



PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF AEGLMARMELLOS LEAVES

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ABSTRACT: Phytochemical analysis conducted on the aeglmarmelos leaves extracts revealed the presence of constituents which are known to exhibit medicinal activities. The Aeglemarmelos possessing significant broad spectrum antibacterial efficacy. From the above research it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. The extracts showed a significantly high antibacterial activity against the microorganisms. The data clearly depicts the presence of compounds used for treating various bacterial diseases, indicating its use in the traditional system of medicine since ancient times. Further, the broad-spectrum activity of aqueous, methanol, and aqueous: ethanol extracts proves to be encouraging in the development of novel antimicrobial formulation in the near future.

KEYWORDS: Medicine, Phytochemicals, Metabolites, Free radicals, Antioxidant

INTRODUCTION: Plants are not only primary source for our food but also they have immense therapeutic potential. Plant metabolites (phytochemicals) are used to cure many of the human ailments. Plant's secondary metabolites have been of interest to man for a long time due to their pharmacological relevance (Arora, Kaur & Kaur, 2003). Oxidation is a natural process in organisms for the production of energy to fuel biological cycles. Conversely, the uninhibited production of oxygen-derived free radicals, this is involved in the onset of

many diseases such as arthritis, atherosclerosis, rheumatoid and cancer as well as in many degenerative diseases related with aging (Halliwell et al., 1984). Most of the antioxidant compounds in a typical diet are derived from plant sources and belong to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates, have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants.

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. The unripe and ripe fruits of *A. marmelos* (L.) are bitter, acrid, sour, astringent, digestive and stomachic and are useful in diarrhea, dysentery and stomachalgia (Khanna et al., 1991). Stem bark is used in fever.

Aegle marmelos commonly known as Bilva or Sriphal or Shivadruma (the tree of shiva) in Sanskrit, Bel or Bael in Hindi, and as wood apple, stone apple, Bengal quince, Indian quince, holy fruit or golden apple in English, is an important medicinal tree in India. Bael trees are indigenous to India and are found growing in abundance in the Himalayan regions, Bengal, Central and South India, as well as in Srilanka, Burma, Thailand, Bangladesh, Nepal, Vietnam, Laos, Cambodia, and Pakistan. Bael leaves are offered to the Indian deity, Lord Shiva, and the tree is planted extensively in temples for this reason (Das and Das, 1995; Maity et al., 2009). (1) *Aegle marmelos* (Linn) correa, commonly known as bael (or bel), belonging to the family Rutaceae, is a moderate sized, slender and aromatic tree. A number of chemical constituents and various therapeutic effects of leaves of *A. marmelos* have been reported by different workers. Extensive investigations have been carried out on different parts of *Aegle marmelos* and as a consequence, varied classes of compound viz., alkaloids, coumarins, terpenoids, fatty acids and amino acids have been isolated from its different parts. Broadly, *Aegle marmelos* leaves contain alkaloids, Phenylpropanoids, terpenoids and other miscellaneous compounds whereas potential pharmacological activity of the leaves are hypoglycemic, anti-inflammatory, antimicrobial, anticancer, radioprotective, chemopreventive and anti-oxidative activity.

It is indigenous to India and is abundantly found in the Himalayan tract, Bengal, Central and South India.

It is extensively planted near Hindu temples for its wood and leaves which are generally used for worship.

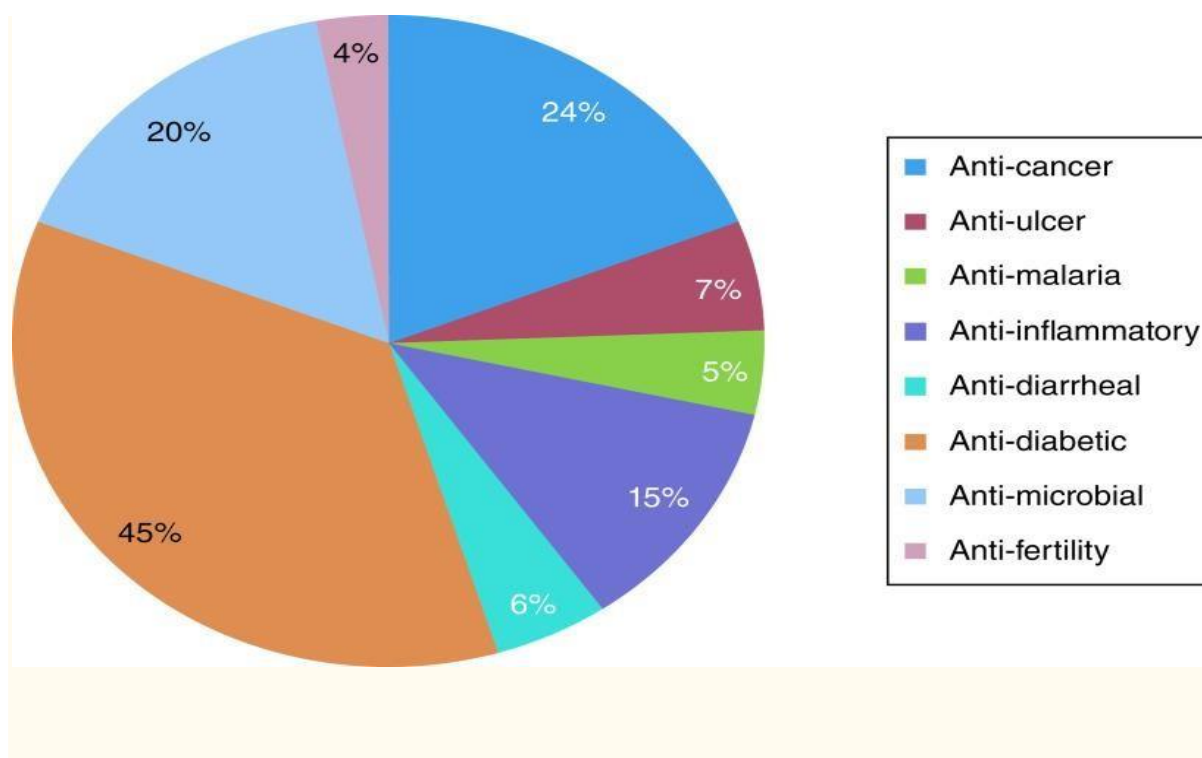
Its branches are armed with sharp straight spines. The bark is soft, light grey and exfoliating in irregular flakes. The bright green leaves are alternate and trifoliate (rarely five-foliate). The flowers are greenish

white and sweet-scented, fruits are yellowish grey and globose with woody rind and seeds are numerous, oblong and compressed. The roots are fairly large, woody and often curved.^{1,2} Fresh leaf juice is used in asthmatic complaints and jaundice. The Chinese used the leaves and young fruits to adulterate Opium. In Bengal it is used for dysentery. In Konkan, small and unripe fruits are used for piles. The juice of bark is a remedy for poverty of seminal fluid.

Medicinal Properties: Different medicinal system like Siddha, Unani and Ayurvedic systems provide information about the potential effects of bael. This is an important medicinal plant having traditional and folk medicines and ethno medicinal applications. For diarrhoea and dysentery treatment, bael fruit is having traditional application. This plant leaves are the causes of abortion or infertility in women. Bael can be considered as an important medicine in Ayurveda for treating chronic diarrhea, dysentery, brain tonic, etc. A good combination of five parts of bael such as root, bark, leaf, flower and fruit can be highly considered as an effective agent for the treatment of certain mental disorders. Fruit powder of bael produces anti-cancer activity. The leaves are supposed to reduce bowel complaints, bleeding piles, diarrhea, and dysentery. Its leaf extract is used to cure ophthalmic, ulcer and intestinal worms by twice daily intake. Treatment of eye diseases requires poultice that are obtained from bael leaf. Leaf juice is having a number of medicinal importance especially for controlling diabetes.

Pharmacological activity

Pharmacological activity is essential in herbal plants. The various acts of *A. marmelos*, which have been reported scientifically and investigated.



MATERIALS AND METHOD:

Table 1: List of reagent and chemicals

S. No.	Reagents and chemicals	Company Name
1.	Glacial Acetic Acid	Clorofilt ind
2.	Petroleum ether	Clorofilt ind
3.	Conc. H ₂ SO ₄	Clorofilt ind.
4.	Ethanol	Clorofilt ind
5.	Mayers Reagents	Clorofilt ind
6.	Sodium Hydroxide	Merk
7.	Ammonia	Merk
8.	Copper Sulphate	Clorofilt ind
9.	Conc. HCl	Clorofilt ind
10.	Dragendroff Reagents	Clorofilt ind
11.	Chloroform	Clorofilt ind.
12.	Lead Acetate	Rankeem
13.	Ferric Chloride	Thermo Fisher Scientific
14.	Ninhydrin reagent	Clorofilt ind

Table 2: List of glassware's

S.No.	Glassware	Company Name
1.	Beakers	Borosilicate
2.	Glass rod	Borosilicate
3.	Volumetric flask	Borosilicate
4.	Graduated pipette	Borosilicate
5.	Test tubes	Borosilicate

The leaf part of *A. marmelos* (L.) was collected and dried under shade (Harborne, 1973). This dried material was mechanically powdered, sieved and stored at a dry place. This powdered material was used for further phytochemical, antimicrobial and antioxidant analysis.

Plant Collection

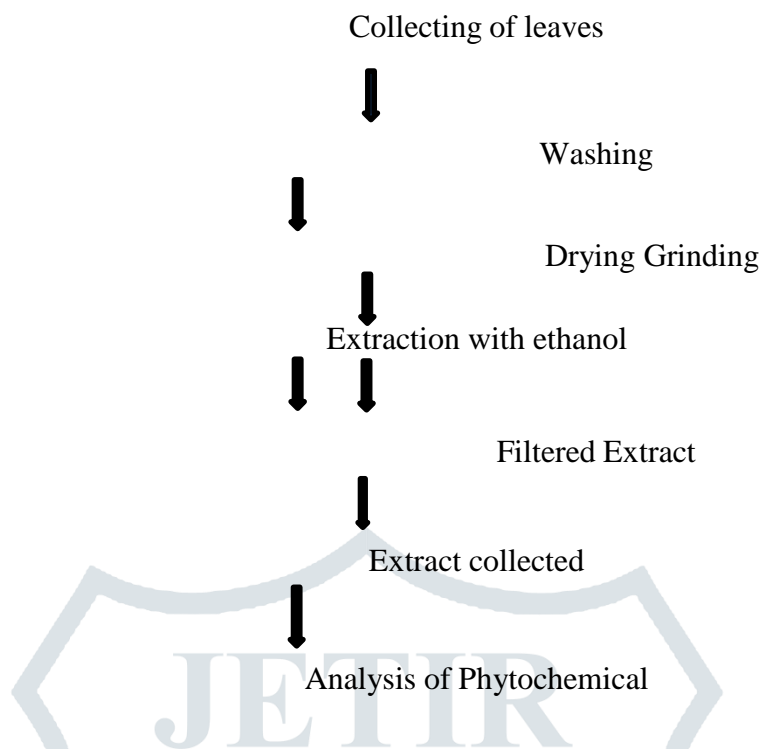
Plant was selected on the basis of literature and indigenous traditional knowledge.

Preparation of plant material:

The collected unripe fruits were cleaned, dried under sunshade in dark room, and powdered by using mechanical mixer. After size reduction fruit powder under sieve No. 40 and sieve No. 60, stored in airtight container at room temperature.

Extraction of the plant material (Maceration Method)

The leaves of the plant were properly washed in tap water and rinsed in distilled water. The rinsed leaves were hot air-dried for 3 days. The dried leaves of each plant were pulverized using pestle mortar to obtain a powdered form which was stored in airtight glass containers at 4°C until used. 10 g of powdered sample was soaked in distilled water and methanol (200 mL and 100 mL) separately for 12 hrs at room temperature. The extracts were then filtered and concentrated to a final volume of 50 mL and subjected to phytochemical analysis.



The fat and plant extract samples were analyzed for the presence of alkaloids, terpenoids, tannins, flavonoids, saponins, and steroids (Adetuyi *et al.*, 2001)

Phytochemical Analysis: Preliminary phytochemical screening done for different active ingredients present in plant for alkaloids, Flavonoids, Tannins, Saponins, Glycosides, Protein & amino acids.

Antioxidant activities

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) assay

The scavenging activity of *A. marmelos* (L.) leaf extracts on DPPH was determined using the method described by [Choi, C.W.; Kim *et al.*]. This method depends on the reduction of purple DPPH to a yellow colored diphenyl picrylhydrazine. The determination of the disappearance of free radicals was done using spectrophotometer [Abdel- Hameed *et al.*]. The remaining DPPH which showed maximum absorption at 518 nm was measured. Each plant extract sample s stock solution (1.0 mg/mL) was diluted to final concentrations of (0.5, 0.4, 0.3, 0.2, and 0.1)mg/mL, in ethanol.

RESULTS AND DISCUSSION

The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses color stoichiometrically depending on the number of electrons taken up. DPPH was used to determine the proton

radical scavenging action of ethanolic extracts of the leaves of *A. marmelos* (L.), because it possesses a proton free radical and shows a characteristic absorbance at 517 nm. The alcoholic extract of the leaves of *A. marmelos* (L.) reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles. A decrease in the concentration of DPPH radical was observed due to the scavenging ability of the soluble constituents in the ethanolic extract of the leaves of *A. marmelos* (L.) and the standard ascorbic acid is taken as a reference compound. Alcoholic extract of leaves of *marmelos* (L.) presented the highest scavenging activity at all concentrations. The highest activity was found to be 95.3% at 0.5mg/ml concentration of alcoholic extract of the leaves of *A. marmelos* (L.), whereas 93.7% of scavenging activity was shown by ascorbic acid at the same concentration.



Table.1 Observation table for phytochemical screening

S. No.	TESTS	PRESENCE AND ABSENCES OF PHTYOCHEMICAL TEST
1.	ALKALOIDS	
	Dragendroffs test	Present
	Mayers reagent test	Present
2.	GLYCOSIDE TEST	
	bortrager test	present
	legals test	Absent
3.	PROTEIN AND AMINO ACIDS	
	biuret test	Present

	ninhydrine test	Absent
4.	FLAVONOIDS TEST	
	shinoda test	Absent
	lead acetate test	Present
5.	TANNIN TEST	
	ferric chloride test	Present
6.	SAPONIN TEST	
	foam test	Absent

Table. 2 Observation table for antioxidant activity of ascorbic acid

S. No.	Concentration (mg/ml)	O.D. (Blank - Test)	% Scavenging capacity
1	0.1	0.05	68.75
2	0.2	0.04	75.00
3	0.3	0.03	81.25
4	0.4	0.02	87.50
5	0.5	0.01	93.75
6	Control	0.16	0

Table. 3 Observation table for antioxidant activity of plant extract

S. No.	Concentration (mg/ml)	O.D. (Blank - Test)	% Scavenging capacity
1	0.1	0.17	73.43
2	0.2	0.13	79.68
3	0.3	0.11	82.81
4	0.4	0.06	90.62
5	0.5	0.03	95.31
6	Control	0.64	0

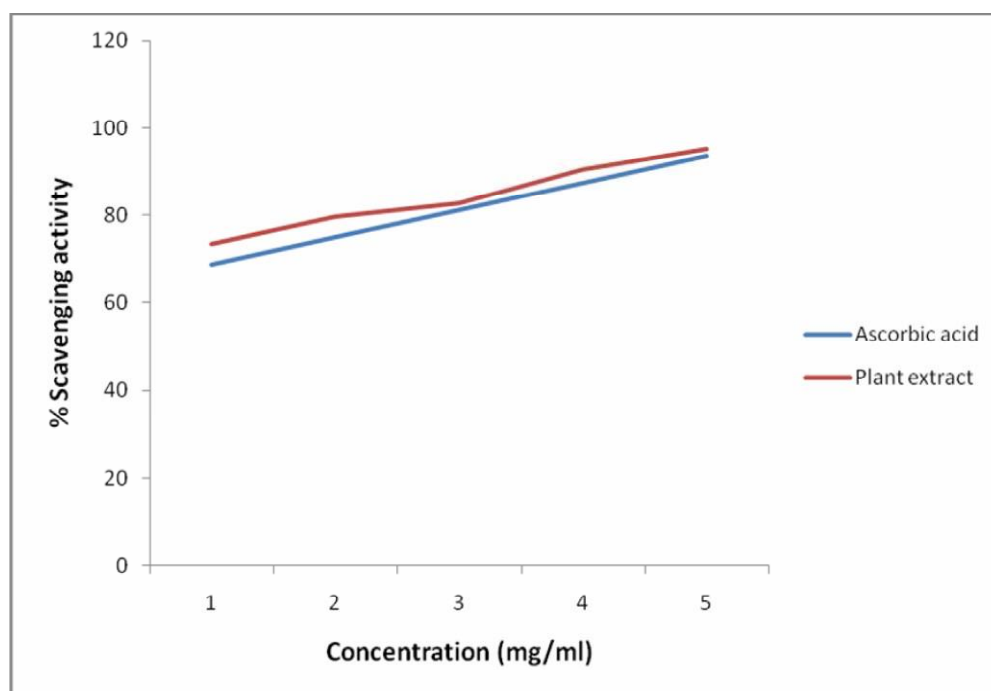


Figure 1 Comparative DPPH scavenging activity

SUMMARY & CONCLUSION

Medicines derived from plants have made immense contribution towards the betterment of human health and act as a source of inspiration for novel drug compounds. From the above research it can be concluded that this plant has immense potential to be used in the area of pharmacology and as a prospective source of valuable drugs. Due to the presence of various compounds that are essential for good health, it can also be used to improve the health status of society. The extracts showed a significantly high antibacterial activity against the microorganisms. The data clearly depicts the presence of compounds used for treating various bacterial diseases, indicating its use in the traditional system of medicine since ancient times. Further, the broad-spectrum activity of aqueous, methanol, and aqueous: ethanol extracts proves to be encouraging in the development of novel antimicrobial formulation in the near future.

A spectrum of compounds showing strong antibacterial, antioxidant, and anti-inflammatory activities was revealed by the GC-MS analysis of the methanolic extract of *Aegle marmelos*.

Antimicrobials derived from plants possess vast curative properties since they have fewer side effects as compared to synthetic antimicrobials drugs. *Aegle marmelos* is of utmost importance for ethnobotanical purposes, and it has been placed in the priority list of thirty-two medicinal plants by

The National Medicinal Plants Board of Govt. of India [62]. The present study contributes to the current knowledge of presence of various phytochemical active compounds in 18 varieties/accessions of *Aegle marmelos* possessing significant broad spectrum antibacterial efficacy. Further fractionation and purification will elucidate the potential compound, which is a pressing

need because of the upcoming resistance of the currently available antibiotics.



REFERENCES

1. Singh, R. N. and Roy, S. K. The Bael: Cultivation and Processing. New Delhi: ICAR (1984).
2. Subhadrabandhu, S. Under-Utilized Tropical Fruits of Thailand. Food and Agriculture Organization of the United Nations Regional Office for Asia and the Pacific, Bangkok, Thailand (2001).
3. Roy, S. K. and Khurdiya, D. S. Other subtropical fruit. In D. K. Salunkhe; S. S. Kadam (ed.), Handbook of Fruit Science and Technology: Production, Composition, Storage and Processing. New York: Marcel Dekker (1995).
4. Arseculeratne, S. N., Gunatilaka, A. A. L. and Panabokke, R. G. Studies on medicinal plants of Srilanka: occurrence of pyrrolizidine alkaloids and hepatotoxic properties in some traditional medicinal herbs. Journal of Ethnopharmacology (1981) 4: 159-177.
5. Karunanayake, E.H., Welihinda, J., Sirimanne, S.R. and Sinnadorai, G. Oral hypoglycaemic Tagad et al RJLBPCS 2018 www.rjlbpcs.com Life Science Informatics Publications © 2018 Life Science Informatics Publication All rights reserved Peer review under responsibility of Life Science Informatics Publications 2018 Sept – Oct RJLBPCS 4(5) Page No.790 activity of some medicinal plants of Srilanka. Journal of Ethnopharmacology (1984)11: 223-231.
6. Singh, Y.N. Traditional medicine in Fiji: Some herbal folk cures used by Fiji Indians. Journal of Ethnopharmacology (1986) 15: 57-88.
7. Nagaraju N, Rao KN. A survey of plant crude drugs of Rayalaseema, Andhra Pradesh Indian. J Ethnopharmacol. (1990) 29:137–158.

8. Morton. J. F. Fruits of Warm Climates. Winterville,N.C.: Creative Resource Systems (1987). 9. S. Rajan, M. Gokila, P. Jency , P. Brindha, R. K. Sujatha antioxidant and phytochemical properties of aeglemarmelosfruit pulp, International Journal of Current Pharmaceutical Research (2011): 65-70.
10. Subhash Chandra, Sarlasaklani In vitro antibacterial, antifungal activity, nutritional evaluation and phytochemical screening of wild edible fruit of Aeglemarmelos, Journal of pharmacy research, (2011)4(12): 6412-4614.
11. Baliga, M. S., Bhat, H. P., Pereira, M. M., Mathias, N., &Venkatesh, P. Radioprotective effects of Aeglemarmelos (L.) Correa (Bael): A concise review. Journal of Alternative and Complementary Medicine, (2010) 16: 1109–1116
12. S. Sharmila, P.A. Vasudara Devi Comparison of invitro antioxidant activity of theethanolic extract of ripe and unripe fruit of Aegle marmelos, Journal of pharmacyresearch, (2011) 4(3): 720-722.
13. Siddhique N A, Munjeeb N. Development of quality standards of aegle marmelos leaves, J phyt, (2010) 36-43.
14. Abdel-Hameed., E.S.S., Total phenolic contents and free radical scavenging activity of certain Egyptian, Ficus species leaf samples. Food Chem. 2008, 11331138.
15. Adetuyi A.O., Popoola A.V., Extraction and dyes ability potential studies of the colourant in zanthoxylum zanthoxyloides plant on cotton fabric. Journal of Science Engineering Technology. 2001, 8 (2): 3291-3299.

