Vol 8, Issue 8, 2025 **Impact Factor: 8.535** DOI: https://doi.org/10.31426/ijamsr.2025.8.8.8628

IJAMSR

International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

Assessment of Bioactive Potential of Aegle Marmelos Leaves Against Microbial Strains and Oxidative Stress

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ABSTRACT

Traditional and folk medicine make extensive use of the Aegle marmelos plant for a variety of purposes. As a cooling agent in particular, A. marmelos is a valuable medicinal herb that is used in the treatment of dysentery and diarrhea. This tree is often planted in homes and is especially beloved in temples dedicated to Shiva and Vishnu. It is also commonly referred to as a temple garden plant. This research delves into the antioxidant and antibacterial capabilities of Aegle marmelos leaf extracts, a medicinal plant that has gained immense recognition for its curative qualities. To extract the bioactive chemicals, the leaves were soaked in organic solvents such acetone and ethanol. The antibacterial activity was tested using conventional laboratory methods on a variety of bacterial and fungal species, including Bacillus subtilis and Aspergillus Niger. The findings showed that the extracts had strong inhibitory effects, with the ethanol extracts exhibiting much more antibacterial activity than the others. Standard tests were used to assess antioxidant activity at different dosages, and the results showed that the ability to scavenge free radicals increased as the dosage was increased. Supporting their long-standing usage in herbal medicine and drawing attention to their potential for pharmaceutical uses, the results indicate that extracts from the leaves of the Aegle marmelos plant have strong antibacterial and antioxidant characteristics.

Keywords: *Aegle Marmelos, Antioxidant Activity, Leaf, Acetone, Ethanol.*

I. INTRODUCTION

There are several different ways in which the Aegle marmelos plant is used in traditional and folk medicine. An important therapeutic plant for dysentery and diarrhea, A. marmelos is known for its cooling properties. This tree is highly prized in temples honoring Vishnu and Shiva, and it is also often planted in homes. The name "temple garden plant" is another popular one. This study

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International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

investigates the antimicrobial and antioxidant properties of Aegle marmelos leaf extracts, a medicinal herb that has received a great deal of acclaim for its therapeutic uses. The leaves were immersed in organic solvents like ethanol and acetone to release the bioactive compounds. Several bacterial and fungal species, such as Bacillus subtilis and Aspergillus Niger, were subjected to traditional laboratory procedures to assess the antibacterial activity. Strong inhibitory effects were shown by the extracts, with the ethanol extracts showing much higher antibacterial activity compared to the others. Using standard procedures, the antioxidant activity was evaluated at various doses. The findings demonstrated that the capacity to scavenge free radicals improved with increasing dosage. The findings show that Aegle marmelos leaf extracts have substantial antibacterial and antioxidant properties, lending credence to their traditional use in herbal therapy and highlighting their potential for pharmaceutical applications.

II. REVIEW OF LITERATURE

Singh, Priyanka et al., (2024). This member of the Fucaceae family is known by several names, including Aegle marmelos, stone apple, wood apple, bale tree, and Bengal quince. Lord Shiva is often offered bale leaves in Hindu mythology. It is recommended to cultivate them near the temples of Lord Shiva. The medicinal practice of ayurveda has a long tradition of using its leaves. Through quality analysis, essential oil extraction, and characterization, this study aimed to discover the potential usefulness of discarded leaves. The research also mainly aimed at understanding the bioactivity and phytochemical composition of oils. We collected and hydro-distilled the leaves given at temples to find out how much oil could be extracted. An average of 0.69018 was determined for essential oil yields. The essential oil extracted from six different regions of the leaves was tested using chromatographic and anti-microbial techniques. When tested against various bacteria, including yeast and Gram-positive and Gram-negative types, the oil showed encouraging results as an antibacterial. The GC and GC/MS studies revealed that limonene was the principal component of the oil. Because of its many phyto-chemical and pharmacological actions, limonene is important for preventing harmful spoilage in both humans and animals.

Timbadiya, Priyanka et al., (2023) this work aimed to test several leaves extracts of Aegle marmelos for phytochemicals, anti-inflammatory, antimicrobial, antioxidant, and physicochemical activities. Hydroxychloroform, hexane, methanol, water, and 90% methanol were among the solvents used to extract medicinal plants. Then, we looked at the antibacterial, antioxidant, oxidative enzyme, and antidiabetic activities. Lastly, the bioactive extracts were used to identify phytochemicals that exhibited antidiabetic action. The findings demonstrate that the polar extracts of Aegle marmelos leaves had the highest concentration of phytochemicals. The plant leaves include phenol at 53.45±0.39 and flavonoids at 63.64±0.45. Potassium (1713.01 ppm), iron (161.08 ppm), and calcium (51861.81 ppm) were abundant in the powdered plant leaves. With the exception of the hexane and chloroform extracts, which had no impact on Bacillus subtilis, all four examined bacteria—Bacillus subtilis, Bacillus cereus, Bacillus megaterium, and Escherichia coli—were inhibited in their growth by different Aegle marmelos-leaf extracts.

Kumar, Vikas. (2023) The Fucaceae family includes the medicinal plant Aegle marmelos, sometimes known as Bale, which is highly prized in the Himalayan region. Scientists in Himachal Pradesh set out to test the antibacterial and antioxidant properties of A. marmelos leaf extracts in n-hexane and ethanol. The broth dilution technique was used to assess the antibacterial activity of the extracts

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against several bacterial and fungal strains, including Gram positive (Staphylococcus aureus), Gram negative (Escherichia coli), and MTCC277 (Candida albicans). Utilising the DPPH radical scavenging and FRAP tests, we also assessed the extracts' antioxidant capabilities. The antioxidant capacity of the ethanolic extract of A. marmelos leaves from eight different locations in Himachal Pradesh was found to be very variable. Compared to the leaf extracts from other districts, the ethanolic extract from the Mandi area (M3) showed more antioxidant ability, as shown by a lower IC50 value in DPPH (14.77µg/ml). In contrast, the leaf extracts from other districts had lower levels of FRAP activity (6.62µM) compared to the hexane extract from Ballarpur (B2). The antioxidant capacity of the ethanol extract was somewhat higher than that of n-hexane across all A. marmelos populations. The ethanolic extracts of Seymour district (SM1) and Mandi (M3) showed the lowest Minimum Inhibitory Concentration (MIC) value of 7.81µg/ml against S. aureus and E. coli, respectively, while the n-hexane extracts of Chambal (CH1) and Una (U2) showed the lowest MIC value (7.81µg/ml) against K. pneumonia.

Dheeba, B. et al., (2010). Natural antioxidants prevent age-related deterioration and its effects by scavenging free radicals and keeping oxidative stress levels constant. The purpose of this study was to identify any antioxidant activity of Aegle marmelos bark extracts in ethanol, ethyl acetate, and water. Aegle marmelos is most often known as Beal in India. This prickly tree is a member of the Fucaceae family. All parts of the Aegle marmelos tree—fruits, leaves, bark, roots, and seeds—are edible. This plant's medicinal properties are detailed in Ayurveda. A coarse powder was made from the collected bark after it had dried in the shade. Using ethanol, ethyl acetate, and water in a stepwise sequence, the powder was extracted using the cold percolation process. In order to concentrate the extracts, the solvent was distilled and then the material was air dried. The phytochemical analysis of crude bark extracts revealed the presence of alkaloids, flavonoids, tannins, glycosides, phenols, sterols, and terpenoids. Three complementary assays were used to assess the antioxidant activity of ethanol, ethyl acetate, and water extracts: DPPH, Phosphomolybdate, and the thiocyanate technique. It seems that the bark of the Aegle marmelos tree contains a natural antioxidant, according to this study.

III. MATERIALS AND METHODS

Leaf Extraction

The Kodangal region of Tamil Nadu, India, was the site of leaf collection for Aegle marmelos. The gathered leaves were rinsed under running water from the faucet and then dried in the shade. Once ground into a fine powder. Two different solvents, acetone and ethanol, were used to dissolve the powder using the soxhlet apparatus. Antimicrobial and antioxidant properties were assessed after the extracts were dried and dissolved in a DMSO (Dimethyl sulfoxide) solution.

Antimicrobial Activity

Microorganisms such as Pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumonia, and Escherichia coli, as well as Aspergillus Niger and Candida albicans, were used to carry out the antibacterial activities. Researchers used the disc diffusion technique to find out how effective the antibiotic was. The three concentrations that were created were 10 mg/ml, 5 mg/ml,

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International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

and 1 mg/ml, respectively. The test extract was put onto 100µl of sterile discs and then placed on agar plates that had been infected with the corresponding bacteria. Prior to incubation, the plates were left for 30 minutes to allow for diffusion. Following that, the plates were placed in an incubator set at 37°C for bacterial cultures and 48 hrs. for fungal cultures. We measured the zones around the discs at the conclusion of the incubation period. Triplicate runs of the research were carried out.

Determination of Antioxidant Activity

The antioxidant activity, or radical scavenging activity, was assessed using a modified version of the technique developed by Hartanto (1988) and Bhuiyan (2009). The leaf extracts were produced at doses of 40, 60, 100, 130, and 160 µg/ml, in that order. Five different concentrations were achieved by diluting the sample stock solution (100 mg/ml). We evaluated duplicate samples of each concentration. At room temperature for 30 minutes with light protection, the 0.5 ml of sample solution was combined with 3.0 ml of 0.1 mM 1, 1-diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol). Spectrophotometric measurements of absorbance were taken at 518 nm. Lower absorbance of the reaction mixture and increased free radical scavenging activity were observed when the samples were quenched with DPPH at the corresponding intensities. The percentage of DPPH radical scavenging was determined by comparing the absorbance of the test and control (DPPH in ethanol). The capacity to neutralize the DPPH radical was determined by use the following equation. The IC50 value, which stands for "Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals," was traditionally used to characterize antioxidants in the DPPH test. The formula is: The percentage of scavenging effect is equal to one minus the sample's absorbance at time zero multiplied by the control's absorbance at time thirty, divided by one hundred.

IV. RESULTS AND DISCUSSION

Table 1: Antimicrobial Activity of Different Extracts of Aegle Marmelos Against Some Pathogens

S. No	Test organism	Zone of Inhibition (mm)					
		Acetone			Ethanol		
		10 mg/ml	5 mg/ml	1 mg/ml	10 mg/ml	5mg/ml	1mg/ml
1	P.aeruginosa	22	10	8	6	-	-
2	B.subtilis	22	15	11	16	10	-
3	S.aureus	16	12	10	15	12	9
4	K.pnemonia	5	-	-	11	-	-
5	E. coli	11	7	6	-	-	-
6	A.niger	18	11	7	-	-	-
7	C. albicans	-	-	-	14	7	6

Antimicrobial Activity

Table 1 displays the results of the antibacterial and antifungal tests conducted on human pathogens using various Aegle marmelos extracts. Antimicrobial activity was quantified by measuring the zone of inhibition surrounding a disc impregnated with plant extract placed over a lawn of bacterial and

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fungal culture plates. As the concentration of crude extracts rose, the results demonstrated that the antibacterial properties of the plant extract were enhanced. When tested against a variety of harmful bacteria and fungi, the extracts demonstrated strong antibacterial action.

Antibacterial Activity

At a concentration of 10 mg/ml, the acetone extract showed the greatest zone of inhibition (22 mm) against P. aeruginosa and Bacillus subtilis, followed by the hexane extract (22 mm) against each strain. In contrast, the acetone extract showed the lowest zone of inhibition (5 mm) against K. Pneumonia. The zones of inhibition measured 20 mm, 19 mm, 16 mm, 11 mm, and 5 mm, respectively, against these bacteria, demonstrating that the acetone extract exhibited strong inhibitory efficacy (Table.1).

Antifungal Activity

The development of Aides Niger could be more effectively controlled by Aegle marmelos, with a zone of inhibition measuring 18 mm in acetone and 17 mm in hexane at a concentration of 10 mg/ml, respectively, as shown in Table 1.

Antioxidant Activity

Table 2: Antioxidant Activity of Different Concentration of in Vitro Leaf Extracts of Aegle Marmelos with The Acetone and Ethanol Extracts

Sl. No	Concentrations of Extracts (µg/ml)	Antioxidant Activity	
		Acetone extracts	Ethanol extracts
1	40	25.2 ±0.03	53.2±0.04
2	60	27.4 ±0.07	56.7±0.06
3	100	30.2 ±0.08	59.9±0.09
4	130	32.5 ±0.05	61.6±0.07
5	160	33.7 ±0.03	63.5±0.05

The antioxidant activity of Aegle marmelos leaf extracts, as shown in Table 2, increases steadily with increasing concentrations of both acetone and ethanol extracts, according to in vitro studies. The antioxidant activity of the ethanol extract (53.2 ± 0.04) is much greater than that of the acetone extract (25.2 ± 0.03) at the lowest concentration (40 µg/ml), suggesting that ethanol is a more effective solvent for extracting antioxidant chemicals. The antioxidant activity of both extracts steadily increases as the concentration rises to 60, 100, 130, and 160 µg/ml. At 160 µg/ml, the ethanol extract consistently displays much greater antioxidant values (63.5 ± 0.05), whereas the acetone extract shows a relatively lower maximum of 33.7 ± 0.03.

V. CONCLUSION

Plants' active components aid the plant and play a role in its defensive system. Undoubtedly, plants have maintained their provision of a wide variety of natural chemicals spanning many molecular families. Significant antibacterial and antioxidant activity were confirmed by the investigation in leaf extracts of Aegle marmelos. When it came to extracting the bioactive chemicals that were

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International Journal of Advanced Multidisciplinary Scientific Research (IJAMSR) ISSN:2581-4281

responsible for these actions, ethanol was the solvent of choice. The fact that the plant's antioxidant activity increases with dosage further highlights its medicinal potential. These results provide credence to Aegle marmelos long history of therapeutic usage and raise the possibility that it might be a natural source of antioxidants and antimicrobials. Its potential use in medication development could be enhanced by future research that aims to isolate and characterize individual bioactive components.

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