of aqueous extracts of bark of bael of Tamil Nadu region. Raja and Khan (2017) reported 89.12% DPPH free radical scavenging activity (at 800 µg/ml) of aqueous extract of *A. marmelos* leaves of Allahabad region. Shrivastava and Shrivastava (2018) reported 43.60 to 77.70% antioxidant activity of aqueous extract of bael leaves of Madhya Pradesh from 20 to 100 µg/ml. Vardhini *et al.* (2018) reported that maximum DPPH free radical scavenging activity of aqueous extract of bael fruit pulp of Chennai region was 60.70% at 300 µg/mL concentration.

Table 6: DPPH free radical scavenging activity (%) of aqueous extracts of leaves, stem- bark, roots and fruit of bael

Part ↓ Conc. (µg/mL) →	DPPH Free Radical Scavenging Activity (%)						
	5000	2500	1000	500	250	100	IC_{50} (µg/ml)
Leaves	82.9 ± 1.02	68.9 ± 0.84	41.8 ± 0.59	28.3 ± 1.19	19.0 ± 1.18	6.2 ± 0.99	1386.4
Stem bark	81.7 ± 1.41	67.8 ± 0.82	39.9 ± 1.03	27.3 ± 1.10	16.9 ± 0.97	5.2 ± 0.89	1441.2
Roots	75.4 ± 1.24	62.1 ± 0.95	38.5 ± 0.50	25.4 ± 0.68	14.5 ± 0.70	4.6 ± 0.63	1632.1
Fruit	86.6 ± 0.92	75.0 ± 1.23	51.2 ± 1.35	35.6 ± 0.80	24.5 ± 0.98	8.5 ± 0.50	1126.8
Range	75.4 – 86.6	62.1 – 75.0	38.5 – 51.2	25.4 – 35.6	14.5 – 24.5	4.6 – 8.5	1126.8-1632.1

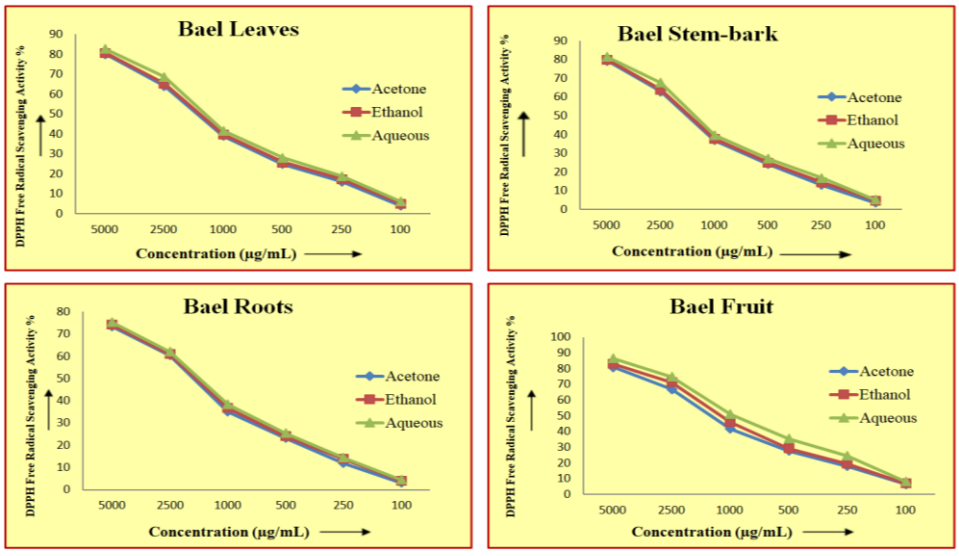

Fig 4: DPPH free radical scavenging activity (%) of acetone, ethanol and aqueous extracts in different parts of bael

3.7 Comparison of DPPH free radical scavenging activity amongst different solvents (acetone, ethanol and aqueous)

Amongst solvents (acetone, ethanol and aqueous), the IC_{50} values (µg/ml) of aqueous extracts were lowest (1126.8-1632.1) followed by ethanol (1310.7-1713.1) and acetone extracts (1436.6-1751.2). The lower values of IC_{50} represent the higher DPPH free radical scavenging activity. Hence, it was observed that aqueous extract has highest activity followed by ethanol and acetone extracts. Prashanth *et al*. (2012) [45] reported that among the five solvents (acetone, chloroform, ethyl acetate, hexane and methanol) methanol extract of bael fruit pulp has given highest activity as compared to ethyl acetate and acetone extracts. Chloroform extracts have shown very less activity and hexane extracts have not showed any activity. Reddy and Urooj (2013) [46] reported that water extract (92%) of *A. marmelos* leaves showed maximum DPPH activity followed by ethanol (88%) and methanol (78%). Raja and Khan (2017) reported that DPPH free radical scavenging activity of aqueous (89.12%) extract of bael leaves was highest followed by ethanol (86.10%) and methanol (79.10%) at 800 µg/ml. Hence, trend of DPPH free radical scavenging activity in different solvents are in consonance with the studies carried out by various researchers.

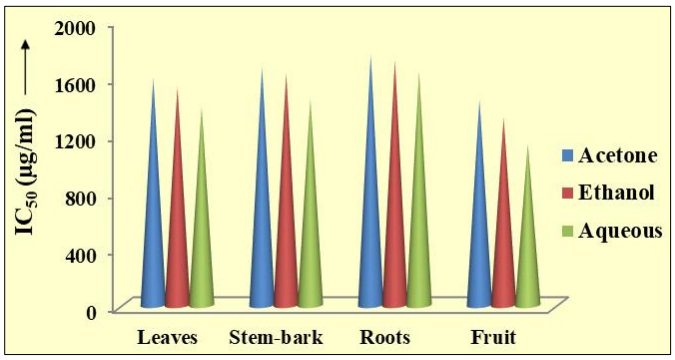

Fig 5: IC_{50} (µg/ml) values of acetone, ethanol and aqueous extracts in different parts of bael

4. Conclusion

Medicinal plants form the backbone of traditional system of medicine in India. Flavonoids and Phenolic compounds widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic. Recent researches have shown that antioxidants of plant origin with free radical scavenging properties have great importance as therapeutic agents for several diseases caused by oxidative stress. *Aegle marmelos* (Rutaceae) is popularly known as bael tree. All parts of this plant namely root, trunk, fruit, and seeds are used for curing one human ailment or another. In our present studies, Among various parts of bael, fruit was found better in terms total phenolics and total flavonoids. All the parts of bael were found to be a rich source of antioxidants and exhibited greater DPPH free radical scavenging activity in aqueous extracts in comparison to ethanol and acetone extracts.

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