ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF AEGLE MARMELOS (LEAF EXTRACT) AGAINST BACTERIAL SPS.

Shyamala Gowri R[1] Meenambigai P[1] Vijayaraghavan R[2]

[1]*Nadar Saraswathi College of Arts and Science, Theni, Tamilnadu,*
[2]*Nehru Arts and Science College, Coimbatore. Tamilnadu*

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Abstract

Every part of Aegle marmelos is known for its numerous medicinal properties. In the present study invitro antibacterial activity of aqueous, methanol, ethanol and acetone extracts from leaves were investigated and the extracts exhibit a broad spectrum of antibacterial activity with the zone of clearance ranging from 6-17mm against Salmonella sp, Staphylococcus sp, Shigella sp and Pseudomonas sp. Extracts of acetone and ethanol shows high susceptible to Salmonella and Shigella sp. The extracted Aegle marmelos have antioxidant and cytotoxic potential were investigated by determined by 1,1-diphenyl -2- picylhydroxyl radical scavenging activity, Hydroxyl radical scavenging activity and scavenging of hydrogen peroxidase. Phytochemical screening was performed to determine the ability of an extracts which inhibit the growth of bacteria.

Keywords: *Aegle marmelos,* antibacterial activity, leaf extracts, Zone of inhibition

1. INTRODUCTION

A. Marmelos is a native plant of India. *A. Marmelos*fit in to Rutaceae family and commonly recognized as wood apple. In India, *A. Marmelos* is grown as a temple garden plant and the leaves are charity to pray Lord Shiva. Bael (*Aegle marmelos*) is one of the most significant tree species used in numerous aboriginal systems of medicine in India, China, Burma and Sri Lanka (1)and has been used by the inhabitants of Indian subcontinent for over 5000 years.In recent years antimicrobial assets of Indian medicinal plants have been increased (2,3). The outmoded treatment approach is of much importance, especially in India due to the endemic presence of infective gastro intestinal diseases which are the major causes of infant and adult mortality (4). Antibiotic principles are the distributed widely among angiosperm plants and variety of components were gathered in plant parts for their constitutive antimicrobial activities (5). Each and every part of *Aegle marmelos* plant like fruits, stem, bark, and leaves exhibits the medicinal property and is used for treating eye and skin infections. Most accumulatory parts of the plant leaf contain bioactive compounds which are produced as secondary metabolites (6). Nearly 100 phytochemical components have been isolated from different parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids and tannins. These components possess both biological and pharmacological activity against chronic diseases such as cancer and cardiovascular and gastrointestinal disorders (7). The

present study was, therefore, aimed at evaluating the antimicrobial and antioxidant activity of *Aegle marmelos* leaf extracts against some pathogenic microbes. Antimicrobial activities are due to the secondary metabolites synthesized by the plants such as phenolic compounds. Limited information is available regarding antimicrobial activity of *Aegle marmelos* leaves; therefore, present study is carried out to investigate antimicrobial activity of serial extracts from leaves of *Aegle marmelos* against various bacterial species. Preliminary phytochemical studies of these extracts are also under-taken to find out bioactive compounds having antimicrobial activity.

2. Materials & methods

2.1.Collection of plant samples

The fresh leaves of *Aegle marmelos* were collected from cultivated farms and the open fields of Theni district. Fresh parts of the plants were recognized and authenticated proceeding for phytochemical analysis. The leaves were rinsed thrice with distilled water followed by double distilled water to remove the dirt and other contaminants then dried at room temperature to remove the wetness for 4 hours.

2.2.Extraction of plant samples

The plant materials was collected, washed with water and gloom dried at room temperature, compressed and busted using a mortar and pestle, and then stored in a cool and dry place.

The materials (20g) were mixed with 100ml of different solvents such as acetone, ethanol, and methanol and aqueous in conical flask and kept on a rotary shaker for 12 hours at 30◦c.

2.3.Phytochemical screening

Preliminary phytochemical screening for possible presence of bioactive compounds such as Alkaloids, Flavonoids, Terpenoids, Tannins, Saponins, Cardiac Glycosides, Steroids, Phlobatannins, Anthraquinones, Reducing sugar, Phenols, Coumarins, Anthocyanin and Betacyanin by standard methods.

A) Mayer's Test: Filtrates were treated with Mayer's reagent (potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) Dragendroff's Test: Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide).Formation of red precipitate indicates the presence of alkaloids.

d) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

e) **Ninhydrin.**

To the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

f)Molisch's Test: Filtrates were treated with 2 drops of alcoholic alpha-naphthol solution in a test tube. Formation ofthe violet ring at the junction indicates the presence of carbohydrates.

g) Benedict's Test: Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitateindicates the presence of reducing sugars.

h)Fehling's Test: Filtrates were hydrolyzed with dil.hcl, neutralized with alkali and heated with Fehling's A&Bsolutions. Formation of red precipitate indicates the presence of reducing sugars.

i) 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.

j)Salkowski test:

To 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.

k) 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.

l) 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.

m) Keller-Kiliani test

2 ml filtrate was treated with 1 ml glacial acetic acid containing few drops of $fecl_3$.Conc. H_2SO_4 was added to the above mixture giving green-blue color depicting the positive results for presence of cardiac glycosides.

n)Liebermann-Burchard reaction

200 mg plant material was added in 10 ml chloroform. Acetic anhydride was added in the ratio of 1 : 1 which resulted into the formation of blue-green ring pointing towards the presence of steroids.

o) Phlobatannins

Take 2ml plant sample in a test tube and add 10ml deionized water and boil at 1000C with few drops of 1%hcl. Deposition of red precipitation gives positive result.

p) Anthraquinones

500 mg of dried plant leaves were 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 2 ml filtrate. The formation of rose-pink color implies the presence of anthraquinones

q) Reducing sugar

Take 1ml or 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 1000C in a water bath. Brick red precipitate shows a positive result.

r) Phenols

(1 ml of extract + 5 ml distilled water + few drops of neutral ferric chloride) appearance of dark green color indicated the presence of phenol.

s) Coumarins

(1 ml of extract + 1ml of ethanol KOH solution) appearance of precipitate indicated the presence of coumarins.

t) Anthocyanin and Betacyanin

About 2ml of test extract was added with 1ml of 2N NAOHand heated for 5 min at 1000 c formation of bluish green color indicates the presence of anthocyanin and formation of yellow color indicates the presence of betacyanin.

2.4. Invitro Antioxidant Assay

Antioxidant potential of *Aegle marmelos* extract was determined by three methods.

a) DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity:

DPPH reduction can be measured directly in the reaction medium by a continuous spectrophotometry assay. DPPH assay give reliable information concerning the antioxidant ability of the tested compound. Aliquots of 20-100 µl (5 mg/10 ml) was added to 2.9 ml of freshly prepared solution of DPPH (6×10-5M in meoh). After incubation at 37◦ C for 1 hour the absorbance was recorded at λmax 517 nm using spectrometer.

Formula:

% inhibition or Scavenging activity of DPPH = (1-B/A) %Where, B = Absorbance taken by control solution A = Absorbance taken by different concentration of solution.

b) Scavenging of Hydrogen peroxide (H2O2):

Hydrogen peroxide assay-The assay for hydrogen peroxide in fruit & root extracts of *A.marmelos* was carried out following procedures. The antioxidant capacity of fruit extract against H2O2 value was expressed as mole of ascorbate equivalent per kg fresh weight. 0.6 ml of 4 mm hydrogen per oxide (in phosphate buffer solution) was added to 4 ml solution of fruits & leaf of *A.marmelos* and incubated for 10 minute. The absorbances of the solutions were measured at λmax 230 nm against a blank solution containing hydrogen per oxide without extract.

2.5. Isolation And Identification Of Organism

The sample was collected from local Lab in Theni. By serial dilution technique the organisms were isolated and undergo for biochemical and morphological identification of organisms. The isolates were maintained in nutrient agar plates for further studies.

2.6. Antimicrobial Activity

Antimicrobial activity was performed using the well-diffusion. The plant extracts were tested on Mullen Hinton agar plates to detect the presence of antibacterial activity.

Bacterial suspensions for each of the tested organism were prepared in 9ml sterile nutrient broth and were incubated at 37^0c for 18hrs to obtain a turbidity of 0.5macfarland.each bacterial suspension corresponding were spread on the surface of Mullen Hinton agar plates with sterile cotton swab and kept to dry. The antimicrobial assay was achieved with agar diffusion technique, consequently five equally distant 6mm wells were made on the inoculated Mullen Hinton agar plates with the help of a sterile cork borer. Each well was loaded with 50µl of different solvent plant extract respectively using a micropipette, the extract was allowed to diffuse for 30minutes at room temperature and the loaded plates were then incubated at 37^0c for 18 to 24hrs.Standard without plant extract used as a control. Appearance of a zone indicated the presence of antibacterial of the plant extract being tested against the bacterial isolates.

3.Results and discussion

3.1. Phytochemical screening

The four extracts revealed the presence of different phytochemicals such as tannins, saponins, flavonoids, alkaloids, trepenoids, phenol, reducing sugars, amino acids and phylobatanninsand the results were tabulated in the table 1. Medicinal plants containing phenolic compounds with tannins as major ingredients are used topically for care and repair of skin wounds (8). Tannins are considered nutritionally unwanted because they precipitate proteins, hinder digestive enzymes and disturb the absorption of vitamins and minerals. Further benefits include the absence of adverse effects and a low incidence of resistance (9)

Table -1: Phytochemical screening of extracts from leaves

Compounds	Methanol Extract	Ethanol Extract	Acetone Extract	Aqueous Extract
Alkaloids	A	A	A	P
Flavonoids	A	P	P	P
Tannins	P	P	P	A
Terpenoids	P	A	A	P
Phenol	P	P	P	A
Steroid	P	P	P	A
Cardiac Glycosides	A	P	P	A
Anthraquinone	A	A	A	A
Reducing sugar	A	A	P	P
Saponins	P	A	P	P
Amino acids	P	P	P	P
Phylobatannins	A	A	A	P

P=PRESENCE, A=ABSENCE

3.2.Antioxidant Activity

The antioxidant activity of *Aegle marmelos* might be due to the inactivation of free radicals on complex formation with the metal ions results the phytochemical screening of aqueous, acetone, methanol and ethanol revealed the availability of multipolar and no polar chemical components. DPPH assay is a stable free radical method. It is an easy, rapid and sensitive path to survey the antioxidant activity of plant extracts. (10) The reduction DPPH takes place due to the presence of hydrogen contribution as antioxidant by the formation diphenypicrylhydrozine. It reduces the color of DPPH with the help of power donating ability. Hydrogen peroxide is not a reactive and it sometimes cause the cytotoxicity and increase the hydroxyl inside the cell thus remove the hydrogen peroxide and scavenging the aqueous, ethanol, methanol and acetone extract. Antioxidant activity of leaf extract in various solvent was tabulated in the table

Table 2: Antioxidant activity

Met- Methanol, Eth- Ethanol, Ace- Acetone, Aqu- aqueous

S.No	Concentration (µg/ml)	DPPH Scavenging Activity				H_2O_2 Radical assay			
		Met	Eth	Ace	Aqu	Met	Eth	Ace	Aqu
1	50	15.5	16.7	13.2	8.2	15	15.9	14.7	9.5
2	100	21	20.8	19.6	13	20.5	19.2	20.2	12.3
3	150	26.3	25.1	22.9	17.3	25	23.6	24.6	18.5
4	200	32.7	28.3	27.5	22.9	35.2	27.8	28.9	23.9
5	250	38	34	30.4	25.7	40.3	30.1	32.5	29.2

3.3.Isolation And Identification Of Organisms

The isolated pathogens were identified as *Salmonella, Shigella, Pseudomonas* and *Staphylococcus sp* by Bergey's manual classification of bacteria.

3.4. Antibacterial Activity

The extract from solvents such as ethanol, methanol, acetone showed unreliable antibacterial activity against the test organisms.[Table 3 (a, b, c, d)]. Well diffusion method revealed maximum inhibition against the *Salmonella, Shigella, Pseudomonas* and *Staphylococcus sp*. It is observed that methanol, ethanol extracts of leaf shows maximum inhibitory effect against *Shigella* and *Salmonella sp.* In the acetone extract *Staphylococcus sp* shows maximum susceptibility when compared to other organisms. Most antimicrobial medicinal plants are more active against Gram-positive than Gram-negative bacteria (11,12).

Table 3 (a) : Antimicrobial activity of Aqueous leaf extract

Concentration	***Staphylococcus sp***	***Salmonella sp***	***Pseudomonas sp***	***Shigella sp***

of extract	Zone of Inhibition in mm			
50µl	2	3	1	2
100µl	5	4	4	3
150µl	6	7	7	6
200µl	9	9	8	9
250µl	11	10	9	11

(b) : Antimicrobial activity of methanol extract

Concentration of extract	***Staphylococcus sp***	***Salmonella sp***	***Pseudomonas sp***	***Shigella sp***
	Zone of Inhibition in mm			
50µl	9	10	10	9
100µl	11	13	10	12
150µl	14	12	12	14
200µl	15	17	13	16
250µl	17	19	16	18

(c) : Antimicrobial activity of ethanol extract

Concentration of extract	***Staphylococcus sp***	***Salmonella sp***	***Pseudomonas sp***	***Shigella sp***
	Zone of Inhibition in mm			
50µl	9	9	8	9
100µl	11	10	12	13
150µl	13	12	13	14
200µl	14	13	14	15
250µl	15	17	16	17

(d) : Antimicrobial activity of acetone extract

Concentration of extract	***Staphylococcus sp***	***Salmonella sp***	***Pseudomonas sp***	***Shigellas p***
	Zone of Inhibition in mm			
50µl	10	9	8	8
100µl	12	10	9	11
150µl	13	12	11	12
200µl	15	14	12	15
250µl	17	16	14	16

4.Conclusion

The activities of aqueous, methanol, acetone and ethanol extracts of *Aegle marmelos* on bacterial pathogens such as *Salmonella, Shigella, Pseudomonas* and *Staphylococcus sp*. as discovered in this present study it can be used as traditional therapy.

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