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Research Article

ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF *AEGLE MARMELOS* FRUIT PULP

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ABSTRACT

Aegle marmelos fruit pulp has been used as a remedy for gastrointestinal infections of human. This study reveals the antioxidant potentials of *Aegle marmelos* fruit pulp extracts. Standard methods were adopted to screen antioxidant and phytochemical nature of *A. marmelos* fruit pulp. Results of Phytochemical screening of the aqueous extract revealed the presence of steroid, terpinoids, saponins, tannis, lignin, flavonoids. Alcoholic extract showed the availability of alkaloids and devoid of saponin. *In vitro* antioxidant activity of the plant extract revealed that both the extracts showed good antioxidant power with IC_{50} value ranges for 37.11±3.50 to 158.99±59.46 µg/ml for aqueous extract and 35.02±8.10 to 283.06 ± 135.80µg/ml for alcoholic extract.

Keywords: Antioxidant activity, Phytochemical, *Aegle marmelos,* Free radicals

INTRODUCTION

Aegle marmelos Linn. is a perenial tree, wild in the sub Himalaya tract, central and South India. This plant is commonly called as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit. It belongs to the family Rutaceae. It is indigenous to India and is used in folk medicines. The Ayurvedic practitioners use almost all of their parts but the greatest medicinal value ascribed to its fruits. Oxidative stress is produced during normal metabolic process in the body as well as induced by a variety of environmental and chemical factors which cause generation of various reactive free radicals and subsequent damage to macromolecules like DNA, Proteins and Lipids. No specific scientific evaluation of antioxidant activity of *A. marmelos* fruit pulp has been reported so far.

Therefore, it was thought worthwhile to evaluate antioxidant activity of *A. marmelos* fruit pulp to confirm its folk medicinal claim. Many naturally occurring products have been reported to contain large amount of antioxidant compounds other than vitamin C, E and carotenoid[1]. These antioxidants play a vital role in delaying, intercepting or preventing oxidative reactions catalyzed by free radical[2]. Antioxidant activity of medicinal plants might be due to the presence of phenolic compounds such as flavonoids[3,4], Phenolic acids and phenolic diterpine[5]. Synthetic antioxidants like butylated hydroxy anisole (BHA) butylated hydroxy toluene (BHT), tertiary butylated hydroxy quinone and gallic acid esters have been suspected to be carcinogenic. Hence, strong limitations have been placed on their use and there is a trend to replace them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity[6]. Hence, search for natural antioxidant has greatly been increased in the recent scenario. In the present investigation, antioxidant activity of alcoholic and aqueous extracts of *Aegle marmelos* fruit pulp was assessed.

MATERIALS & METHODS

Plant material

The Fruit of *Aegle marmelos* was collected from Tiruchirappalli, Tamilnadu, India during the month of June 2010. The plant material was identified by Dr. John Britto, Professor, Department of Botany, St. Joseph's College, Tiruchirappalli, Tamilnadu, India and specimen was deposited in department of Microbiology, Srimad Andavan Arts and Science College, Tiruchirappalli, Tamilnadu, India.

Preparation of Extracts

The powdered plant material (150gm) was extracted with water and alcohol using cold maceration method. Both the extracts were filtered with a muselin cloth and the filterate was concentrated in vacuum evaporator. Dried extracts were used for further studies[7].

Phytochemical analysis

The aqueous and alcoholic extracts of *Aegle marmelos* fruit pulp were studied for their phytoconstituents using different phytochemical tests[8].

Quantitative phytochemical analysis

Determination of tannins

The total tannin content in the lyophilized plant extract was determined by standard method[9]. The water and ethanolic extract (0.1mL) was mixed with 0.5mL of Folin- Denis reagent followed by 1mL of Na_2CO_3 (0.5% w/v) solution and made up to 10mL with distilled water. The absorbance was measured at 755nm within 30 minutes of the reaction against the reagent blank. Standard curve was prepared using 20, 40, 60, 80 and 100µL/ml tannic acid. Total tannins in extracts were expressed as equivalent to tannic acid (g TE/g extract).

Total flavanoid determination

Aluminum chloride colorimetric method was used for flavonoids determination[10]. Each plant extracts (0.5mL of 1:10g/mL-1) in methanol were separately mixed with 1.5mL of methanol, 0.1mL of 10% aluminum chloride, 0.1mL of 1M potassium acetate and 2.8mL of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415nm with a single beam Systronics UV/Visible spectrophotometer (India). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100mg/mL in methanol.

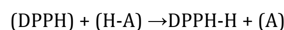
Total phenols determination

Total phenols were determined by Folin Ciocalteu reagent[11]. A diluted plant extract (0.5mL of 1:10g/mL-1) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5mL, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4mL, 1M). The mixtures were allowed to stand for 15 minutes and the total phenols were determined by colorimetry at 765nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250mg/L solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

In-vitro antioxidant assay

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

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as



(DPPH) + (H-A) →DPPH-H + (A)

(Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH-H and as consequence the absorbance were decreased from the DPPH radical to the DPPH-H form. The degree of discolouration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

Antioxidant activity by DPPH staining

An aliquot (3μL) of each sample and standard (Quercetin and Ascorbic acid) were carefully loaded onto a 10cm X 10cm Silica gel plate (silica gel 60 F254; Merck) and allowed to dry for 3 minutes. Drops of each sample were loaded in an order of decreasing concentration along the row. After 5 minutes, the TLC plate was sprayed with 0.2% DPPH in methanol. Discolouration of DPPH indicates scavenging potential of the compound tested[12].

DPPH assay by TLC

This preliminary test was performed with a rapid TLC screening method using the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) as a spray reagent[13,14]. Analytical TLC silica gel plate (10cm X10cm) was developed using chloroform : methanol : water (61:32:7) after application of 5μL of each test compound solution (1mg/mL), dried and sprayed with DPPH solution (0.2%, MeOH). After 5 minutes, the active compounds were appeared as yellow spots against a purple background. The purple stable free radical 2, 2-diphenyl-1-picrylhydrazyl was reduced to yellow diphenylpicryl hydrazine. Quercetin was used as a positive control.

DPPH radical scavenging activity (Spectrophotometer)

The free radical scavenging capacity of the extracts of *Aegle marmelos* aqueous and alcoholic extract was determined using DPPH. DPPH solution (0.004% w/v) was prepared in 95% methanol. Extracts of *Aegle marmelos* fruit pulp was mixed with 95% methanol to prepare the stock solution (10mg/100mL). The concentration of extract solution was 10mg/100mL or 100μg/mL. From stock solution 2mL, 4mL, 6mL, 8mL and 10mL of the solution were taken in five test tubes and serially diluted, this was made up to final volume of each test tubes to 10mL whose concentration was then 20μg/mL, 40μg/mL, 60μg/mL, 80μg/mL and 100μg/mL respectively. Freshly prepared DPPH solution (0.004% w/v) was added in each of these test tubes containing extracts and after 10 minutes, the absorbance was taken at 517nm using a spectrophotometer (Systronics UV-Visible Spectrophotometer 119, INDIA). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (10mg/100mL or 100μg/mL) of extracts. Control sample was prepared containing the same volume without any extract and reference ascorbic acid. 95% methanol was used as blank[15].

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 ferric ferrous complex that has an absorption maximum at 700nm. This experiment was carried out as described previously[16]. 1mL of plant extract solution (final concentration 100-500mg/L) was mixed with 2.5mL phosphate buffer (0.2M, pH 6.6) and 2.5mL potassium ferricyanide [$K_3Fe(CN_6)$] (10g/L), then the mixture was incubated at 50^{0}C for 20 minutes. To this 2.5mL of trichloroacetic acid (100g/L) was added, and centrifuged at 3000rpm for 10 minutes. Finally, 2.5mL of the supernatant solution was mixed with 2.5mL of distilled water and 0.5mL $Fecl_3$ (1g/L) and the absorbance was measured at 700nm in UV-Visible Spectrophotometer (Systronics UV-Visible Spectrophotometer 119, India). Ascorbic acid was used as standard and phosphate buffer as blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. Increased absorbance of the reaction mixture indicates stronger reducing power.

$$\%\text{ increase in Reducing Power} = \frac{A\ test}{A\ Blank} - 1 \times 100$$

A_{test} is the absorbance of test solution; A_{blank} is absorbance of blank. The antioxidant activity of the root extract was expressed as IC_{50} and compared with standard.

Nitric oxide scavenging activity

Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess reagent. Scavengers of NO compete with oxygen leading to reduce production of NO. Nitric oxide was generated from sodium nitroprusside, which at physiological pH liberates nitric acid. This nitric acid gets converted to nitrous acid and further forms nitrite ions (NO_2^-) which diazotize with sulphanilic acid and couple with naphthylethylenediamine (Griess reagent), producing pink color, which can be measured at 546nm. Sodium nitroprusside (10mM, 2mL) in phosphate buffer saline was incubated with the test compounds in different concentrations at room temperature for 30 minutes. After 30 minutes, 0.5mL of the incubated solution was added with 1mL of Griess reagent and the absorbance was measured at 546nm[12].

Superoxide radical scavenging activity (PMS-NADH System)

Superoxide anions were generated using PMS / NADH system. The superoxide anions are subsequently made to reduce nitroblue tetrazolium, which yields a chromogenic product, which is measured at 560nm. Phenazine methosulfate-nicotinamide adenine dinucleotide (PMS-NADH) system was used for the generation of superoxide anion. It was assayed by the reduction of nitroblue tetrazolium (NBT). About 1mL of nitro blue tetrazolium (156μM), 1mL NADH (468μM) in 100mM phosphate buffer of pH 7.8 and 0.1mL of sample solution of different concentrations were mixed. The reaction started by adding 100μl PMS (60μM). The reaction mixture was incubated at 25°C for 5 minutes and absorbance of the mixture was measured at 560nm against blank samples. The percentage inhibition was determined by comparing the results of control and test samples[17].

ABTS radical scavenging assay

ABTS (2, 2'-azinobis-3-ethylbenzothiozoline- 6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABTS radical was green and it was quantified spectrophotometrically at 734nm. ABTS radical cations were produced by reacting ABTS and APS. The ABTS scavenging capacity of the extract was compared with that of BHT and ascorbic acid and percentage inhibition was calculated. The stock solutions included 7mM ABTS solution and 2.4mM potassium per sulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowed them to react for 14 hrs at room temperature in the dark. The solution was then diluted by mixing 1mL ABTS solution with 60mL methanol to obtain an absorbance of 0.706 ± 0.01 units at 734nm using a spectrophotometer. Fresh ABTS solution was prepared for each assay. Plant extracts (1mL) were allowed to react with 1mL of the ABTS solution and the absorbance was taken at 734nm after 7 minutes using a spectrophotometer. All determinations were performed in triplicate (n = 3)[18].

H_2O_2 scavenging activity

H_2O_2 scavenging ability of aqueous and alcoholic extracts of *Aegle marmelos* Root was determined according to the method of Ali *et al.*,[19]. A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). The aqueous and alcoholic extracts at the 30µg/mL concentration in 3.4mL phosphate buffer were added to a H_2O_2 solution (0.6mL, 40mM). The absorbance value of the reaction mixture was recorded at 230nm. Blank solution was containing the phosphate buffer without H_2O_2.

Assessment of % inhibition and IC_{50}

Radical scavenging activity of extracts and standard were expressed in terms of % inhibition. It is calculated by using the formula $[(A_{Control}-A_{Sample}) / A_{Control}] \times 100$. Where $A_{Control}$ is the absorbance of the control, and A_{Sample} is the absorbance in the presence of the sample of aqueous and alcoholic extracts. The IC_{50} value is defined as the concentration (in µg/mL) of extracts that produced 50% antioxidant effect. IC_{50} = Concentration of extract / % inhibition X 50.

Statistical analysis

All data were expressed as Mean±SD. Statistical analysis was performed by Oneway ANOVA using Origin version 6.0 software and $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSIONS

Antioxidants may guard against reactive oxygen species (ROS) toxicities by the prevention of ROS construction, by the description of ROS attack, by scavenging reactive metabolities & converting them to less reactive molecules[20]. The antioxidant activity of *Aegle marmelos* might be due to inactivation of free radicals or complex forming with metal ions or combination thereof. The results of preliminary phytochemical screening of aqueous and alcoholic extracts of *Aegle marmelos* fruit pulp revealed the availability of multiple polar and non-polar chemical constituents (Table 1). Steroids, terpenoids, flavonoids, phenolic compounds, lignin, fat and oil, inulin, proteins, carbohydrates were found in both extracts. Alkaloids present only in alcoholic extracts whereas saponins and cardiac glycosides present only in aqueous extract. Flavonoids and Tannins are a major group of compounds that act as primary antioxidants or free radical scavengers[21].

The antioxidative characteristics might be attributed to the presence of phytochemical such as flavonoids and other polyphenolic compounds. Poly phenols have been known to show medicinal activity as well as exhibiting physiological activity. The compounds such as flavonoids; which contain hydroxyls are responsible for the radical scavenging activity in plant. Table 2 revealed the quantitative nature of flavonoids, tannins and phenolic acids.

DPPH assay is a stable free radical method. It is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract[22]. The principle of DPPH method is based on the reduction of DPPH in the presence of a hydrogen donating antioxidant due to the formation of diphenyl picryl hydrozine. Extracts reduce the colour of DPPH due to the power of hydrogen donating ability[15]. Both aqueous and alcoholic extracts produced more or less similar DPPH anion scavenging power (44.36±2.09% & 40.12±5.36% respectively) at 100µg/ml concentration with 92.648±30.68µg/ml of IC_{50} for aqueous extract & 106.15 ±25.33µg/ml of IC_{50} value for alcoholic extract and 63.99 ±25.24µg/ml for Ascorbic acid (Table 1). Figure 1 and 2 depicted DPPH anion scavenging power of extracts. Discolouration of violet DPPH to Yellow clearly demonstrated the effect of extracts as an antioxidant.

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity[23]. Reducing power is to the measure of the reductive ability of antioxidant and it is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of extracts[24]. The reduction power of aqueous and alcoholic extracts were summarized in Table 4. The data showed that reducing power of the extracts increased with increased concentration of extracts. The extracts showed potent ferric reducing power. The aqueous & alcoholic extract showed 50.33±2.08% and 28.7±12.05% reducing power at 100µg/ml concentration respectively. However, the activity was found to be less when compared to the standard. IC_{50} value for aqueous extract was found to be 158.99±59.46µg/ml & alcoholic extract was 283.06± 135.80µg/ml and 34.62 ± 9.37µg/ml for standard.

The aqueous and ethanol extract of fruit pulp of *Aegle marmelos* showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentration 100µg/ml . Aqueous extract showed 63.74±5.54% inhibition, alcoholic extract yielded 52.02±5.37 % inhibition but Ascorbic acid showed 28.64±0.67% of inhibition (Table 5). The available nitric oxide radical is linked with various carcinomas and inflammatory conditions[25]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract directly competes with oxygen to react with nitric oxide and thereby inhibits nitrite formation. The present study proved that the nitric oxide scavenging activity of the extract is better than the standard.

Superoxide anion is a harmful reactive oxygen species as it damages cellular components in biological systems [26]. Standard (Ascorbic acid) showed better superoxide radical quenching activity (21.19± 9.36%) at 50µg/ml concentration. The aqueous and ethanol extracts showed potent superoxide radical scavenging activity, 91.41±17.36% for aqueous and 147.89±44.86% for alcoholic extract at 100µg/ml concentration. The results suggested that the plant extract is a superoxide radical scavenger but efficiency is low compared to standard. PMS-NADH coupling reaction accelerates the yield of superoxide radicals from dissolved oxygen (Table 6).

Effective ABTS radical scavenging process was exhibited by both the extracts of *A. marmelos* fruit pulp 94.36±1.42% of radical scavenging activity was exhibited by aqueous extract of *Aegle marmelos*. Similarly ethanol extract showed 95.12±4.37% inhibition at 100µg/ml concentration (Table 7). The extracts were also capable of scavenging Hydrogen peroxide in a dose dependent manner & reached 73.77±3.67% for aqueous extract & 69.0±16.40% for alcoholic extract at a concentration of 100µg/ml (Table 8). Hydrogen peroxide itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout the systems of human[27]. IC_{50} for scavenging of H_2O_2 were 56.53±12.44 for aqueous extract, 52.19±18.37 for alcoholic extract and 26.67±7.51µg/ ml for standard. Scavenging of H_2O_2 by extracts may be attributed to their phenolic compounds, which can donate electrons to H_2O_2, thus neutralizing it to water[28].

Table 2: Quantitative determination of important phytoconstituents of *Aegle marmelos* fruit pulp

S. No.	Phytoconstituents	Concentration in mg/g	
		Aqueous extract	Alcoholic extract
1	Flavonoids	129.00±07.00*	166.33±09.60*
2	Tannins	098.66±12.22*	193.33±22.03*
3	Phenolic compounds	147.66±11.06*	158.66±28.67*

*p<0.05

Table 1: Qualitative Phytochemical analysis *Aegle marmelos* fruit pulp extracts

S. No	Test	Result	
		Aqueous Extract	Alcoholic extract
1	Alkaloids	Negative	Positive
2	Steroids	Positive	Positive
3	Terpenoids	Positive	Positive
4	Flavonoids	Positive	Positive
5	Saponins	Positive	Negative
6	Phenolic compounds	Positive	Positive
7	Tannins	Negative	Positive
8	Lignin	Positive	Positive
9	Phlobatannins	Negative	Negative
10	Fat and Oil	Positive	Positive
11	Inulin	Positive	Positive
12	Cardiac glycosides	Positive	Negative
13	Proteins	Positive	Positive
14	Carbohydrates	Positive	Positive
15	Aminoacids	Positive	Positive
16	Reducing sugars	Positive	Positive

Table 3: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by DPPH method

Percentage Scavenging (mean±SD) of Triplicates						
Concentration	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml	IC_{50} μg/ml
Aqueous extract	21.33±2.08**	25.06±1.00*	30±4.35**	32.3±3.78**	44.36±2.09*	92.648±30.368
Alcoholic extract	13.40±4.01***	22.48±2.04***	28.35±8.95***	29.25±9.47***	40.12±5.36***	106.158±25.332
Concentration	10μg/ml	20μg/ml	30μg/ml	40μg/ml	50μg/ml	
Ascorbic acid	4.94±6.85***	12.77±0.96**	27.96±1.41*	47.72±5.37**	55.67±2.32*	63.997±25.244

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Table 4: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by reducing power assay

Percentage Scavenging (mean±SD) of Triplicates						
Concentration	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml	IC_{50} μg/ml
Aqueous extract	5.35±0.66**	8.47±0.81**	17.44±1.36**	31.13±0.80*	50.33±2.08*	158.995±59.463
Alcoholic extract	1.95±1.33***	7.04±2.19***	11.94±1.79***	20.73±8.42***	28.7±12.05***	283.0678±135.801
Concentration	10μg/ml	20μg/ml	30μg/ml	40μg/ml	50μg/ml	
Ascorbic acid	25±5.26***	32.91±2.00**	37.31±0.97*	48.92±2.15*	60.00±1.60*	34.627±9.377

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Table 5: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by nitric oxide scavenging assay

Percentage Scavenging (mean±SD) of Triplicates						
Concentration	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml	IC_{50} μg/ml
Aqueous extract	19.3±0.8*	21.75±1.4**	26.75±1.0*	25.15±2.4**	63.74±5.54**	98.680±40.238
Alcoholic extract	11.02±2.08***	17.11±1.83**	19.33±0.51*	55.35±1.43*	52.02±5.37**	106.243±31.651
Concentration	10μg/ml	20μg/ml	30μg/ml	40μg/ml	50μg/ml	
Ascorbic acid	43.29±2.94**	37.16±2.06**	31.52±1.02*	30.23±1.34*	28.64±0.67*	47.899±30.195

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Table 6: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by superoxide radical scavenging assay method

Percentage Scavenging (mean±SD) of Triplicates						
Concentration3	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml	IC_{50} μg/ml
Aqueous extract	15.8±1.05**	22.41±1.0*	28.28±1.1*	37.99±2.6**	53.68±1.7*	91.410±17.365
Alcoholic extract	12.34±2.72***	14.74±1.03**	20.48±1.58**	22.43±2.13**	25.26±1.13*	147.85±44.86
Concentration	10μg/ml	20μg/ml	30μg/ml	40μg/ml	50μg/ml	
Ascorbic acid	52.61±2.63***	64.93±5.77**	72.54±0.80*	73.69±1.85*	75.21±1.43*	21.192±9.36

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Table 7: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by ABTS radical scavenging assay method

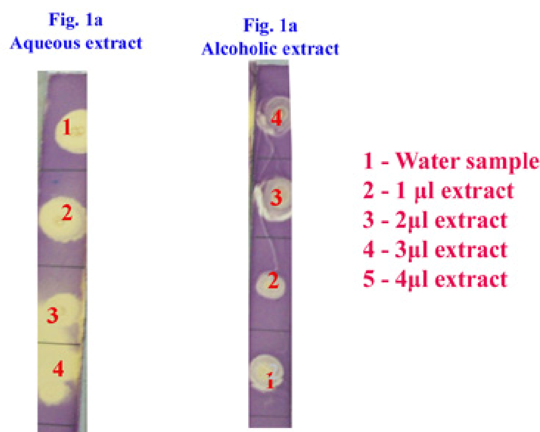
Percentage Scavenging (mean±SD) of Triplicates						
Concentration	20μg/ml	40μg/ml	60μg/ml	80μg/ml	100μg/ml	IC_{50} μg/ml
Aqueous extract	93.01±2.12***	93.54±3.31 ***	93.31±0.73 ***	95.40±1.34***	94.36±1.42 ***	37.11±13.50
Alcoholic extract	85.77±7.11***	90.53±5.97***	91.57±1.40***	71.34±24.10***	95.12±4.37***	35.02±19.16
Concentration	10μg/ml	20μg/ml	30μg/ml	40μg/ml	50μg/ml	
Ascorbic acid	78.99±6.83***	89.58±2.11***	89.18±4.43***	90.51±3.6***	94.45±3.77***	16.575±8.10

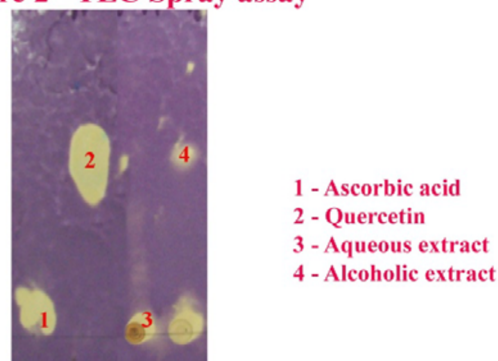
* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level

Table 8: *In vitro* Free Radical scavenging effect of *Aegle marmelos* fruit rind by H2 O2 method

Percentage Scavenging (mean±SD) of Triplicates						
Concentration	20µg/ml	40µg/ml	60µg/ml	80µg/ml	100µg/ml	IC_{50} µg/ml
Aqueous extract	39.38±2.66**	51.51±1.19*	51.40±1.56*	65.39±3.02*	73.77±3.67*	56.535±12.44
Alcoholic extract	31.70±14.79***	55.76±2.46***	56.39±2.19***	58.93±0.53***	69.0±16.40***	52.190±18.36
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	
Ascorbic acid	33.25±2.71**	43.06±2.27*	49.11±2.91**	62.88±2.42*	76.27±7.90**	26.677±7.51

* Significant at p<0.001 level ** Significant at p<0.01 level *** Significant at p<0.05 level





CONCLUSION

Based on the result in the study, it was concluded that extracts of *Aegle marmelos* fruit pulp were found to be a good natural antioxidant. Further studies are required to identify specific active principles of this plant for the significant antioxidant effect.

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