Comparative Assessment of Antioxidant Activity in *Aegle marmelos* and *Piper betle*: Phytochemical Composition and Potential Health Benefits

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Abstract

This study aims to compare the antioxidant activity, phytochemical composition, and potential health benefits of *Aegle marmelos* (bael) and *Piper betle* (betel leaf), two widely used plants in traditional medicine. Both plants are known for their bioactive compounds, which contribute to their therapeutic properties. The antioxidant potential of extracts from both plants was assessed through *in vitro* assays, including DPPH and ABTS free radical scavenging tests, Nitric oxide scavenging activity and Total Antioxidant activity. The results demonstrated that the antioxidant properties of *Aegle marmelos* were demonstrated by IC_{50} values of 110 and 102 µg/ml for DPPH radical scavenging, 125 and 115 µg/ml for ABTS radical scavenging, and 190 and 198 µg/ml for nitric oxide scavenging activity, respectively. In comparison, the IC_{50} values for the methanolic and aqueous extracts of *Piper betle* were 18 and 26 µg/ml for DPPH radical scavenging, 22 and 29 µg/ml for ABTS radical scavenging, 62 and 75 µg/ml for nitric oxide scavenging activity, and 260 µg/ml and 180 µg/ml for total antioxidant activity (ascorbic acid), respectively. The comparative analysis suggests that both plants offer promising health benefits. This study underscores the therapeutic potential of *Aegle marmelos* and *Piper betle* in promoting health and preventing chronic diseases.

Introduction

Aegle marmelos[(1)] (Bael) and *Piper betle*[(2)] (Betel leaf) are two plants with significant medicinal value, widely used in traditional medicine across Asia. Both are known for their potent antioxidant properties, which contribute to their therapeutic potential in preventing oxidative stress-related diseases such as cancer, cardiovascular diseases, and neurodegenerative disorders. Oxidative stress, caused by an imbalance between free radicals and the body's antioxidant defense system, is implicated in the pathogenesis of numerous chronic health conditions. Therefore, plants with strong antioxidant activity are of great interest for their potential to mitigate these risks. *Aegle marmelos*, native to the Indian subcontinent, is a plant used in Ayurvedic medicine for its anti-inflammatory, antimicrobial, and antioxidant effects. Its bioactive compounds, including flavonoids, alkaloids, phenolic acids, and triterpenoids, contribute to its antioxidant activity by scavenging free radicals and reducing oxidative damage[(3)]. Studies have shown that *A. marmelos* extracts effectively inhibit oxidative stress and have protective effects against several oxidative stress-related diseases[(4)]. *Piper betle*, another plant with medicinal importance in Southeast Asia, is commonly used in betel chewing. The leaves of *P. betle* are rich in polyphenolic compounds, particularly flavonoids, tannins, and essential oils, which contribute to their antioxidant properties[(5)]. The antioxidant activity of *P. betle* has been well-documented, with studies highlighting its ability to reduce oxidative stress and prevent cellular damage[(6)].

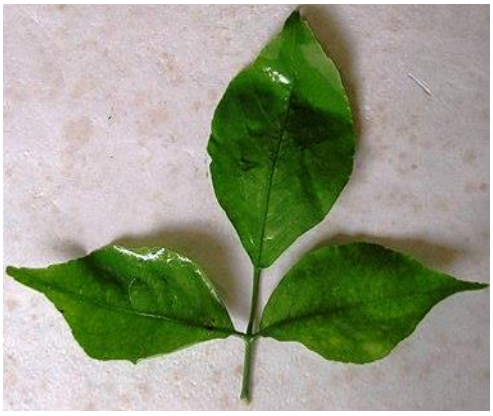

Figure 1: *Aegle marmelos* leaf

Figure 2: *Piper betle* leaf

This research aims to compare the antioxidant activity of *Aegle marmelos* and *Piper betle*, focusing on their phytochemical composition and evaluating their potential health benefits. Understanding the mechanisms behind their antioxidant properties could help in the development of natural products for health promotion and disease prevention.

Materials and Methods

Plant Material

(a) Leaves of *Aegle marmelos* were collected from Herbal Garden of Rajasthan College of Agriculture Rajasthan, India-313001.

(b) Leaves of *Piper betle* were collected from Herbal Garden of Rajasthan College of Agriculture Rajasthan, India-313001.

Chemicals

All chemicals and solvents were of analytical grade and were obtained from Nice Chemicals, Mumbai. 1,1- Diphenyl-2- Picryl Hydrazine (DPPH), 2, 2 – Azino bis (3-ethyl Benzo Thiazoline – 6 – Sulphonic acid (ABTS), quercetin was obtained from Sigma Chemicals, USA and gallic acid from Nice Chemicals, Mumbai. The other chemicals used were sodium nitroprusside, sulphanilic acid, naphthylethylene diamine hydrochloride. Ascorbic acid (Ranbaxy Fine Chemicals Ltd.) was used as standard for whole study.

Plant extracts

Methanolic extract- The leaves and fruits of *Aegle marmelos* and leaves of *Piper betle* were shade dried, powdered and about 100 g of powder was extracted with methanol by hot extraction process (soxhlet) for 72 h. After completion of the extraction the solvent was recovered by distillation and concentrated *in vacuo.*

Aqueous extract (Chloroform: water – 1:99) - The leaves and fruits of *Aegle marmelos* and leaves of *Piper betle* were shade dried, powdered and macerated with chloroform water for seven days. It was then filtered, solvent removed and concentrated *in vacuo*

In vitro Antioxidant Activity

1. DPPH radical scavenging activity- The antioxidant activity for the plant extracts was assessed on the basis of the radical-scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The concentration of DPPH was kept at 300 µM in MeOH. The extracts were dissolved in MeOH. 10 µl of each extract solution was allowed to react with 200 µl DPPH at 37 °C for 30 min in a 96-well microliter plate. After incubation, decrease in absorption for each solution was measured at 490 nm using a microplate reader. Ascorbic acid was used as standard[(7)].

2. ABTS Assay- For ABTS (2, 2-Azinobis(3-ethylbenzothiazoline-6-sulfonicacid) diammoniumsalt) assay, the stock solutions of 7.4 mM ABTS and 2.6 mM potassiumpersulfate were prepared and the working solution was then prepared by mixing the two stock solutions

in equal quantities and allowing them to react for 16 h at room temperature in a dark place. The solution was then diluted by mixing $ABTS^{-+}$ solution with methanol to obtain an absorbance of 1.00 ± 0.02 units at 734 nm using the spectrophotometer. Fresh $ABTS^{-+}$ solution was prepared for each assay. Different concentrations of standard solution of ascorbic acid were prepared. The crude and purified extracts (200 ml) were allowed to react with 4 ml of the $ABTS^{-+}$ for 2 h in a dark place. Then, the absorbance was noted at 734 nm[7,8].

3. Nitric oxide scavenging activity- Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations and the mixture incubated at 25 °C for 150 min. From the incubated mixture 0.5ml was taken out and added into 1ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally 1.0 ml naphthylethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance at 540 nm was measured with a spectrophotometer. The nitric oxide radical scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_0 - A_1) / A_0 \times 100]$$

Where A_0 was the absorbance of the control (blank, without extract) and A_1 was the absorbance in the presence of the extract/Standard[9].

4. Total antioxidant capacity- Total antioxidant capacity was measured with slight modifications. In brief, 100 µg of extract and 100 µg of ascorbic acid (as standard) were taken in 0.1 ml of alcohol, combined separately in an eppendroff tube with 1.9 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. A typical blank solution contained 1.9 ml of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under the same conditions as the rest of the samples. For samples of unknown composition, water-soluble antioxidant capacities are expressed as equivalents of ascorbic acid. Ascorbic acid equivalents were calculated using standard graph of ascorbic acid. The experiment was conducted in triplicate and values are expressed as ascorbic acid equivalents in µg/ml of extract[10].

Result and Discussions

It has long been recognized that naturally occurring substances in higher plants have antioxidant activity. Several mechanisms have been proposed to be involved in the antioxidant activity such as hydrogen donation, termination of free radical mediated chain reaction, prevention of hydrogen abstraction, chelation of catalytic ions, and elimination of peroxides. Owing to the complex reactive nature of phytochemicals, the antioxidant activities of plant extracts cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity. Therefore in the present study, the antioxidant activity of methanolic and aqueous extracts were analyzed by DPPH free radical scavenging activity, ABTS radical cation scavenging activity, nitric oxide scavenging activity and total antioxidant activity.

Table-1: Scavenging of DPPH free radical by various extracts

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	12.36	16.95	37.69
10	21.82	26.17	56.21
25	30.72	35.32	80.57
50	38.63	42.76	88.55
100	46.79	49.80	92.09
200	57.38	61.34	95.22

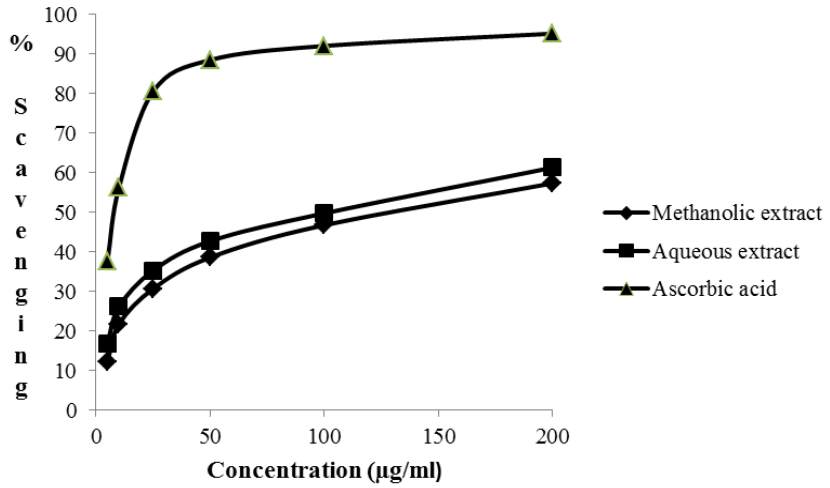

Figure-3: Scavenging of DPPH free radical by extracts

Various methods of monitoring the amount of DPPH in the antioxidant test system have been reported: electron spin resonance spectroscopy (ESR)/plant powders, NMR/catechins and UV spectrophotometry/polyphenols. However, monitoring DPPH with a UV spectrophotometer has become the most widely and commonly used method recently because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple).

A stable ABTS radicalcation, which has blue-green chromophore absorption, was produced by oxidation of ABTS with potassium persulfate prior to the addition of antioxidants. The antioxidant activity of the natural products, including carotenoids, phenolic compounds, and some plasma antioxidants, is determined by the decolorization of the ABTS, by measuring the absorbance at 734 nm.

Table-2: Interaction of different extracts with ABTS radical

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	9.60	13.53	28.67
10	17.76	22.32	45.51
25	25.85	31.41	65.32
50	33.41	40.76	77.67
100	44.65	47.91	85.45
200	53.45	56.47	89.76

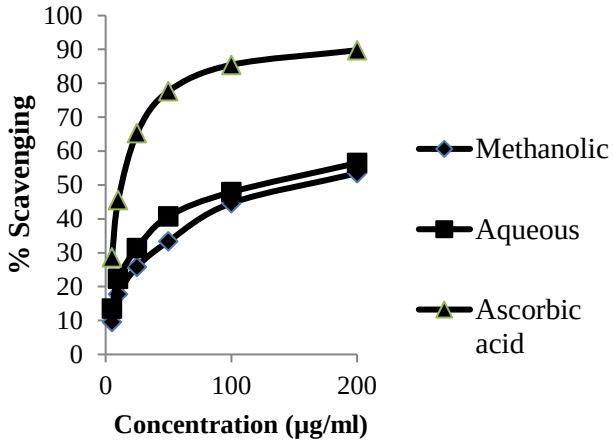

Figure-4: Interaction of different extracts with ABTS radical

In the present study several concentrations ranging from 5-200µg/ml of methanolic and aqueous extracts of *Aegle marmelos* and *Piper betle* were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the test extracts in a concentration dependent manner in all the models. The antioxidant activity was estimated by IC_{50} value.

Table-3: Nitric oxide scavenging activity of various extracts

Concentration (µg/ml)	Methanolic extract % scavenging	Aqueous extract % scavenging	Standard (Ascorbic acid) % scavenging
5	1.19	1.10	4.83
10	2.25	2.38	18.06
25	8.37	6.19	48.22
50	21.26	18.80	68.13
100	40.20	36.18	82.78
200	52.09	50.96	88.33

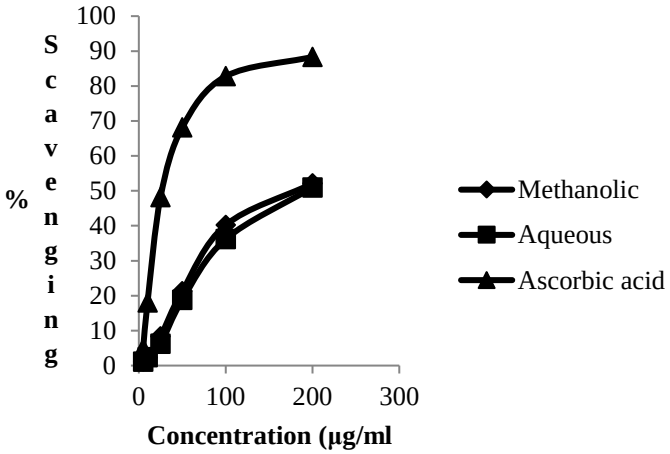

Figure-5: Nitric oxide scavenging activity of various extracts

The antioxidant activity of *Aegle marmelos* showed IC_{50} values of 110 and 102µg/ml (DPPH radical scavenging); 125 and 115µg/ml (ABTS radical scavenging); 190 and 198 µg/ml (Nitric oxide scavenging activity) respectively and the IC_{50} values of methanolic and aqueous extracts of *Piper betle* were 18 and 26 µg/ml (DPPH radical scavenging); 22 and 29 µg/ml (ABTS radical scavenging); 62 and 75 µg/ml (Nitric oxide scavenging activity) and 260 µg/ml and 180 µg/ml ascorbic acid (Total antioxidant activity) respectively.

Table-4: Total antioxidant activity

Concentration (µg/ml)	Absorbance
25	0.167
50	0.324
100	0.604
200	1.084
400	2.005
800	3.489

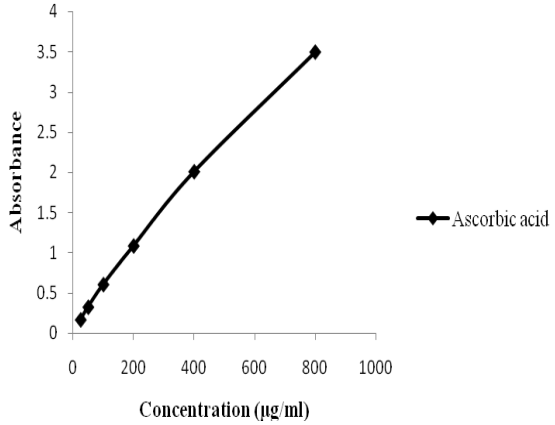

Figure-6: Standard plot of ascorbic acid

Reference

1. Mali SS, Dhumal RL, Havaldar VD, Shinde SS, Jadhav NY, Gaikwad BS. A systematic review on Aegle marmelos (Bael). Research Journal of Pharmacognosy and Phytochemistry. 2020;12(1):31-6.
2. Depi S. Review of traditional use, phytochemical and pharmacological activity of Piper betle L. Galore International Journal of Health Sciences and Research. 2020;5(3):59-66.
3. Venthodika A, Chhikara N, Mann S, Garg MK, Sofi SA, Panghal A. Bioactive compounds of Aegle marmelos L., medicinal values and its food applications: A critical review. Phytotherapy Research. 2021 Apr;35(4):1887-907.
4. Singh AK, Singh S, Saroj PL, Krishna H, Singh RS, Singh RK. Research status of bael (Aegle marmelos) in India: A review. The Indian Journal of Agricultural Sciences. 2019 Oct 1;89(10):1563-71.
5. Siregar KA, Hamzah H, Kustiawan PM, Wirnawati LC. Bioactivity and Phytochemical Compound Test on Black Betel Leaves (Piper betle var. nigra) A Literature Review. Int J Med Sci Dent Res [Internet]. 2022;5(1):37-43.
6. Depi S. Review of traditional use, phytochemical and pharmacological activity of Piper betle L. Galore International Journal of Health Sciences and Research. 2020;5(3):59-66.
7. Rajan S, Gokila M, Jency P, Brindha P, Sujatha RK. Antioxidant and phytochemical properties of Aegle marmelos fruit pulp. Int J Curr Pharm Res. 2011;3(2):65-70.

8. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free radical biology and medicine. 1999 May 1;26(9-10):1231-7.
9. Ebrahimzadeh MA, Nabavi SF, Nabavi SM, Pourmorad F. Nitric oxide radical scavenging potential of some Elburz medicinal plants. African journal of Biotechnology. 2010;9(32):5212-7.
10. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry. 1999 May 1;269(2):337-41.