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Phytochemical screening, antioxidant, antibacterial and anti-inflammatory activity of ethanolic extract of *Aegle marmelos* leaf

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Abstract

The present investigation was aimed to focus on the screening of phytochemical constituents of ethanolic extract of *Aegle marmelos* leaf. In addition, it was also evaluated for its potential antioxidant, antibacterial and anti-inflammatory activity. The ethanolic extract of *Aegle marmelos* leaf showed the presence of several phytochemicals, such as carbohydrates, alkaloids, flavonoids, tannins, quinones, terpenoids, and steroids. The ethanolic leaf extract contained a high level of protein, phenolic compounds, and antioxidant content. The ethanolic extract of *Aegle marmelos* leaf showed potential antioxidant activity in the evaluation of DPPH assay. It has also been found to exhibit significant antibacterial activity against different bacterial species, including both Gram-positive and Gram-negative bacteria, as concluded using bacterial activity tests. Further, the ethanolic extract from *Aegle marmelos* leaf also exhibited significant anti-inflammatory activity as confirmed using the BSA denaturation assay. Additional research, including in vivo experiments, is needed to further explore its medicinal potential

Keywords: Phytochemicals, *Aegle marmelos*, ethanolic extract antioxidant and antibacterial activity.

1. Introduction

Plants or their various parts are employed to cure various physical and mental disorders and assist us in living successfully. Ancient scriptures such as the Rigveda, Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also speak about the utilisation of plants to cure various health disorders [1]. With the relevance of plants and their use as medicine in mind, many research studies are ongoing to isolate the active chemical compounds. The traditional use of plants or their parts for topical application suggests their potential effectiveness in treating various diseases and is studied extensively by sophisticated scientific methods and documented for a range of medicinal activities such as anticancer, antifungal, antidiabetic, antioxidant, hepatoprotective, haemolytic, larvicidal, and anti-inflammatory activity etc [1]. Traditional herbal treatments, combined with dietary practices, are commonly prescribed in Ayurveda and other alternative medical sys-

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tems for managing various health conditions in both humans and animals. A considerable number of individuals, including those in highly developed countries, have engaged in alternative therapies such as herbal medicine at least once [2]. Given the widespread use of these herbal formulations across both developed and developing regions, it is crucial to regulate their quality through rigorous scientific research. This includes chemical standardisation, biological evaluations, and well-structured clinical trials [2]. Ensuring quality control and assurance is essential, as the composition and properties of herbal products can vary due to factors such as harvesting time, storage conditions, processing methods, and formulation techniques, etc. [3]. Oxidative stress is a by-product of normal metabolic processes and can also be activated by environmental and chemical exposure. Scientific evidence suggests that oxidative stress is a major factor in the initiation and progression of diabetes and its related complications [4]. Also, alloxan, a substance commonly used to induce diabetes in laboratory animals, has been found to destroy insulin-producing pancreatic cells by generating reactive oxygen species [5]. Patients with diabetes are found to have lower antioxidant levels, suggesting a correlation with disease progression. Plant bioactive compounds reduce oxidative stress and also help in the regulation of high blood sugar and alleviate diabetic complications. In previous studies, it was shown that *Aegle marmelos* extract, which is one of the most popular folk medicines used for the control of blood sugar and it also potentially acts as an antioxidant in vitro [6].

The present research intends to explore the phytochemical content, antioxidant activity, anti-inflammatory activity, and antibacterial activity of ethanolic extract from *Aegle marmelos* (Indian Bael) leaves.

2. Experimental

2.1 Collection of *Aegle marmelos*

The leaves of *Aegle marmelos* are collected from in and around Tirupattur district, Tamil Nadu. The leaves are removed carefully, washed with water, and dried in the absence of sunlight to remove the water molecules present in the leaves. Then the fine powder is stored properly in an airtight container for future use [10].

Fig. 1. Collection and preparation of *Aegle marmelos*

2.2 Methodology of Extraction of *Aegle marmelos*

Aegle marmelos leaf is finely ground to 14 g of powder to increase the surface area, which enhances the extraction process. This ground sample is then placed inside a thimble, a porous container that allows the solvent to pass through. 140ml of ethanol is poured into a round-bottom

flask. Ethanol is chosen due to its high polarity for the extraction of numerous bioactive constituents. The ethanol in the flask is heated, causing it to vaporise. The ethanol vapour rises through the Soxhlet apparatus. The vapour then enters a condenser where its cooled and condenses back into liquid ethanol. This liquid ethanol drips into the thimble containing the solid sample. This cycle of vaporisation, condensation and siphoning repeats continuously, ensuring that the solid sample is repeatedly washed with fresh ethanol. The process continues for a set amount of time, and the ethanol now containing the compounds is collected in the round-bottom flask. And the sample is collected in petri plates, allowed to dry and collected in the Eppendorf tube for further analysis. [7].

The concentrated extracts were then allowed to dry completely for two or three days at room temperature. After the botanical extracts had dried, they were stored in sterile bottles until needed again.

2.3. Preliminary Phytochemical Screening

The leaf extract was tested for detecting various phytochemicals present in the ethanolic extract of *Aegle marmelos* using the standard procedure [8], [9].

1. Detection of Alkaloids

Mayer's test

The leaf extract underwent treatment with Mayer's reagent. The presence of alkaloids is indicated by the formation of a yellow cream precipitate.

Wagner s test

The leaf extract was treated with Wagner s reagent. Formation of brown/reddish brown precipitate indicates the presence of alkaloids.

2. Detection of Flavonoids

Lead acetate test

Leaf extract was treated with a few drops of lead acetate solution. Formation of yellow-coloured precipitate indicates the presence of flavonoids.

Sulphuric acid test

Leaf extract was treated with a few drops of H_2SO_4. Formation of orange colour indicates the presence of flavonoids.

3. Detection of Steroids

Two ml of acetic anhydride was added to five ml of the leaf extract, and then two ml of H_2SO_4 was added to each. The colour was changed from violet to blue or green, indicating the presence of steroids

4. Detection of Terpenoids

Salkowski s Test

The leaf extract was combined with chloroform at a ratio of 5 ml to 2 ml, and then 3 ml of concentrated H_2SO_4 was added slowly to create a distinct layer. The presence of terpenoids is indicated by a reddish-brown colour on the inner surface.

5. Detection of Phenols

Ferric chloride test

Ten ml of the leaf extract was treated with a few drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenol.

Lead acetate test

Ten ml of the leaf extract was treated with a few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of phenol.

6. Detection of Saponins

About 0.5ml of the extract was shaken with five mL of distilled water. Formation of frothing (appearance of creamy or small bubbles) shows the presence of saponins.

7. Detection of Tannins

After dissolving a little amount of extract in water, it was heated on a water bath. Following filtering, ferric chloride was added to the resulting mixture. The result was a shade of dark green. The presence of tannins is indicated by it.

8. Detection of Carbohydrates

About 0.5ml of extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test the presence of carbohydrates.

2.4. Antioxidant activity

Assay of antioxidant activity by DPPH (1, 1-diphenyl 1-2picrylhydrazyl) free radical scavenging activity:

The free radical scavenging potential of the ethanolic extract of *Aegle marmelos* leaf was determined using DPPH. The DPPH solution (0.006% w/v) was prepared in 95% methanol. Different concentrations of the ethanolic extract of (20, 40, 60, 80, and 100 µg/ml) were prepared using ethanol. 3 ml of different concentrations of ethanolic leaf extract of *Aegle marmelos* were mixed with 1 ml of DPPH solution in the dark. Ascorbic acid, which is a strong antioxidant, is taken as standard, prepared in different concentrations using ethanol (20, 40, 60, 80 and 100µg /ml). 3 ml of different concentrations of standard solution of ascorbic acid was mixed with 1 ml of DPPH solution in the dark.

The prepared solution of ascorbic acid and leaf extract samples was incubated for 30 minutes, and then absorbance was measured using a UV-Spectrophotometer at 517 nm. Ethanol serves as a blank, and the experiment was expressed as the inhibition percentage of free radicals by the sample and was calculated using the following formula [10].

DPPH radical scavenging activity (%) = (Control OD-Sample OD)/ Control OD x 100

2.5. Antibacterial activity

Antibacterial activity was analysed with the standard agar well diffusion method. Different concentrations of ethanolic extract (50, 100, 150 µg/ml) were prepared by using 2% DMSO (dimethylsulphoxide). 10µl of 24-hour test cultures, such as Pseudomonas, *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli,* were spread onto respective Mueller-Hinton agar medium by the spread plate method. Gentamicin is used as the positive control for this experiment. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was analysed by measuring the diameter of the zone of inhibition formed around the well [11].

2.6. Anti-inflammatory activity

Bovine Serum Albumin Denaturation Assay,

Dissolved in 8 g of sodium chloride (NaCl), 0.2g of potassium chloride (KCl), 1.44 gm of disodium hydrogen phosphate (Na2HPO4), and 0.24 g of potassium dihydrogen phosphate (KH2PO4) in 800 ml of distilled water. The pH was adjusted to 6.3 using hydrochloric acid (HCl) and made the volume up to 1000ml with distilled water.

Method:

Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (BSA) (0.5% W/V aqueous solution) and 0.05 ml of test solution of various concentrations.

Test control solution (0.5ml) consists of 0.45ml of bovine serum albumin (0.5% W/V aqueous solution) and 0.05ml of distilled water.

Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (0.5%w/v aqueous solution) and 0.05ml of Diclofenac sodium as a standard of various concentrations.

Procedure:

About 0.05 ml of various concentrations (20, 40, 60, 80 and 100 µg/ml) of test drugs and standard drug Diclofenac sodium (20, 40, 60, 80 and 100 µg/ml) were taken, respectively, and 0.45 ml (0.5% W/V BSA) was mixed. The samples were incubated at 37°c for 20 minutes, and the temperature was increased to keep the samples at 57°C for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using a UV-Visible spectrophotometer at 255 nm. The control represents the result without the addition of a sample or a standard. The results were compared with Diclofenac sodium. The percentage inhibition of protein denaturation can be calculated as: [12], [13].

Percentage Inhibition = 100 [(optical density of test solution- optical Density of

control)/optical density of test)/x 100]

3. Results and Discussion

A range of phytochemical constituents and their corresponding activities have been synthesised and reported using modified methodologies from the literature, enabling multifarious characterisation studies. Plants are a crucial reservoir of dynamic characteristic components that vary extensively in aspects of structures, organic characteristics and tools of activity. Various phytochemical fractions include carbohydrates, flavonoids, tannins, alkaloids, quinones, terpenoids and steroids. Subjective phytochemical considerations of carbohydrate showed an excellent characteristic colour and speed in the ethanolic extract [14].

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The consequences of subjective phyto-chemicals are exhibited in Table I.

Table 1: Phytochemical analysis of ethanolic extract of *Aegle marmelos* leaf

S.No.	Phytochemicals	Ethanolic Extract of *Aegle marmelos*
1	Carbohydrate	+
2	Tannins	+
3	Saponins	-
4	Alkaloids	+
5	Flavonoids	+
6	Glycosides	-
7	Quinones	+
8	Phenols	-
9	Terpenoids	+
10	Steriods	+

High content of flavonoids in the ethanolic extract was confirmed by the yellow colouration test, and minor proximity in all the other extracts was seen. Flavonoids and terpenoids are polar compounds effective in their biological activities.

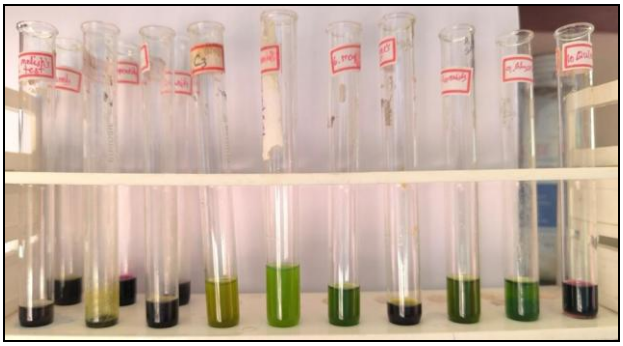

Fig. 2. Phytochemical analysis of the ethanolic extract of *Aegle marmelos* leaf

Auxiliary metabolites were present in high proportion in ethanolic extracts. These supporting metabolites can be used for various pharmacological effects of the ethanolic extract of *Aegle marmelos* [15].

3.2 Antioxidant studies

DPPH scavenging activities:

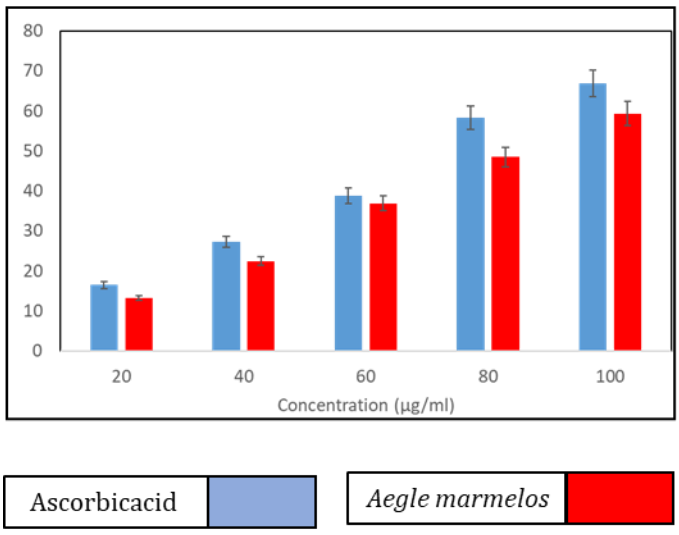

Fig. 3. Antioxidant activity of *Aegle marmelos* leaf extract. All experiments were performed in triplicate (n=3).

The result of the DPPH scavenging assay carried out in the present study indicated that the leaves of *Aegle marmelos*

were potentially active. This suggests that the leaf extract does contain compounds that are capable of donating hydrogen to a free radical to remove an odd electron, which are responsible for the radical's reactivity [16]. This implies that the *Aegle marmelos* leaf extract could be useful for treating radical-related pathological damage, especially at higher concentrations.

3.3 Anti-bacterial activity of *Aegle marmelos* leaf extract:

Fig. 4. Antibacterial activity of *Aegle marmelos* leaf extract.

All experiments were performed in triplicate (n=3).

The effectiveness of *Aegle marmelos* leaf extract against harmful bacteria was assessed using its antibacterial activity. This required evaluating the extract's capacity to inhibit the development of a number of bacterial strains, both Gram- positive and Gram-negative. The agar well diffusion method was utilised to determine the lowest concentration of the extract required to halt bacterial growth, with gentamicin serving as a reference standard drug for comparison [17].

Table 2: Inhibition Zone of bacterial species

S.No	Organisms	Positive control	Zone of inhibition		
			50 µg/ml	75 µg/ml	100 µg/ml
1	*Pseudomonas*	Gentamicin	15 mm	17 mm	20mm
2	*Bacillus subtilis*	Gentamicin	14 mm	16 mm	17 mm
3	Staphylococcus aureus	Gentamicin	18 mm	19 mm	20 mm
4	*Escherichia coli*	Gentamicin	15 mm	18 mm	19 mm

3.4 Anti-inflammatory activity of *Aegle marmelos* extract

In the inflammatory process, protein denaturation is essential because it disrupts protein structures and may result in tissue damage [18]. Therefore, finding compounds that can stop or lessen protein denaturation is essential for creating anti- inflammatory treatments. Bovine serum albumin (BSA) was chosen as the model protein in this case because it is widely accessible and simple to evaluate denaturation, which was measured using a col-

ourimetric assay at 600 nm [19]. The extract from the leaf of *Aegle marmelos* was tested for its anti-inflammatory properties using diclofenac sodium as a reference compound. Interestingly, the leaf extract showed strong anti-inflammatory properties compared to the standard drug [20].

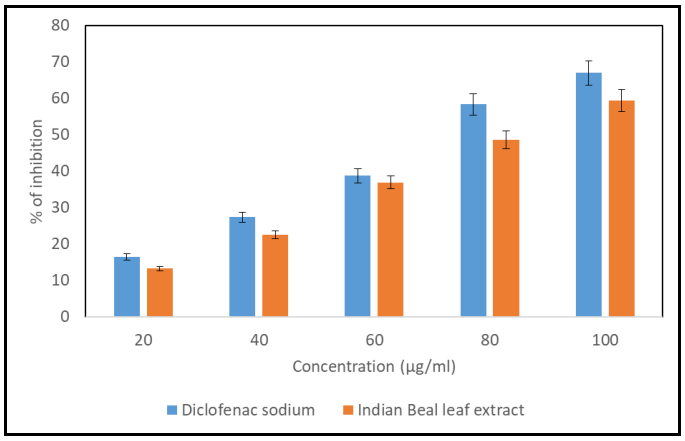

Fig. 5. Anti-inflammatory activity of *Aegle marmelos* leaf extract. All experiments were performed in triplicate (n=3).

4. Conclusion

The ethanolic extract of *Aegle marmelos* leaf showed the presence of several phytochemicals, such as carbohydrates, alkaloids, flavonoids, tannins, quinones, terpenoids, and steroids. The ethanolic leaf extract contained a high level of protein, phenolic compounds, and antioxidant content. The ethanolic extract of *Aegle marmelos* leaf showed potential antioxidant activity in the DPPH assay. It has also been found to exhibit significant antibacterial activity against different bacteria, including both Gram-positive and Gram-negative bacteria, as concluded using bacterial activity tests. Additionally, the ethanolic extract from *Aegle marmelos* leaf also exhibited significant anti-inflammatory activity as confirmed using the BSA denaturation assay. Additional research, including *in vivo* experiments, is needed to further explore its medicinal potential.

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Conflict of Interest: Nil

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