IJRAR.ORG E-ISSN: 2348-1269, P-ISSN: 2349-5138

INTERNATIONAL JOURNAL OF RESEARCH AND ANALYTICAL REVIEWS (IJRAR) | IJRAR.ORG

An International Open Access, Peer-reviewed, Refereed Journal

Phytochemical Evaluation and Antimicrobial Activity of Leaves of *Aegle marmelos* (Bael)

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ABSTRACT

Therapeutic value of *Aegle marmelos*, commonly known as Beal has been recognized as a component of traditional medication for the treatment various human ailment. The plant, though, being highly explored, still lack sufficient evidences for the best variety possess the highest degree of medicinal values. The present study is focused on phytochemical screening of ethanolic and methanolic leaf extract of Beal plant. The crude extracts of *Aegle marmelos* revealed the presence of several biologically active phytochemicals with the highest quantity of alkaloids, flavonoids and phenols. The antibacterial efficacy was investigated against pathogenic bacterial strains and the highest inhibitory activity of methanol extract was obtained against *S. typhi and B.cereus* and MIC was observed as 7.8µg/ml whereas ethanolic extract were found to be potent against *B. cereus* and MIC was observed as 15.62 µg/ml. the MIC for standard antibiotics ampicillin was 1.29 µg/ml. The leaves of *Aegle marmelos* showed potent antimicrobial activity against pathogenic microorganisms.

Keywords: Antimicrobial, MIC, Phytochemicals, *Aegle marmelos.*

INTRODUCTION

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs [1]. Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO [2]. Because of the growing recognition of natural products, the demand for medicinal plants has been increasing all over the world. They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects [3]. Side effects by antibiotic-resistant microorganisms have urged scientists to search for compounds which have potential antimicrobial activity [4]. The ability to synthesize compounds by secondary metabolism possessing antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products [5]. The effectiveness of plant extracts on microorganism has been studied worldwide [6–9].

Bael (*Aegle marmelos*) has been known to be one of the most important medicinal plants of India since Charak (1500 B.C) [10]. More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders [11–14]. Antioxidant, antiulcer, antidiabetic, anticancer, anti hyperlipidemic, anti- inflammatory, antimicrobial, ant spermatogenic effects have also been reported on various animal models by the crude extracts of this plant [14]. Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possess medicinal property and is used for treating various eye and skin infections. Leaf is considered to be one of the highest accumulator parts of the plant containing bioactive compounds which are synthesized as secondary metabolites. The present study was, therefore, aimed at evaluating accumulator and methanolic leaf extracts.

MATERIALS AND METHODS

Collection and Identification of plant material

The fresh leaves of *Aegle marmelos* were collected from various farms from Latur district (MS).

Extraction of Phytochemicals:

The leaves of the plants were properly washed under 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 air tight glass containers at 4^{o}C until used. 10 gm of powdered sample was soaked in methanol and ethanol (100ml & 100ml) separately for 12 hours at room temperature. And concentrated to a final volume of 50mL and subjected to phytochemical analysis. For antibacterial screening the leaf extracts were prepared from Pant Aparna variety by following the protocol of Harborne [15] in Soxhlet apparatus using 180 mL of ethanol and methanol. Every extraction was carried out for 24hrs and the extract was then dried, weighed, and stored in refrigerator at4◦C.

Bacterial Strains:

Pure cultures of five test organisms, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhi* were procured from MTCC Chandigarh, India Laboratory. Stock cultures were maintained at 4◦C on agar slants of nutrient media. Active cultures for experiment were prepared by transferring a loop full of microorganism from the stock cultures to 50 mL of sterile nutrient broth.

Phytochemical Analysis:

Qualitative phytochemical analyses of both the extracts were performed by following the protocol.

Tannins: 200 mg of plant material was boiled in 10 mL distilled water and few drops of $FeCl_3$were added to the filtrate; a blue-black precipitate indicated the presence of Tannins.

Alkaloids: 200 mg plant material was boiled in 10 mL methanol and filtered.1% HCL was added followed by 6 drops of Dragen dorff reagent, and brownish-red precipitate was taken as evidence for the presence of alkaloids.

Saponins: (Frothing test). 5 mL distilled water was added to 200 mg plant material. 0.5 mL filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable foam indicates the presence of saponins.

Terpenoids: (Salkowski test) .The 200 mg plant material 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated sulphuric acid (H_2SO_4) were carefully added. A reddish brown colouration signified the presence of terpenoids.

Flavonoids: To the aqueous filtrate 5 mL of dilute ammonia solution was added, followed by concentrated H_2SO_4. A yellow colouration indicated the presence of flavonoids.

Phlobatannins: The deposition of a red precipitate denoted the presence of Phlobatannins when 200 mg of plant material was dissolved in 10 mL of aqueous extract and few drops of 1% HCL were added in the boiling tube.

Anthraquinones: 500 mg of dried plant leaves was boiled in 10% HCL for 5 min and filtrate was allowed to cool. Equal volume of $CHCl_3$with few drops of 10% $NH_3$was added to 2ml filtrate, formation of rose-pink color indicates presence of Anthraquinones.

Reducing Sugars: To the 10 mL of aqueous extract a few drops of Fehling's solution A and B were added; an orange red precipitate suggests the presence of reducing sugars.

Antibacterial Activity:

The extracts mentioned above were tested against five pathogenic bacterial strains, three gram-positive bacteria (*B. cereus*, *S. epidermidis*, and *S. aureus*), and two gram-negative bacteria (*E. coli*, *S. typhi*). Antibacterial screening was done using agar well diffusion method. For this 20 mL of sterile Mueller- Hinton Agar (Hi-media) was poured in sterile autoclaved petri plates. After solidification, the sterile cotton swab was dipped into the bacterial culture. The entire agar surface of each plate was evenly inoculated by swabbing. The four uniform wells were prepared with the help of sterile 6 mm diameter cork-borer. Each well was filled with the various concentrations of both the methanol and ethanol extract (10, 20, 30, and 40mg/mL), respectively, whereas, in case of aqueous: ethanol, (40, 80, 100, and 120 mg/mL) concentrations were used and allowed for diffusion for 45 minutes. The plates were then incubated at 37°C for 24 hrs. Triplicate plates were prepared for each treatment and the average zone of inhibition excluding well was recorded. DMSO was used as negative control. Turbidity of bacterial culture was maintained up to 1×10^7 CFU/ml. The antibacterial potential of extracts was compared with standard antibiotic Ampicillin (10μg/disc) with paper disc (Hi-media) method.

RESULTS AND DISCUSSION:

The study was carried on ethanolic and methanolic extracts of *Aegle marmelos* to investigate the presence of medicinally important phyto-chemicals in the leaves.

Both the extracts revealed the presence of various phytochemicals such as tannins, saponins, flavonoids, alkaloids, terpenoids, carotenoids, cardiac glycosides, and reducing sugars while paleobotanies and antho-cyanins were absent (Table.1).

The presence of different phytochemicals and the antimicrobial activity of ethanolic and methanolic extracts of *Aegle marmelos* leaves have been done. In case of methanolic extract the maximum antibacterial activity was seen against *B. cereus* and *S. typhi at* highest concentration of i.e. 40 mg/ml & they showed both 19 mm zone of inhibition. Followed by *B. cereus* (18 mm) and *S. aureus* (16mm) at 30 mg/ml concentration. In case of ethanolic extract, the maximum antibacterial activity was seen against *S. aureus* at highest concentration of 120 mg/ml it showed 23 mm zone of inhibition. Followed by *E. coli* (22 mm) and *S. typhi* (18 mm) The antibiotic susceptibility showed that among all the bacterial strains *S. epidermidis* (35 mm) was found to be more susceptible towards ampicillin followed by *S. aureus* (28 mm) (Figure.1). The lowest MIC of methanolic leaf extract was found 7.8 µg/ml against *S. typhi* and *B. cereus* whereas, for ethanolic leaf extract it was found to be 15.62 µg/ml against *B. cereus* (Table.2).

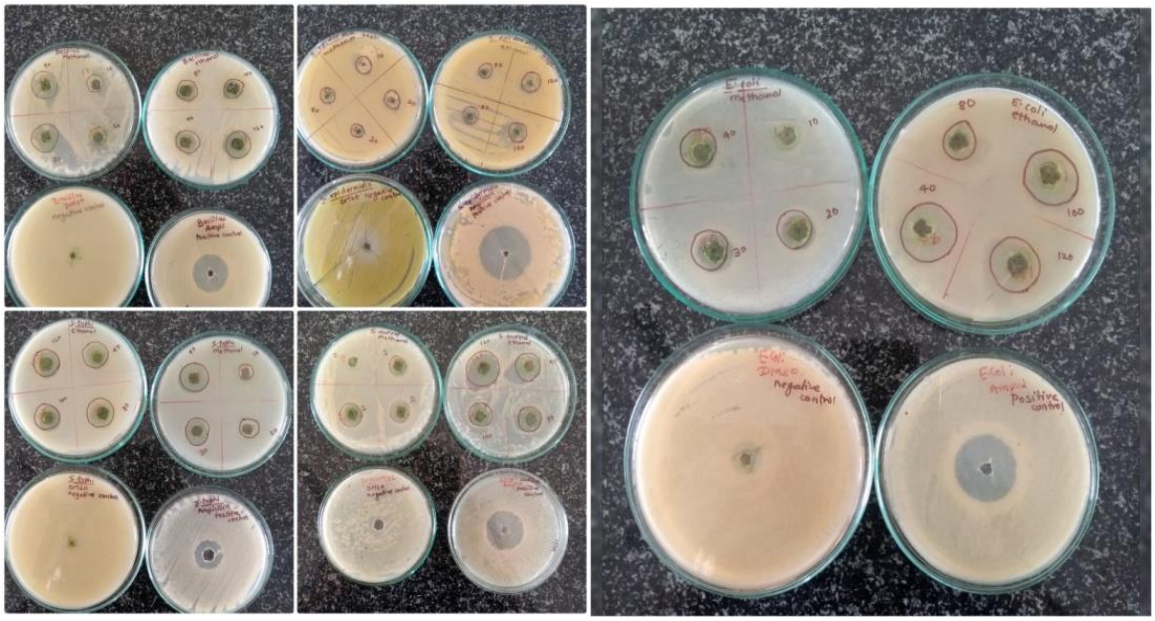

Figure.1. Antimicrobial activity of methanolic and ethanolic leaf extract by agar well diffusion method.

Table.1: Qualitative analysis of phytochemicals in methanol and ethanol leaf extract of different varieties of Bael (*Aegle marmelos*).

Diff.Bael variety/accessions	[a]TA	[b]PHL	[c]SAP	[d]TER	[e]FLA	[g]ANTH	[h]CAR	[i]RED	[j]ALK
NB-17	+	−	+	+	+	−	+	+	+
Pant Aparna	+	−	+	+	+	−	+	+	+
NB-9	+	−	+	+	+	−	+	+	+
NB-5	+	−	+	+	+	−	+	+	+
AM-4	+	−	+	+	+	−	+	+	+
NB-7	+	−	+	+	+	−	+	+	+
AM-3	+	−	+	+	+	−	+	+	+
NB-1	+	−	+	+	+	−	+	+	+
Kaghzi	+	−	+	+	+	−	+	+	+

[a]TA:tannins;[b]PHL:hlobatannins;[c]SAP:saponins;[d]TER:terpenoids;[e]FLA:flavonoids;;[g]ANTH:combinedanthraquinones;[h]CAR:carotenoids; [i]RED: reducing sugar; [j]ALK: alkanoids; +: present; −: absent.

Table. 2: Antimicrobial activity of selected plant leaf extracts (Pant Aparna).

Bacterial strain	Methanolic conc. (mg/ml)	Zone of inhibition (mm)	Ethanolic conc. (mg/ml)	Zone of inhibition (mm)
E.coli	10	09	40	22
	20	14	80	13
	30	15	100	21
	40	15	120	19
MIC	10	31.25 (µg/ml)	10	62.50 (µg/ml)
B.cereus	10	12	40	14
	20	12	80	14
	30	18	100	15
	40	19	120	17
MIC	10	7.8 (µg/ml)	10	15. 62(µg/ml)
S. typhi	10	09	40	17
	20	18	80	18
	30	16	100	17
	40	19	120	18
MIC	10	7.8 (µg/ml)	10	62.50 (µg/ml)
S. aureus	10	No zone	40	18
	20	12	80	17
	30	14	100	17
	40	16	120	23
MIC	10	31.25 (µg/ml)	10	62.50 (µg/ml)
S. epidermidis	10	10	40	09
	20	11	80	11
	30	09	100	12
	40	10	120	13
MIC	10	31.25 (µg/ml)	10	62.50 (µg/ml)
Ampicillin Zone	18	16	15	18
Ampicillin MIC	1.29(µg/ml)	1.29(µg/ml)	3.90(µg/ml)	1.29(µg/ml)

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 work 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 extract 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: ethanol and aqueous: methanol extracts prove to be encouraging in the development of novel antimicrobial formulation in the near future.

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