ISSN-Online: 2320-7876
ISSN-Print: 2319-1775

Original Article

Phytochemical screening and *in vitro* antioxidant activity of *Aegle marmelos* leaf extract

Sheeja T Tharakan[1]*, Neethu KM [1]

[1]Department of Botany, Vimala College (Autonomous),Thrissur- 680009, Kerala, India

Abstract

The present work is based on the study of the antioxidant properties of *Aegle marmelos* (L). correa. This plant sometimes recognized as bael, is widely renowned for its medical uses and is utilized to cure a variety of illnesses. Several kinds of phytochemicals such as tannins, alkaloids, phenols, resins, coumarins, etc. are present in the leaf extract of *A. marmelos*. To find the existence of secondary metabolites in the leaf extracts, phytochemical screening was carried out. The ABTS and DPPH radical scavenging activity were applied to assess the antioxidant properties of *A. marmelos*. Diverse extracts of *A. marmelos* possess antioxidant activity.

Address for correspondence: Sheeja T Tharakan, Department of Botany, Vimala College (Autonomous),Thrissur- 680009, Kerala, India .
E-mail: sheejatharakan@gmail.com
Submitted: 14-Aug-2021 **Revised:** 06-Dec-2021 **Published:** 19-Jan-2022

Introduction

Medicinal plants have been a vital source of medical therapy preparation for human beings. The discovery and use of herbs with medicinal properties were closely linked with human civilization from ancient times (1). Since there are around 500,000 plants in the globe and the majority of them have not yet been explored in medical practice, current research, or future studies, medicinal herbs have a bright future. Medicinal plants are used as a medicinal resource in almost all cultures (2). Medicinal plants are used for treating leprosy, intestinal worms, anorexia, skin, urinary disorder, diabetes, wounds, asthma, piles, blood morbidity, itching, and burning sensation and as curative of fever.

Various medicinal herbs and plants are used in certain nations to deter ants, flies, and other insects from entering buildings such as houses and workplaces. The existence of specific phytochemicals in medicinal plants is the primary cause of their therapeutic effects. Primary and secondary metabolites are two categories for these phytochemicals. Primary metabolites include amino acids, carbohydrates, chlorophyll, and proteins. Secondary metabolites include saponins, alkaloids, steroids, tannins, and flavonoids (3).

A. marmelos (L.) Correa, a plant from the Rutaceae family, is a significant medicinal herb. It is generally called sriphal or Bilva or shivadruman, wood apple, bael, or golden apple (4). Different A. marmelos components have various therapeutic uses. The medicinal oil made from the plant's leaves not only aids in the prevention of common cough, cold, and other respiratory illnesses. When combined with cumin seeds and used on the scalp in a massage, *Aegle* is regarded as an excellent hair tonic (5).

ISSN-Online: 2320-7876
ISSN-Print: 2319-1775

In the current work, the antioxidant activity of *A. marmelos* leaf extract and phytochemical screening were examined.

Materials and Methods

Plant materials

Aegle marmelos (L.) Correa was collected locally during the January-February periods.

Preparation of extract

The rinsed leaves were hot air-dried. Using a mixer grinder, the dried leaves of each plant were ground into powder. Then stored in airtight glass containers until used. The weighed sample was individually extracted using water, petroleum ether, and ethyl acetate. The extracts were filtered and the residue of each extract was stored in a glass beaker and covered with aluminum foil.

Extractive Value

The extraction yield is given as a percentage of the mass of the material utilized divided by the total mass of the extracts (Mext) (6).

Qualitative Phytochemical Screening

Qualitative phytochemical analysis of different leaf extracts was done by standard procedure (6).

Determination of antioxidant activity

Utilizing the ABTS DPPH and radical scavenging activity, the antioxidant activity of *A. marmelos* leaf extracts was assessed.

DPPH radical scavenging activity

The amount of DPPH radical scavenging activity was calculated by adding 1.25mL of the extracts and standard (gallic acid) at various concentrations to 1ml of 0.135mM DPPH radical in methanol. The mixture was vortexed and maintained at 25°C in the dark for 30 minutes. The absorbance was then assessed spectrophotometrically at 517 nanometer using methanol as a reference. DPPH inhibition % was calculated by applying the given formula: [absorbance of (control-sample)/absorbance of control]x100 (7).

ABTS radical scavenging activity

A 7µM concentration of ABTS was prepared by dissolving it in water. The reaction of 2.45µM potassium per sulphate with ABTS stock solution resulted in $ABTS^+$, which was then preserved at room temperature for 12 to 16 hours in the dark. When kept in the dark, the radical remained stable in this state for over 2 days. For the infusion investigation, samples including the $ABTS^+$ mixture was diluted with redistilled water to an absorbance of 734nm and equilibrated at 30°C. A0 was recorded as the reagent blank. The absorbance measurement was obtained precisely 6 minutes after the initial adding (A_t) when 3ml of diluted $ABTS^+$ was added to 30µl of polyphenolic extracts. Every determination was carried out in triplicate (8). The formula used to determine the ABTS inhibition% was [absorbance of (control-sample)/absorbance of control]x100.

Results

Preliminary phytochemical analysis of various extracts of *A. marmelos*

Alkaloids, flavonoids, triterpenoids/steroids, coumarins, tannins, resins, and phenols were discovered in distilled water, ethyl acetate, and petroleum ether extract of *A. marmelos* during preliminary phytochemical screening (Table 1).

ISSN-Online: 2320-7876
ISSN-Print: 2319-1775

Table 1: Phytochemical analysis of different *A. marmelos* extracts

Phytochemical constituents	*A. marmelos* extracts		
	Petroleum ether	Distilled water	Ethyl acetate
Alkaloids	+	+	+
Flavonoids	+	+	+
Steroids/triterpenoids	+	+	+
Tannins	+	+	+
Coumarins	+	+	+
Phenols	+	+	+
Resins	+	+	+

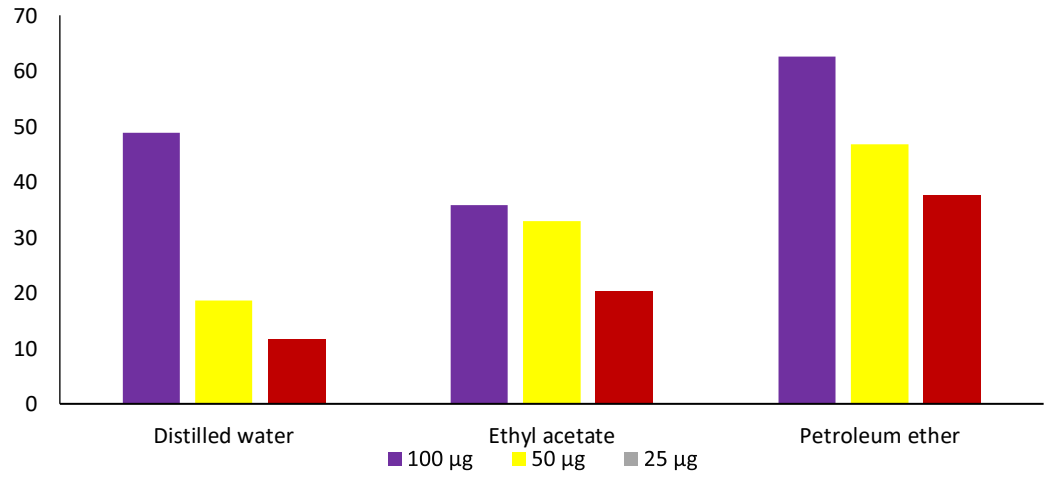
***In vitro* antioxidant activity of various *A. marmelos* extracts**

The antioxidant activity of *A marmelos* was investigated by the DPPH as well as ABTS radical scavenging activity.

DPPH radical scavenging activity in different extracts of *A. marmelos*

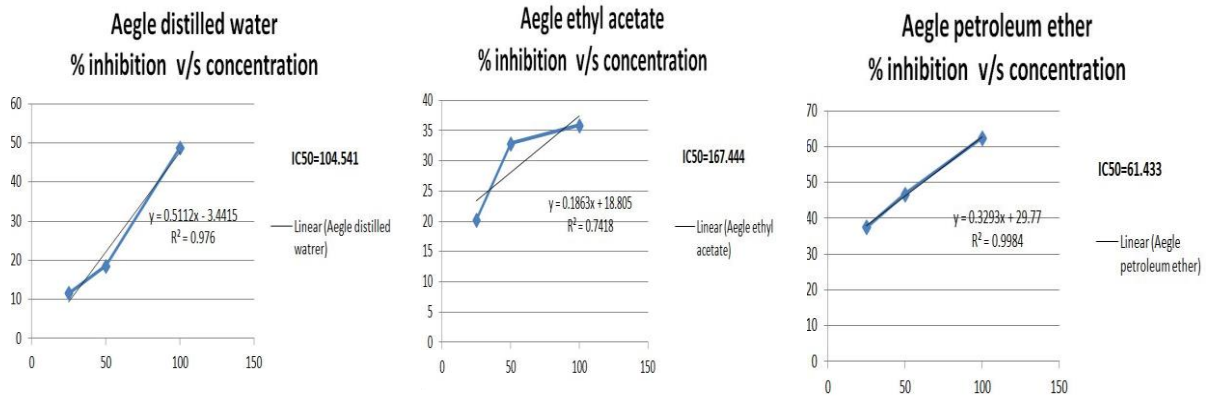
The existence of antioxidant effects in the different extracts of *A. marmelos* was discovered by an in vitro antioxidant experiment using the given activity (Fig. 1). Petroleum ether, distilled water, and ethyl acetate extract exhibited percentages of inhibition of 62.52%, 48.83%, and 35.85%, respectively, in the DPPH radical scavenging activity in *A. marmelos*. In the case of petroleum ether extract, *Aegle* shows the highest percentage of inhibition. The plant extract scavenged free radicals depending on their concentration, as shown by the inhibition % seen in all the extracts. IC_{50} values of different extracts were calculated (Figure 2).

Figure *1. In vitro* antioxidant activity by DPPH radical scavenging activity in various extracts of *Aegle marmelos*



ISSN-Online: 2320-7876
ISSN-Print: 2319-1775

Figure 2: IC50 for DPPH radical scavenging activity



In vitro* ABTS radical scavenging activity in different extracts of *Aegle marmelos

In the ABTS radical scavenging activity, in A. *marmelos*, petroleum ether, distilled water, and ethyl acetate extract showed a percentage of inhibition of 5.85%, 41.14%, and 56.85%, respectively (Table 2). When compared to conventional Rutin, the experiment revealed that the ethyl acetate extract of *Aegle marmelos* at a 30µg/mL concentration had strong antioxidant activity.

Table 2. *In vitro* antioxidant activity by ABTS radical scavenging activity in different *A. marmelos* extracts

Extract	OD	% Inhibition	mg equivalents of Rutin
Distilled water	0.412	41.14	330
Ethyl acetate	0.302	56.85	25.
Petroleum ether	0.659	5.85	470

Discussion

A medicinal plant known as *A. marmelos* may be found all across the sub-Himalayan jungles. The tree has therapeutic properties in all of its parts. The minerals in wood ash are abundant, includes pectin as well as volatile oils, and is utilized in many different products such as squash, coffee, candy, etc. (9). Different leaf extracts of *A. marmelos* were subjected to phytochemical analysis, which confirms the existence of flavonoids, alkaloids, triterpenoids, steroids, coumarins, tannins, phenols, and resins. DPPH as well as ABTS radical scavenging assay revealed that Aegle possesses antioxidant activity.

Various reports demonstrated that the presence of phytochemicals gives the plants under study pharmacological and physiological qualities that may be used to treat different conditions. The presence of the discovered phytochemicals may be the cause of the extracts' antioxidant action. Plant phenolics, which include flavonoids, tannins, and other phenolic substances, are a significant group of substances that function as main free radical scavengers or antioxidants (10). Terpenoids function similarly to vitamins in that they control metabolism and serve as antioxidants for protection. The leaves are pharmacologically active due to the known compounds (11). Alkaloids, tannins, and terpenoids are examples of phytochemicals

that are known to possess bioactive qualities such as anticancer and analgesic activities. Triterpenoids have anticancer and analgesic effects, whereas saponins are said to have antidiabetic and hypocholesterolemia characteristics (12).

The extracts' antioxidant potential may have vital contribution in the uses of the plants in the diagnosis, prevention, and treatment of numerous conditions. Free radicals harm cells and essential biomolecules during oxidative stress, which is prevented by antioxidants. By eliminating free radical intermediates, they interrupt chain reactions caused by free radicals and prevent additional oxidation processes (13). According to the literature, the existence of phytochemical components suggests the medicinal behavior of plants, such as their anti-oxidant action.

References

1. Jamshidi-Kia, F., Lorigooini, Z., & Amini-Khoei, H. Medicinal plants: Past history and future perspective. *Journal of Herbmed Pharmacology*, 2017, *7*(1), 1-7.
2. Refaz Ahmad Dar, Mohd Shahnawaz, Parvaiz Hassan Qazi. General overview of medicinal plants: a review. *The Journal of Phytopharmacology* 2017; 6(6): 349-351.
3. Hussein, R. A., & El-Anssary, A. A. (2019). Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. *Herbal Medicine*, 2019, 1, 13-17.
4. Baliga MS, Thilakchand KR, Rai MP, Rao S, Venkatesh P. Aegle marmelos (L.) Correa (Bael) and its phytochemicals in the treatment and prevention of cancer. Integr Cancer Ther. 2013 May;12(3):187-96.
5. Dutta, A., Lal, N., Naaz, M., Ghosh, A., & Verma, R. (2014). Ethnological and Ethnomedicinal importance of Aegle marmelos (L.) Corr (Bael) among indigenous people of India. *American Journal of Ethnomedicine*, *1*(5), 290-312.
6. Khandelwal K R. Practical pharmacognosy, techniques and experiments 2002 9th edition, 23.10-25.
7. Yen, G. C., & Chen, H. Y. (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agricultural and Food Chemistry*, *43*(1), 27-32.
8. Pellegrini, R., Proteggente N., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231-1237.
9. Reddy, V. P., & Urooj, A. (2013). Antioxidant properties and stability of Aegle marmelos leave extracts. *Journal of Food Science and Technology*, *50*(1), 135-140.
10. Potterat, O. (1997). Antioxidants and free radical scavengers of natural origin. *Current Organic Chemistry*, *1*(4), 415-440.
11. Agbafor, K. N., & Nwachukwu, N. (2011). Phytochemical analysis and antioxidant property of leaf extracts of Vitex doniana and Mucuna pruriens. *Biochemistry*, *4*(5), 68-80.
12. Akhtar, N., & Mirza, B. (2018). Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant

ISSN-Online: 2320-7876
ISSN-Print: 2319-1775

properties of 61 medicinal plant species. *Arabian Journal of Chemistry*, *11*(8), 1223-1235.

13. Sies, H. (1997). Oxidative stress: oxidants and antioxidants. *Experimental Physiology*: *Translation and Integration*, 82(2), 291-295.