

PHYTOCHEMICAL AND ANTIOXIDANT PROPERTIES OF TENDER STEM EXTRACT OF AEGLE MARMELLOS

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ABSTRACT:

Aegle marmelos is a semitropical plant commonly known as bilva or vilvam belongs to family Rutaceae, is well known among the medicinal plants because of its diverse medicinal properties. The scientific curiosity about the plant is increased due to its potential for various medicinal properties in almost all parts of the plant. Leaves, stem and root of *Aegle marmelos* contains significant amount of tannins, alkaloids, saponins, flavanoids, proteins, carbohydrates, coumarins and steroids. It is used in preparation of several medicines as it has potential to cure various diseases. Flavones, iso flavones, flavonoids, eugenol, marmesinin and other compounds contribute its antioxidant properties. The reports on tender stem are few so in the present study the tender stem extract of *Aegle marmelos* is evaluated for its phytochemical constitution and for its antioxidant properties. The antioxidant property of the tender stem extract is estimated by 2, 2, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, the ABTS radical scavenging assay, and the super oxide radical scavenging assay. Results revealed the presence of phytochemicals like saponins, tanins, alkaloids, proteins, anthraquinones, steroids, terpenoids, phenols and carbohydrates. Also, antioxidant properties are found in the extract of tender stem.

KEYWORDS: *Aegle marmelos*, Antioxidant properties of *Aegle marmelos*, Medicinal plants, Phytochemicals.

INTRODUCTION:

Aegle marmelos Linn, commonly known as Bael, corresponding to the family Rutaceae, has been traditionally used as an indigenous medicinal product due to its wide medicinal properties. In India it is worshiped and its mention is made in Jain literature, in puranas like Garuda, Vama, Skanda, Koorma and Shiva (1). In India, it is distributed mostly in Northern India, but also found in Indian Peninsula, Burma, Bangladesh, Ceylon, Thailand and Indo-china (2). *A. marmelos* can grow to 12-15 meters, has thorny branches, thick trunk, soft peeling bark (3-4). Various phytochemicals have been extracted from the different parts of the plant like leaves, root, bark, flower, fruit and seed. These are attributed to different medicinal properties and used to treat fever, nausea, vomiting, swellings, dysentery, dyspepsia, seminal weakness, urinary problems, reducing heart palpitations, cough. The extracts are used as anti-diabetic, diaphoretic, hypolipidemic agent, antiviral, antibacterial, hepatoprotective, antioxidant, free radical scavenger (5). Bioactive substances like carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids, and terpenoids, have been found in the pulp of bael fruit. The best solvents to extract the components of this plant are methanol and water, and then ethanol [6-9]. *A. marmelos* has been studied extensively for its phytochemistry and it is found to contain various biologically active compounds. The major phytochemicals found in *A. marmelos* are Alkaloids, Tannins, Flavonoids, Terpenoids, Saponins, Glycosides. (6-9)

Although *Aegle marmelos* has been extensively studied for its medicinal properties across various plant parts such as the fruit, leaves, roots, and bark, there is limited scientific literature focusing on the tender stem. The therapeutic potential of the tender stem remains largely unexplored despite its possible significance in traditional medicine. Therefore, the present study aims to evaluate the phytochemical composition and antioxidant activity of the crude extract of the tender stem of *A. marmelos*.

MATERIALS AND METHODS:

Collection and Identification of Plant Material:

Tender stems of *Aegle marmelos* were collected from a mature and healthy tree growing in Madeenaguda, Hyderabad, Telangana, India. The collected plant material was taxonomically identified and authenticated by the Botanical Survey of India (BSI), Koti, Hyderabad (Authentication No. 145, Dated 05.06.2023). Only healthy, disease-free stems were selected to ensure the accuracy and reliability of the experimental results.

Preparation of Plant Extract:

The collected tender stems were separated from the leaves and thoroughly washed to remove adhering dust and other surface contaminants. The cleaned stems were air-dried at room temperature on blotting/buffer paper until complete dehydration was achieved. The dried stems were then cut into small pieces and pulverized into a fine powder using a traditional stone grinder.

The powdered plant material was used for the preparation of crude extracts and subsequent phytochemical investigations. The prepared samples were submitted to Life Teck Research Centre, Chennai, for phytochemical screening and evaluation of antioxidant activity.

Phytochemical Screening of Tender Stem Extract of Aegle marmelos:

The crude extract of the tender stem of Aegle marmelos was subjected to qualitative phytochemical analysis to identify the presence of various bioactive compounds. Standard procedures were followed for the detection of key phytochemical constituents (10), and the results were visually confirmed based on characteristic color changes or precipitate formation.

ABTS Radical Scavenging Activity Assay:

The ABTS radical scavenging activity of the plant extracts was evaluated using the ABTS radical cation (ABTS^{•+}) decolorization assay. The ABTS^{•+} radical was generated by reacting 7 mM ABTS solution with 2.45 mM ammonium persulfate. The reaction mixture was incubated in the dark at room temperature for 12–16 hours to allow complete radical formation. Prior to analysis, the ABTS^{•+} solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.05 at 734 nm.

Plant extracts were prepared at different concentrations. Butylated hydroxytoluene (BHT) was used as the reference standard, while methanol served as the control. For the assay, a fixed volume of the ABTS^{•+} solution was mixed with the sample extract or standard solution and incubated for a specified period at room temperature. The reduction in absorbance was measured at 734 nm using a UV–Visible spectrophotometer.

The percentage inhibition of the ABTS^{•+} radical by the samples was calculated using the following formula:

ABTS radical scavenging activity (%) = $(A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}) \times 100$
where

^A control represents the absorbance of the control reaction, and

^A sample represents the absorbance of the sample extract or standard.

DPPH (1,1- diphenyl -2-picrylhydrazyl) Radical Scavenging Assay:

To each test tube, 3.7 mL of absolute methanol was added, while 3.8 mL was added to the blank. Then, 100 µL of BHT (standard) or 100 µL of plant extract (test samples) was added accordingly. Afterward, 200 µL of DPPH reagent was added to all tubes, including the blank. The mixtures were incubated in the dark at room temperature for 30 minutes. Absorbance was measured at 517 nm using a spectrophotometer.

Calculation: % Antioxidant activity = $(A_{\text{blank}} - A_{\text{test}} / A_{\text{blank}}) \times 100$

Where: ^A blank = Absorbance of the control (methanol + DPPH),

^A test = Absorbance of the sample or standard.

Catalase Activity Assay:

Catalase activity was estimated by the method of Sinha (15), based on the decomposition of hydrogen peroxide (H₂O₂). The residual H₂O₂ reacts with dichromate in acetic acid to form chromic acetate, producing a blue-green color upon heating, which was measured spectrophotometrically at 620 nm. A 20% tissue homogenate was prepared, and 0.1 mL of the supernatant was mixed with 0.9 mL phosphate buffer (pH 7.0) and 0.4 mL of 0.2 M H₂O₂. After 60 seconds of incubation, the reaction was terminated by adding 2.0 mL of dichromate–acetic acid reagent (1:3). The mixture was heated in a boiling water bath for 10 minutes, cooled, and the absorbance was recorded. A control without homogenate was used for baseline correction. Catalase activity was expressed as µmol of H₂O₂ decomposed per minute per mg of protein.

Superoxide Dismutase (SOD) Activity Assay:

Superoxide dismutase (SOD) activity was assayed based on its ability to inhibit the reduction of nitroblue tetrazolium (NBT) by the NADH–phenazine methosulphate (PMS) system. The reduction of NBT forms a colored formazan product, which was extracted in n-butanol and measured at 560 nm. Tender stem tissue was homogenized in potassium phosphate buffer and centrifuged at 2000 g for 10 minutes, and the supernatant was used as the enzyme source. The reaction mixture consisted of sodium pyrophosphate buffer (pH 8.3), PMS, NBT, enzyme extract, and distilled water (final volume 2.8 mL). The reaction was initiated by adding NADH and incubated at 30°C for 90 seconds, then terminated with glacial acetic acid. After extraction with n-butanol and centrifugation, the absorbance of the butanol layer was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction per minute, calculated as:

% Inhibition = $(A_{\text{blank}} - A_{\text{test}} / A_{\text{Blank}}) \times 100$

Phenolic Content Estimation:

The total phenolic content (TPC) of the sample was estimated using the Folin–Ciocalteu spectrophotometric method. In this assay, 1 mL of the sample (1 mg/mL) was mixed with 1 mL of Folin–Ciocalteu's reagent. After 5 minutes, 10 mL of 7% sodium carbonate was added, followed by 13 mL of deionized distilled water. The mixture was thoroughly mixed and incubated in the dark at 23°C for 90 minutes. Absorbance was then measured at 750 nm using a spectrophotometer. The TPC was calculated by extrapolation from a standard calibration curve prepared using gallic acid, and results were expressed as milligrams of gallic acid equivalents (GAE) per gram of sample.

Ferric Acid Reducing Antioxidant Power (FRAP) Assay:

The FRAP (Ferric Reducing Antioxidant Power) assay measures the antioxidant capacity of a sample based on its ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), forming a blue-colored Fe^{2+} -TPTZ complex that absorbs at 593 nm. To perform the assay, a working FRAP reagent is freshly prepared by mixing acetate buffer (pH 3.6), TPTZ solution in HCl, and ferric chloride solution. Samples or standards are incubated with prewarmed FRAP reagent at 37°C , and after 4 minutes, the absorbance is measured at 593 nm. A ferrous sulfate standard is used to generate a calibration reference, and antioxidant capacity is calculated using the absorbance values relative to the standard. The FRAP value (in mmol/L) is determined using the formula:

FRAP value (mmol/L) =

Absorbance of test $\times$ Fe^{2+} standard concentration / Absorbance of Fe^{2+} standard

In this assay, a Fe^{2+} standard concentration of 1000 $\mu\text{mol/L}$ with an absorbance of 0.336 at 593 nm was used.

RESULTS:

The extract of the tender stem of *Aegle marmelos* was subjected to qualitative phytochemical analysis to identify the presence of various bioactive compounds. The results were visually confirmed based on characteristic color changes or precipitate formation:

Tannins:

The addition of 0.1% ferric chloride to the extract resulted in a brownish-green or bluish-black coloration, indicating the presence of tannins.

Saponins:

On vigorous shaking of the extract with water in a graduated cylinder, the formation of a persistent foam layer confirmed the presence of saponins.

Flavonoids:

In the alkaline reagent test, the extract turned deep yellow with NaOH, which turned colorless upon adding concentrated HCl, indicating flavonoids. In Shinoda's test, the addition of dilute HCl and magnesium produced a pink color, further confirming flavonoids.

Alkaloids:

The addition of Dragendorff's reagent to the extract resulted in a yellow precipitate, indicating the presence of alkaloids.

Proteins:

Millon's reagent added to the extract led to the formation of a white precipitate, confirming the presence of proteins.

Steroids:

The addition of concentrated sulfuric acid to the extract resulted in a brown coloration, suggesting the presence of steroids.

Anthraquinones:

Upon treatment with aqueous ammonia, the appearance of a pink, red, or violet color in the aqueous layer indicated the presence of anthraquinones.

Phenols:

The addition of 10% lead acetate to the extract resulted in a bulky white precipitate, confirming the presence of phenolic compounds.

Terpenoids:

The formation of a red-brown coloration at the interface upon adding chloroform and concentrated sulfuric acid confirmed the presence of terpenoids.

Carbohydrates:

The extract was mixed with Benedict's reagent and heated in a water bath; the formation of a colored precipitate confirmed the presence of reducing sugars. (Figure 1)

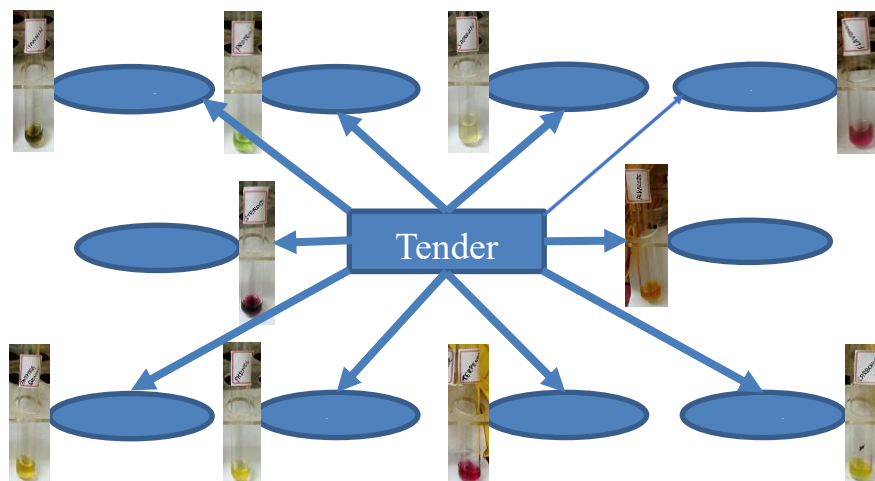


Figure 1: Depicting the various phytochemicals found in the extract of tender stem of *Aegle marmelos*.

ABTS Radical Scavenging Assay:

The results indicate that as the concentration of the extract increases (from 200 to 1000 µg/mL), the average absorbance (O.D.) decreases steadily from 0.591 to 0.357. This causes a corresponding increase in % ABTS activity (radical scavenging / inhibition) from 8.08% to 44.42% (Table 1). The relationship is dose-dependent: higher extract concentrations provide stronger antioxidant activity.

Table 1: Showing the Concentration-Dependent ABTS Radical Scavenging Activity of the extract of tender stem of *Aegle marmelos*.

Concentration (µg/mL)	Avg. O.D.	% ABTS (Scavenging)	Activity	Interpretation
200	0.591	8.08%		Low activity
400	0.533	17.10%		Mild activity
600	0.472	26.59%		Moderate activity
800	0.421	34.52%		Good activity
1000	0.357	44.42%		Strongest in this range

DPPH (1,1- diphenyl-2-picrylhydrazyl) Radical Scavenging Assay:

The results with standard BHT (Butylated Hydroxytoluene, a synthetic antioxidant) shows Control O.D. as 0.252, % Scavenging increases from 60.31% (200 µg/mL) to 86.90% (1000 µg/mL), while with tender bark extract shows Control O.D.: 0.589, Average O.D. decreases from 0.532 (200 µg/mL) to 0.198 (1000 µg/mL) and % DPPH activity (% scavenging) increases from 9.84% to 66.38% reflecting a clear concentration-dependent (dose-dependent) radical scavenging (Table 2).

Table 2: Show results from the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay

Concentration (µg/mL)	O.D	BHT % Scavenging	O.D	Sample % Scavenging
200	0.100	60.31%	0.532	9.84%
400	0.086	65.87%	0.445	24.44%
600	0.072	71.42%	0.364	38.20%
800	0.053	78.96%	0.281	52.29%
1000	0.033	86.90%	0.198	66.38%

Catalase activity assay:

The results of the study indicate that as the concentration of the extract increases (200 to 1000 µg/mL), the average O.D. increases from 0.109 to 0.332. This results in a corresponding increase in catalase activity (%) from 0.858% to 2.614% (Table 3). The response is clearly dose-dependent: higher extract concentrations lead to greater apparent catalase-like activity.

Table 3: Showing Catalase Activity of the tender stem extract at Varying Concentrations (µg/mL)

Concentration (µg/mL)	Avg. O.D.	Catalase Activity (%)
200	0.109	0.858%
400	0.171	1.346%
600	0.221	1.740%
800	0.283	2.228%
1000	0.332	2.614%

Superoxide Dismutase (SOD) activity assay:

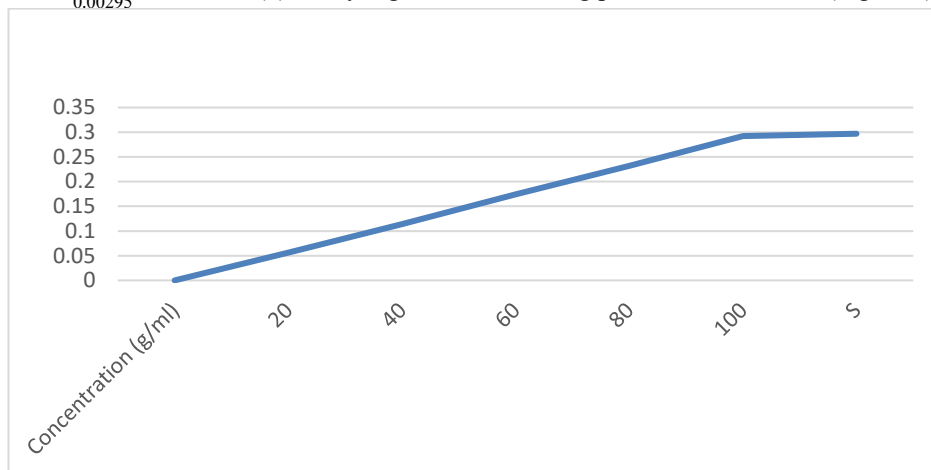
SOD assay results show that the average O.D. decreases from 0.449 to 0.181 as the extract concentration increases (200 to 1000 µg/mL or equivalent). This leads to a progressive increase in SOD activity from 6.06% to 62.13% (Table 4). The response is strongly dose-dependent: higher concentrations yield greater superoxide radical quenching.

Table 4: Show results from a Superoxide Dismutase (SOD) activity assay for the tender stem of *Aegle marmelos*.

Concentration (µg/mL)	Avg. O.D.	SOD Activity (%)
200	0.449	6.06%
400	0.399	16.52%
600	0.332	30.54%
800	0.256	46.44%
1000	0.181	62.13%

Phenolic Content Estimation:

The results reveal the tender stem extract O.D. as 0.297. This indicates the phenolic content as 102 µg/mL GAE (from equation: $x = \frac{0.297 - (-0.0034)}{0.00295} \approx 101.8$.) a very high value indicating phenolic-rich extract (Figure 2).



X - axis: Std Conc., Y – axis: Std O.D, S: Sample with O.D. 0.297

Figure 2: Shows the Phenolic content with respect to standard and tender stem extract.

Ferric Acid Reducing Antioxidant Power (FRAP) assay:

The assay results indicate absorbance increases from 0 min (A_0) to 4 min (A_4), with the change in absorbance ($\Delta A = A_4 - A_0$) rising proportionally with sample concentration. ΔA increases from ~0.030 to ~0.092 as concentration rises from 200 to 1000 µg/ml. FRAP values rise linearly (Table 5), indicating strong dose-dependent ferric reducing capacity.

Table 5: Show dose-dependent ferric reducing antioxidant power (FRAP) values of the tender stem extract at various concentrations.

Sl.No.	Concentration (µg/ml)	0 min (A_0)	4th min (A_4)	ΔA (increase)	FRAP value
1	200	0.450	0.480	0.030	85
2	400	0.378	0.427	0.049	110
3	600	0.289	0.340	0.051	135
4	800	0.230	0.296	0.066	160
5	1000	0.158	0.250	0.092	185

DISCUSSION:

The qualitative phytochemical screening of the tender stem extract from *Aegle marmelos* demonstrates the presence of various bioactive secondary metabolites, like tannins, saponins, flavonoids, alkaloids, proteins, steroids, anthraquinones, phenols, terpenoids, and carbohydrates. These findings are consistent with the plant's traditional use in Ayurvedic and folk medicine for treating ailments such as gastrointestinal disorders, inflammation, and infections. *A. marmelos* is renowned for its rich phytochemical profile across various plant parts, including stems and bark, which contribute to its therapeutic potential. The detected compounds align with prior phytochemical evaluations of *A. marmelos* bark and stem extracts, which have reported similar constituents. Below, we interpret these results by discussing the role of each compound class, their potential pharmacological activities based on established literature, and the overall implications for health and further research (11-13).

1. Tannins

In *A. marmelos*, tannins contribute to antimicrobial and antidiarrheal effects by binding to proteins and inhibiting microbial enzymes. Studies on bark extracts have linked tannins to wound-healing and anti-inflammatory activities, potentially through free radical scavenging and modulation of pro-inflammatory cytokines. Their presence supports the use of bael stems in treating dysentery and skin infections (14).

2. Saponins

Saponins in *A. marmelos* stem extracts are associated with expectorant, anti-inflammatory, and immunomodulatory effects, often by disrupting microbial cell membranes or enhancing immune responses. Research highlights their role in cholesterol-lowering and anticancer activities. This aligns with the plant's ethnopharmacological applications for respiratory and digestive issues (11).

3. Flavonoids

In *A. marmelos*, flavonoids like rutin and quercetin are prevalent in stem tissues and contribute to anti-diabetic, hepatoprotective, and cardioprotective effects by inhibiting enzymes like α -glucosidase and reducing lipid peroxidation.

Their high content in extracts correlates with strong DPPH radical scavenging activity, supporting bael's use in managing chronic diseases like diabetes and inflammation (15 – 17).

4. Alkaloids

Alkaloids in *A. marmelos* stem and bark are linked to analgesic, antiviral, and antidiabetic properties, potentially through interactions with neurotransmitter systems or viral proteins like hemagglutinin-neuraminidase (11, 18).

5. Proteins

In *A. marmelos* extracts, proteins could enhance nutritional value or contribute to immunomodulatory effects, though they are less emphasized in phytochemical literature compared to other compounds. Their detection suggests potential for protein-derived therapeutics, but further isolation is needed to explore specific activities (19).

6. Steroids

Steroids in *A. marmelos* bark, such as β -sitosterol, exhibit anti-ulcer and wound-healing properties by modulating prostaglandin synthesis. This supports the plant's role in treating gastric issues, with studies showing gastroprotective effects in animal models (14).

7. Anthraquinones

In *A. marmelos*, anthraquinones contribute to purgative effects and may aid in detoxification, aligning with traditional uses for constipation. Limited studies on stem-specific anthraquinones suggest broader antimicrobial synergy with other phytoconstituents (20).

8. Phenols

Phenolic acids like gallic acid in *A. marmelos* stem extracts provide hepatoprotective and anti-cancer benefits by neutralizing free radicals and inducing apoptosis in cancer cells. Their abundance correlates with high total phenolic content (TPC) in assays, reinforcing bael's antioxidant profile (21).

9. Terpenoids

Red-brown interface coloration confirms terpenoids, volatile compounds with anti-inflammatory and antimicrobial properties. Terpenoids in *A. marmelos* extracts exhibit analgesic effects and inhibit bacterial growth, supporting uses in pain management and wound care (11).

10. Carbohydrates (Reducing Sugars)

In *A. marmelos*, carbohydrates like glucose contribute to antidiabetic effects by influencing gut microbiota, though they are more prominent in fruit than stems (11, 20).

ABTS Radical Scavenging Assay:

The results are indicative of clear Dose-Dependent Antioxidant Activity. The extract exhibits moderate to good ABTS radical scavenging activity, with scavenging increasing linearly with concentration. This is typical for plant extracts rich in polyphenols, flavonoids, tannins, and other phenolics (22). Our qualitative phytochemical screening results have shown the presence of tannins, flavonoids, phenols, terpenoids, etc. which confirms the radical scavenging activity of the extract. These classes are well-known ABTS scavengers via electron/hydrogen donation mechanisms.

The extract shows good, progressive DPPH radical scavenging that rises steadily with increasing concentration. This is characteristic of plant extracts rich in polyphenolic compounds like flavonoids, tannins, phenols, terpenoids, etc., which correlates with the phytochemical screening results. Further, strong dose-dependent antioxidant activity of the extract is clearly seen. This DPPH activity complements ABTS assay. DPPH shows slightly higher % scavenging than ABTS for phenolic-rich extracts, which is observed in the present study. The presence of tannins, flavonoids, phenols, terpenoids, etc., explains this solid free radical quenching via electron/hydrogen donation (22 -23).

Catalase activity assay:

The extract exhibits catalase-like activity, meaning it can mimic or enhance the decomposition of H_2O_2 in a dose-dependent manner. This is not true enzymatic activity from purified catalase but rather non-enzymatic or mimetic decomposition facilitated by bioactive compounds in the extract. These compounds can act as catalysts or scavengers for H_2O_2 (24).

The activity correlates with phytochemical content (tannins, flavonoids, phenols, terpenoids, saponins, etc.), which are known to exhibit H_2O_2 -scavenging or catalase-mimetic properties via electron donation or metal chelation. This complements ABTS (~44% at 1000 $\mu\text{g/mL}$) and DPPH (~66% at 1000 $\mu\text{g/mL}$) results: the extract shows broad-spectrum antioxidant action, including direct radical scavenging (DPPH/ABTS) and potential enzymatic-mimetic decomposition of H_2O_2 (catalase-like). *Aegle marmelos* is traditionally used in Ayurveda for digestive, anti-inflammatory, and antioxidant purposes, and modern studies support its role in reducing oxidative stress in models of liver toxicity, diabetes, etc., (23, 25).

The extract demonstrates good to strong SOD-like activity, effectively scavenging superoxide radicals in a concentration-dependent manner. This is typical for plant extracts rich in polyphenolics, flavonoids, tannins, and terpenoids, which act as non-enzymatic scavengers or mimetics by donating electrons/hydrogen or chelating metals involved in radical generation. The direct radical scavenging and enzyme-mimetic activity correlates with the diverse phytochemicals like tannins, flavonoids, phenols, terpenoids, etc., known for superoxide quenching (23, 26).

Aegle marmelos extracts are well-documented for enhancing/restoring SOD activity in oxidative stress models (e.g., diabetes, hepatotoxicity) (27).

High Total Phenolic Content of the extract shows exceptionally high TPC, confirming the extract's richness in polyphenols (tannins, flavonoids, phenols — all positive in phytochemical screening). This is superior to many Aegle marmelos reports (e.g., leaf/fruit methanol extracts often 16–185 mg GAE/g DW), especially for bark, which is less studied but known for tannins/phenolics (21, 23).

This may explain the traditional uses of Aegle marmelos as antioxidant, anti-inflammatory, antidiabetic via gallic acid, rutin, tannins.

The FRAP assay result clearly demonstrates potent, concentration-dependent antioxidant activity through the single electron transfer (SET) mechanism. In the FRAP assay, antioxidants in the sample donate electrons to reduce the Fe^{3+} -TPTZ complex (pale/yellow) to the intensely blue Fe^{2+} -TPTZ complex, causing the observed increase in absorbance at 593 nm over the 4-minute reaction period (28).

The nearly linear increase in ΔA and FRAP value with concentration (no saturation up to 1000 $\mu\text{g/ml}$) indicates high reducing (antioxidant) potency. Also, indicates that the extract is rich in polyphenolic compounds (e.g., phenolic acids, flavonoids, tannins), which are excellent electron donors and the primary contributors to ferric reducing power in plant/natural extracts (29).

All these compounds are found to be present in the extract as per qualitative screening results.

ACKNOWLEDGEMENTS:

the authors express sincere gratitude to BSI in providing the authentication of the plant, Life Teck Research Centre, Chennai, for phytochemical screening and evaluation of antioxidant activity, and to the persons who extended their metaphoric support in this work.

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