Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
https://www.plantsjournal.com
JMPS 2024; 12(1): 139-143

Received: 25-12-2023
Accepted: 03-02-2024

Ajay Singh
Student, Institute- Dreamz College of Pharmacy Sundernagar, Himachal Pradesh, India

Vishal Koundal
Assistant Professor, Department of QA, Institute- Dreamz College of Pharmacy Sundernagar, Himachal Pradesh, India

Kashish Bhardwaj
Assistant Professor, Department of Pharmacology, Institute- Dreamz College of Pharmacy Sundernagar, Himachal Pradesh, India

Corresponding Author:
Ajay Singh
Student, Institute- Dreamz College of Pharmacy Sundernagar, Himachal Pradesh, India

Aegle marmelos leaves: A natural antioxidant powerhouse

Ajay Singh, Vishal Koundal and Kashish Bhardwaj

Abstract
This article navigates the phytochemical profile of *Aegle marmelos*, endeavoring to furnish a comprehensive insight into its remedial potential. The inquiry employs a systematic methodology, encompassing the extraction of phytochemicals, validation assays, organoleptic characteristics, microscopic characteristics, and an evaluation of physiochemical parameters. Validation assays confirms the existence of pivotal phytochemicals, comprising alkaloids, flavonoids, tannins, saponins, and terpenoids. Organoleptic characteristics bestows sensory revelations concerning taste, aroma, appearance. Physiochemical parameters, encompassing moisture content, and ash values, furnish insights for standardization and quality assurance. In conclusion, this exploration of *Aegle marmelos*, illuminating its phytochemical constitution through the process of extraction, validation, organoleptic valuation, microscopic analysis, and physiochemical illustration. These revelations deepen our comprehension of the plant's therapeutic potency and chart a course for subsequent research and advancement in the field of natural therapeutics.

Keywords: *Aegle marmelos*, phytochemical extraction, anti-oxidant assay, identification test, microscopic, organoleptic properties, physicochemical parameters

Introduction
The journey to uncover the antioxidant activity of *Aegle marmelos* begins with the exploration of its leaves. *Aegle marmelos Corr.* (Rutaceae), commonly known as *Bael*, is a tree of Indian origin, well known from ancient period and prescribed for various ailments in Ayurveda [1-3]. Usage of *Bael* fruit in everyday life has a great nutritional, environmental as well as commercial importance. Every part of *Aegle marmelos* including stem, bark, root, leaves, fruit and seeds at all stages of maturity possess medicinal virtues and has been used in Ethno medicine to exploit its medicinal properties [4-6]. Objective of this review was undertaken to examine the antioxidant activity for the extract (methanol, ethanol) of *Aegle marmelos* (leaves) this indigenous part is trifoliate, having a round base and pointed tip [7]. Young leaves are light green and matured leaves are in dark green color. The antioxidant activity was done by using several assays like Radical Scavenging Assay (RSA) DPPH free radicals (*In vitro*) [1-2], Reducing Power Assay, inhibition of Lipid peroxidation (*In vitro*) [1], ABTS Assay 2, 2′-azinobis (3-ethylbenzothiazoline-6 sulfonic acid), β-carotene bleaching assay, ferric reducing/antioxidant power (FRAP) assay, Thiobarbituric Acid Assay (TBA) [1-3], Oxygen Radical Absorption Capacity (ORAC) test, Hydroxyl Radical Antioxidant Capacity (HORAC) test, Total Peroxyl Radical Trapping Antioxidant Parameter (TRAP) test, Total Oxyradical Scavenging Capacity (TOSC) test, the tests based upon the electron transfer Cupric Reducing Antioxidant Power (CUPRAC) test, Ferric Reducing Antioxidant Power (FRAP) test, Folin Ciocalteu test [4]. These seemingly unassuming fruits hold a remarkable tendency they possess the potential to combat free radicals. Antioxidants are the body's natural defense system against free radicals, which are unstable molecules that can cause damage to our cells and DNA [8-11]. In essence, antioxidants neutralize these harmful molecules, protecting us from oxidative stress and its associated health issues. Behind this extraordinary antioxidant activity lie *Aegle marmelos* hidden treasures phytochemical compounds [12-4]. The various phytochemical compounds are present Alkaloids, Saponins, Tannins and phenolics, Fixed oils and fats, Steroids and sterols, Flavonoids. Among these Flavonoids, phenols, and tannins are the key players contributing to the fruit's impressive antioxidant capabilities. In a world where the pursuit of well-being is a constant endeavor, *Aegle marmelos* stands as a testament to nature's wisdom [15, 16].

Its antioxidant properties make it a gift not just to India but to the world. In this article the methods and assays are selected for the detailed description of *Aegle marmelos* (leaves) for anti-oxidant activity.

Taxonomical classification: [7]

Table 1: Shows the Taxonomical classification

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Family	Rutaceae
Genus	Aegle
Species	*Aegle marmelos*

Material and Methods

Phytochemical Extraction

The leaves of plant *Aegle marmelos* has to be collected freshly during the September to November and dried by using hot air oven at temperature 55 °C or by shade drying the leaves, then ground those leaves once they are dried and passed through the sieve and contained in the air tight container. A 15 g dried powder was extracted with 50 ml solvent (methanol, ethanol and in aqueous solvent) for 6 hrs. in a mechanical shaker. After 6 hrs. the solvent and powder sample were filtered and dried. The residue of different solvent was stored in air tight container. Or 15 g of dried powder was extracted by using Soxhlet apparatus using a solvent (methanol) for 4 hrs. The extract was reduced to thick residue by evaporating the solvent on water bath and stored in an air tight container as specified [17-20].

Anti-oxidant components of *Aegle marmelos*, extracted yield from leaves according to [1]:

Table 2: Shows the Anti-oxidant components Per 100 g

Antioxidant component	Per 100 g
α- Tocopherol (mg)	27
β- carotene (µg)	8600
Glutathione (m. moles)	580
Ascorbic acid (mg)	260
Total Flavonoids (mg)	2.4
Total polyphenols (g)	2.4

Anti-Oxidant Assay

1. Radical Scavenging Assay [DPPH]

Anti-oxidant activity of the extract of *Aegle marmelos* can be determined by the radical scavenging assay, this method is based on the radical scavenging, involves the stable free radical (DPPH) 2, 2-diphenyl-1-picrylhydrazyl. The ability of extracts to scavenge DPPH radicals was determined according to the method. Briefly, 1 ml of 0.1 mM DPPH solution was mixed with 3 ml of extract (containing 100-1000 µg) in methanol. The mixture was then vortex mixed vigorously and left for 30 min at room temperature in the dark, the absorbance was measured at 517 nm [1-3].

The percentage inhibition was calculated using

Percent Inhibition = [(Ac - As)/Ac] x 100

Where, AC is absorbance of control,
As is the absorbance of sample.
The IC50 (The concentration of sample required to scavenge 50% of DPPH free radical) was calculated by plotting graph between % inhibition vs concentration.

2. Reducing Power Assay

The ability of extracts to reduce iron (III) to iron (II) was determined in this method. The extract of *Aegle marmelos* leaves (200 µg) was mixed with phosphate buffer adjusted to 6.6 ph. and potassium ferricyanide, corresponding with the solvent used. Then the mixture was incubated at 50 °C for 30 min, after the 30 min added the 2.5 ml of the trichloroacetic acid to the mixture which was then subjected to centrifuged for 10 min. The obtained supernatant fluid (2.5ml) was mixed with the distilled water (2.5ml) and $FeCl_3$ (0.5ml). the absorbance measured at 700nm as specified [1, 3]. Increased absorbance of the reaction mixture indicates increase in reducing power.

3. Lipid peroxidation (*In vitro*)

The protective action of the extracts against oxidation of a lipid system was analyzed using the method outlined by [9]. In a 30 ml test tube, an aqueous solution containing Linseed oil at a concentration of 1.5 mg/ml was introduced. This was then diluted with 5 ml of Trizma-buffer solution, which consisted of 0.25 mM Tris buffer (pH 7.4), containing 0.2% sodium dodecyl sulfate (w/v), and 0.75 mM potassium chloride. The Trizma buffer was prepared by diluting 6.075 g of Tris (Trihydroxy methyl) amino methane and 11.184 g of potassium chloride with distilled water to make up 1 liter, after which the pH of the solution was adjusted to 7.4. Lipid peroxidation was induced by adding Fenton's reagent, consisting of 1 µm FeCl3 and 0.5 µm H2O2. The mixture was then incubated for 16 hours at 37 °C in a dark environment. The reaction was halted by adding 50 µl of a 1% BHT (butylated hydroxytoluene) alcoholic solution. The resulting solution was utilized for the antioxidant activity assay [1, 3].

4. TBA Assay

The degree of oil oxidation was determined using the 2-thiobarbituric acid (TBA) assay, as described by. The reacted solution, which was mentioned earlier (1 ml), was combined with 3 ml of a 0.2% (w/v) TBA solution and 2.5 ml of 0.05 M sulfuric acid. This mixture was heated for 30 minutes in a 95 °C water bath. Subsequently, the solution was cooled in an ice bath for 5 minutes. The colored substances formed during the reaction were then extracted using 4.0 ml of 1-butanol. The absorbance of the 1-butanol layer was measured at a wavelength of 532 nm [1, 3]. The antioxidant activity (AOA) was expressed as a percentage inhibition of lipid peroxidation relative to the control, and this was calculated using the following equation:

AOA % = [(Ac - As)/Ac] x 100

Where the, A_C is absorbance of control,
A_s is the absorbance of sample.

Identification test

1. **Cardiac Glycosides:** To initiate the examination, 5 milliliters of botanical extract underwent treatment with 2 milliliters of glacial acetic acid infused with a single droplet of ferric chloride solution. Subsequently, an overlay with 1 milliliter of highly concentrated sulfuric

acid revealed the presence of a deoxy sugar, indicated by the emergence of a brown ring at the interface—a characteristic hallmark of cardioactive glycosides. Occasionally, a violet ring may appear just beneath the brown ring, and within the acetic acid layer, a gradual greenish ring formation can be observed [5].

2. **Saponins:** Approximately 2 grams of the powdered sample were boiled in 20 milliliters of distilled water using a water bath. The resulting mixture was meticulously filtered, and 10 milliliters of the filtrate were combined with 5 milliliters of distilled water. Vigorous agitation incited the formation of a resilient froth, subsequently blended with three drops of olive oil. Upon further agitation, the formation of an emulsion was closely scrutinized [5].
3. **Fixed Oils and Fats (Spot Test):** A minute quantity of the powdered material was compressed between two sheets of filter paper. The presence of oil stains on the filter paper indicated the existence of fixed oils within the sample [5].
4. **Detection of Alkaloids:** The botanical extract was mixed with a few drops of acetic acid and meticulously combined with dragendorff's reagent. The ensuing consequence was the distinctive formation of an orange-red precipitate, signaling the presence of alkaloids [5].
5. **Flavonoids:** A solution comprising five milliliters of dilute ammonia was gently introduced to the aqueous filtrate of the botanical extract. This was followed by the addition of concentrated H2SO4. The emergence of a yellow coloration within the extract indicated the presence of flavonoids. It's noteworthy that this yellow hue dissipates over time [5].
6. **Tannins and Phenolic Compounds:** For this investigation, approximately 0.5 grams of the desiccated powdered sample were boiled in 20 milliliters of water within a test tube. After filtration, a few drops of 0.1% ferric chloride were added, and careful observation detected the manifestation of a brownish-green or a blue-black coloration. Additionally, the combination of a few drops of alcohol with ferric chloride solution, when mixed with the plant extract, revealed a blue-green or red color, indicative of phenolic compounds [5].

Thin Layer Chromatography (TLC): Thin layer chromatography was carried out to know the chemical profile of ethanolic extract of *Aegle marmelos* leaves. Preparation of TLC plates The TLC plates were prepared, 25 g of silica gel-G mixed with 50 ml of distilled water and the slurry formed was uniformly. The plates were allowed to dry at room temperature and heated in an oven at 100^0c for 1 hr. Sample of the extract *Aegle marmelos* leaves. The prepared TLC plates were marked 1 cm from bottom and sample was applied on TLC plates at equal distance with the help of capillary tubes. For separation of maximum bands on TLC plates different solvent systems were used according to polarity and from that petroleum ether: ethyl acetate (02:01v/v) was selected as standard solvent system [5].

Result and Discussion

Organoleptic Properties: These properties are observed by the according to their size, shape, odor, taste and color of *Aegle marmelos* leaves are [6-16]:

Table 3: Shows the macroscopic structure and Observation

Description of the macroscopic structure	Observation
External Color	Green
Size	7- 8 cm
Shape	Lanceolate
Apex	Acute
Surface	Smooth and shiny
Odor	Characteristic
Taste	Bitter

Fig 1: The Macroscopic Characteristics of the *Aegle marmelos*

Microscopic Properties

The microscopic parameters such as stomatal index, stomatal number and palisade ratio of plant *Aegle marmelos* are [6]:

Table 4: Shows the microscopic parameters and observation

Parameters	Observation
Stomatal number	6-11
Stomatal index	16.0
Palisade ratio	7-10

Physicochemical Parameters

The physiochemical parameters are determined for those are plant *Aegle marmelos* leaves [6]:

Table 5: Shows the physiochemical parameters and Observation

Parameters	Observation
Loss on drying	0.7430
Total ash	6.012
Water Soluble ash	1.379
Acid Insoluble ash	2.372

Identification test: Ethanolic extract of plant *Aegle marmelos* leaves [5, 6]:

Table 6: Shows the Identification test and Observation

Tests	Observation
Alkaloids	Positive
Saponins	Positive
Tannins and phenolics	Positive
Fixed oils and fats	Positive
Cardio glycosides	Negative
Flavonoids	Positive

Fig 2: Shows the tests performed for the identification of the phytochemicals, the positive confirmation of the Alkaloid, Saponins, Tannins, Flavonoids and not confirmation for Cardio Glycosides.

Fig 3: Shows the confirmation of Fixed Oils Fats

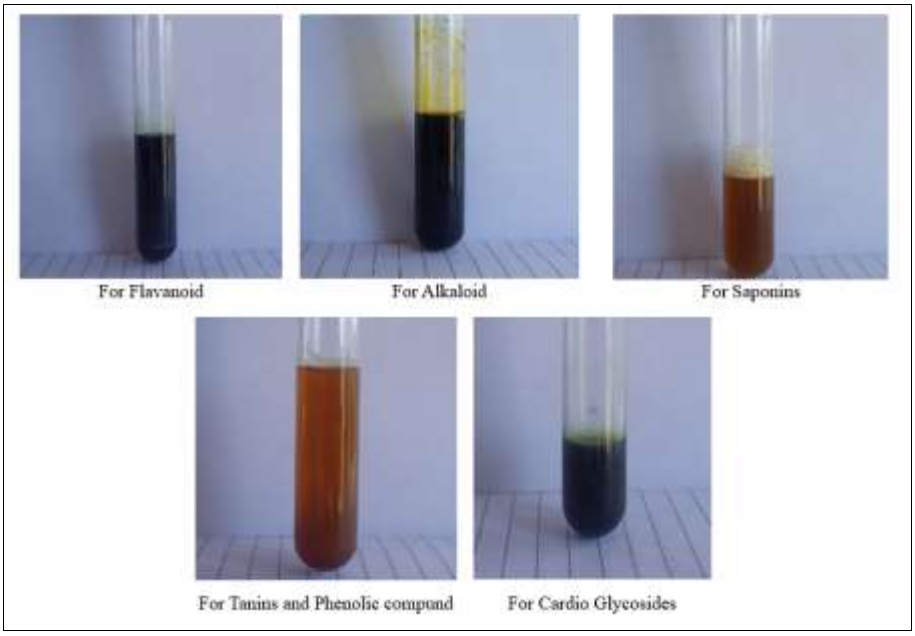

Fig 4: Thin Layer Chromatography

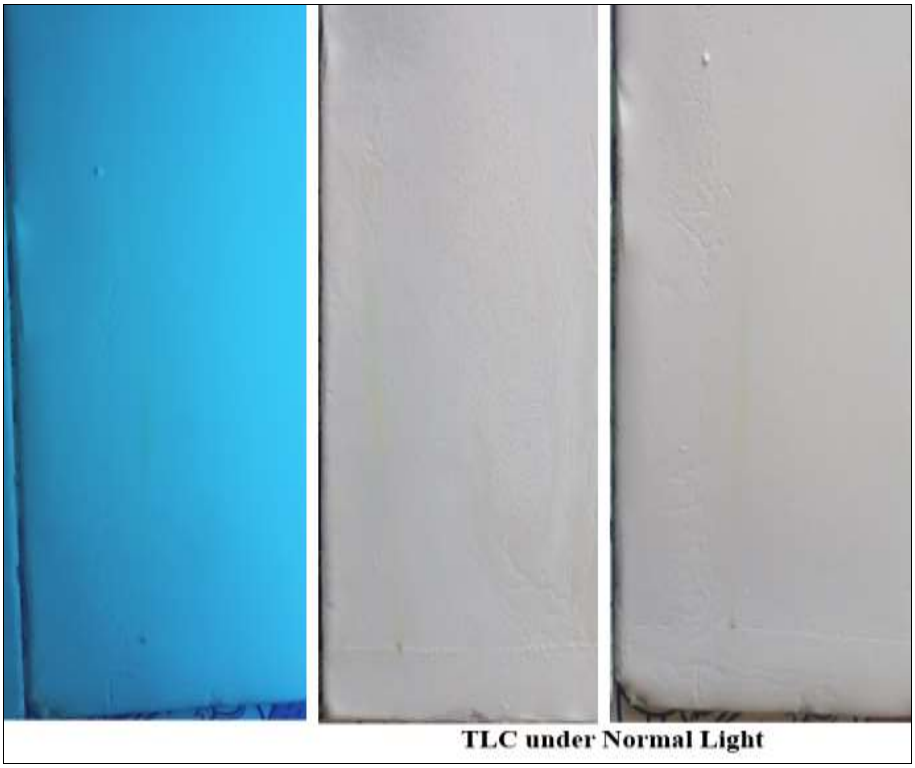

Fig 5: TLC plate Under U.V

$$\textbf{The } R_f \textbf{ value} = \frac{\textbf{distance travelled by solute}}{\textbf{distance travelled by solvent}} = 0.80$$

Reference

1. Reddy VP, Urooj A. Antioxidant properties and stability of *Aegle marmelos* leaves extracts. Journal of food Science and Technology. 2013 Feb;50:135-140.
2. Gupta D, John PP, Kumar P, Jain J. Evaluation of antioxidant activity of unripe *Aegle marmelos* corr. Fruits. Journal of Applied Pharmaceutical Sciences and Research; c2018. p. 4-7.
3. Moon JK, Shibamoto T. Antioxidant assays for plant and food components. Journal of agricultural and food chemistry. 2009 Mar 11;57(5):1655-1666.
4. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. International Journal of Molecular Sciences. 2021 Mar 25;22(7):3380.
5. GADE R, Shitole A, Wavare SH. Phytochemical investigation and thin layer chromatography of *Aegle marmelos* leaves Methanolic extract.
6. Siddique NA, Mujeeb M, Ansari SH, Ahmad S, Ali B, Aslam J. Development of quality standards of *Aegle marmelos* L. leaves. Journal of Phytology, 2010, 2(2).
7. Bhar K, Mondal S, Suresh P. An eye-catching review of *Aegle marmelos* L. (Golden Apple). Pharmacognosy Journal. 2019, 11(2).
8. Balandrin MF, Kinghorn AD, Farnsworth NR. Plant-derived natural products in drug discovery and development: An overview.
9. Srivastava J, Lambert J, Vietmeyer N. Medicinal Plants: An Expanding Role in Development. The World Bank. Washington, DC; c1996. JAPSR,
10. Chase MW, Morton CM, Kallunki JA. Phylogenetic relationships of Rutaceae: A cladistic analysis of the subfamilies using evidence from RBC and ATP sequence variation. American Journal of Botany. 1999 Aug;86(8):1191-1199.
11. Chopra RN, Chopra RN. Supplement to glossary of Indian medicinal plants.
12. Anonmyous. The Wealth of India, Council of Scientific and Industrial Research, New Delhi, 1:85-91.
13. Banquar SR, Sindinga I, Nyaigatti-Chacha C, Kanunah MP. The role of traditional medicine in a rural medicine. Traditional Medicine in Africa. English Press Ltd, Nairobi; c1993.
14. Parichha S. Bael (*Aegle marmelos*): Nature's most natural medicinal fruit. Orissa Review. 2004 Sep;9:16-17.
15. Dhankhar S, Ruhil S, Balhara M, Dhankhar S, Chhillar AK. *Aegle marmelos* (Linn.) Correa: A potential source of Phytomedicine. J Med Plant Res. 2011 May 4;5(9):1497-1507.
16. Sharma N, Dubey W. History and taxonomy of *Aegle marmelos*: a review. International Journal of Pure and Applied Bioscience. 2013;1(6):7-13.
17. Bell EA, Charlwood BV. The possible significance of secondary compounds in plant in: Secondary Plant Products. Springer-Verlag, New York; c1980. p. 11-21.
18. Chander SP, Vivek B, Nitin B. A review on bael tree. Natural Product Radiance. 2007;6(2):171-178.
19. Tiwari L, Pant D, Sharma KN, Sarkar M, Lohar DR. Pharmacognostical evaluation of *Aegle marmelos* (L) Correa ex Roxb. leaf. Journal of Scientific Speculations and Research. 2010;1(1):13-18.
20. Constabel F, Gamborg OL, Kurz WG, Steck W. Production of secondary metabolites in plant cell cultures. Planta medica. 1974 Mar;25(02):158-65.
21. Mangla M, Shuaib M, Jain J, Kashyap M. *In-vitro* evaluation of antioxidant activity of *Curcuma caesia* Roxb. Int J Pharm Sci Res. 2010;1:98-102.
22. Rishabha M, Ajay K, Anupama S, GT K. Pharmacological screening, Ayurvedic values and commercial utility of *Aegle marmelos*. Int. J Drug Dev. & Res. 2012 Jan;4(1):28-37.
23. Hema CG, Lalithakumari K. Screening of pharmacological actions of *Aegle marmelos*. Indian Journal of Pharmacology. 1988 Apr 1;20(2):80-5.