Phytochemical Analysis, Antifungal and Antibacterial Screening of Aegle marmelos: A Guyana Floral Extract

ABSTRACT

The plant material, leaves of Aegle marmelos were collected from Cove and John Ashram, Georgetown, Guyana. Leaves are dried in oven at 50-55 ºC for 72 h. The moisture content is calculated. The dried leaves were grounded and extracted in each acetone, ethanol and methanol solvents. Extracts were collected and evaporation of solvent was done on rotavapour. The antimicrobial and antifungal activity of leaf extract were examined by well diffusion, poison plate, paper disc plate and streak plate methods. In Aegle marmelos leaves extract studies, maximum and minimum antimicrobial potential was observed for methanol and acetone solvents, respectively. Antimicrobial potential of leave extract were also found to be maximum and minimum in Candida albicans and Escherichia coli, respectively in most assay studies. The phyto constituents, tannins, flavonoids, alkaloids, terpenoids, phenol, steroids and phytosteroids were found to present in methanol leave extract of Aegle marmelos. Present study is focus on phytochemical analysis, antifungal and antibacterial screening of Aegle marmelos leaves extracts.

Keywords: Aegle marmelos; Leaves extract; Antimicrobial potential; Antifungal potential; Phytochemical screening

1. INTRODUCTION

Classification of Aegle marmelos (bael)

Kingdom - Plantae – Plants

Subkingdom : Tracheobionta – Vascular Plants

Superdivision : Spermatophyta – Seed Plants

Division : Mangoliophyta – Flowering Plants

Class : Mangoliopsida – Dicotyledons

Subclass : Rosidae

Order : Sapindales

Family : Rutaceae – Rue Family

Genus : Aegle corr – serr – aegle P

Species Aegle marmelos (L)

"Aegle marmelos (L) commonly known as bael , golden apple, wood apple, stone apple, Bengal quince, Japanese better orange is a species of tree native to Indian subcontinent and south East Asia. It is present in Sri Lanka, Thailand and Malaysia as a naturalized species" [1, 2]. "Bael is a small to medium sized tree up to 13 m tall. Bark is pale brown or grayish smooth armed with long straight spines, 1.2 – 2.5 cm singly or in pairs. The leaf is trifoliate, alternate, each leaflet 5 - 14 x 2- 6 cm ovate with tapering or pointed tip and rounded base. The flowers are 1.5 to 2.0 cm, pale green are yellowish, sweetly scented bisexcial at the end of twings and leaf axils. Bael fruits typically has a diameter of between 5 – 12 cm. It takes about 11 months to ripen on the tree. This is considered a sacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship" [3]. "The bael tree contain Furocoumarins, including xanthotoxol and the methyl ester of alloimperatorin as well as flavonoids, rutin and marmesin. It also contains a number of essential oils and alkaloids such as o – isopentenylhalfordinal, o – methylhafoxdinol. Angeline is

a constituent that can be extracted from bael leaves" [4, 5]. "Anglemarmelosine (C_{16} H_{15} NO_2) has been isolated as an orange viscous oil" [6].

The various proved therapeutic values of Aegle marmelos are such as anti-diabetic activity [7], hepatoprotective activities [8], antimicrobial activity [9], analgesic anti-inflammatory [10], antifungal activity [11], anticancer activity [12], antiulcer activity [13], antithyroid activity [14] etc. Jannat et al. [15] has presented a review to examine the potential of the plant Aegle marmelos in the treatment of Alzheimer's disease. Guleria et al. [16] has determined the antibacterial activity of variant of vilram oil (AB -1 and AB -2) against selected pathogenic bacteria (B. subtilis, E. coli, S. aureus).

A summarized information concerning the morphology, distribution, phytochemistry, pharmacological and ethno-botanical uses of Aegle marmelos has been reported by Singh et al. [17]. Kumar et al. [18] has investigated "significant increase in acidic protein concentration in semen of Aegle marmelos treated mice may add more negative charges on sperm surface membrane which affect capacitation and fertilizing ability of spermatozoa that may cause infertility among treated group of mice". "The qualitative and quantitative analysis of photochemical in different solvents extracts of Aegle marmelos was described by Nayaka and Landonkar" [19]. "Primary metabolites like total soluble carbohydrates, proteins and secondary metabolites such as flavonoids, total phenols and tannins were estimated using standard protocols". [19] Hameed et al. [20] has examined "anti-pyretic, anti-diarrheal activities of n-hexane and aqueous extracts of the leaves of Aegle marmelos". "The result of the study supported the traditional use of the plant as a crude anti-pyretic and anti-diarrheal drug. Phytochemical screening, spectroscopic examination and antimicrobial evaluation of methanolic leaf extract of Aegle marmelos was conducted" by Manorama et al. [21]. The phytochemical

screening has been studied by using UV, IR, TLC and AAS. “A review consists of all the updated information and secondary metabolites, medicinal properties and tissue culture studies on Aegle marmelos” described by Gupta et al. [22]. Ankita et al. [23] has evaluated “anti-microfilarial, antifungal, analgesic, anti-inflammatory, antipyretic, hypoglyecamic, antidyslipidemic, immunomodulatory, antiproliferative, wound healing, anti-fertility and insecticidal abilities of bael fruit. Other aspects of potential use of Aegle marmelos such as phytochemical, ethonobotanical and pharmacological evaluations have been reported in this review”.

The management of floral temple waste such as yellow flowers of chrysanthemum marifolium and leaves of Aegle marmelos discussed by Shrivastava and Shrivastava [24]. This study also deals with the phytochemical screening and thin layer chromatographic separation of plant material. Warkhade and Gupta [25] has studied “antimicrobial activity and phytochemical analysis of leaves and fruit extract of Aegle marmelos in combination with commercial antibiotic tetracycline and streptomycin. The antibacterial activity of tetracycline and streptomycin was enhanced against the test organism in the presence of ethanolic, acetone, aqueous extract of leaves and fruits of bael”. Determination of volatile bioactive compound from Aegle marmelos root, stem, leaves, bark, fruit peel and pulp was done by Sharma and Dubey [26]. GC-MS analysis revealed chromatogram of methanol extract of A. marmelos were found to have a number of phytochemicals.

Perumal et al. [27] has evaluated “the antioxidant activities of leaves of Aegle marmelos and identify the bioactive compounds by performing GC-MS analysis resulting in presence of volatile and semi volatile compounds. It was concluded that plant might be promising as a curative for many diseases associated with free radicals”. “The macroscopic, microscopic,

powder study, physicochemical and florescence analysis of the seed were carried out" by Pande et al. [28]. The data generated in present work could be used as reference for the standardization and quality control of A. marmelos seed. It will help to identifying and preventing intentional or unintentional adulteration of this plant material.

Timbadiya et al. [29] has discussed phytochemical screening, physicochemical activities, oxidative enzyme activities, anti-inflammatory properties from hexane, chloroform, methanol and aqueous bael leaf extracts. A review on potential antidepressant property of traditional plants described by Rahaman et al. [30]. "The purpose of this review is to further outcome should come to light. The assessment of antifungal activities of acetone, ethanol, methanol and chloroform leaf and fruit extracts of Aegle marmelos, Syzygium cumini and Pangamia pinnata against the soil borne fungi, Pythium debaryanum described" by More et al. [31]. "The methanol extract revealed strongest antifungal activity against P. debaryanum, followed by ethanol extract and lowest antifungal activity was found in chloroform extract. Antibacterial activity of benzene extracts of three plants namely Abutilon indicum, Plectronthum amboinicus and Aegle mormelos were determined using agar disc diffusion method at different concentration from 5-30 µg/µl against two gram –positive Staphylococcus aureus , Enterococcus faecalis and two fungal strains Aspergillus niger , Aspergillus fumigatus and compared with standard drugs norfloxacin and fluconazole" , respectively studied by Sasikala et al. [32]. Benzene extract of fruit from A. indicum inhibited S. aureus, E. faecalis at 30 µg/µl and leaves of P. amboinicus showed considerable inhibiting activity against the A. niger , A. fumigatus at 30 µg/ µl. Hence these plants can be further used for determine the bioactive natural products that may provide a leads in the development of new drugs.

Laddha et al. [33] has investigated "nutritional and phytochemical analysis of ripened fruits of Aegle marmelos a wild edible plant of Bhiwapur Tahsil, Nagpur district, India. The study includes estimation of ash content, protein, carbohydrate, vitamins and mineral contents (Cu, Fe, Mn, Zn, Ca, K) of bael fruit. The nutritional and phytochemical analysis reveals that the fruit are not only acting supplementary fruits, but is the tonic requirements of the tribals and deprived of poor Bhiwapur Tahsil".

Antibacterial property of Aegle marmelos (L.) correa methanolic and chloroform leaves extract was evaluated by Yadav et al. [34]. Using agar disc diffusion method. A. marmelos extracts are found to be potential antibacterial agent against both gram-positive and gram-negative bacteria. Aegle marmelos aqueous, acetone, ethyl acetate leaf extracts were screened enteric pathogens such as Eshcherichia coli, Salmonella spp. And Shigella spp by Selaraj et al. [35]. The study proves that compounds from Aegle mermelos will be a good source for diarrhea causing organism. Present studies report phytochemical analysis, antifungal antibacterial screening of acetone, ethanol and methanol leaves extract of Aegle marmelos.

2.0 METHODOLOGY

2.1 Collection of plant materials

The plant material leaves of Aegle marmelos were collected from Cove and John Ashram, Cove and John village, East Coast Demerara, Guyana.

2.2 Preparation of plant materials:

The collected leaves sample of Aegle marmelos is weighted on Citizen CTG 3000E electronic balance. The leaves dried in oven (Gallenhamp Incubator Model IH-150) at 50-55ºC. The

dried leaves were cooled at room temperature and weighted again on same citizen electronic balance. Weight of green leaves, dried leaves and value of percentage moisture content in various samples of Aegle marmelos is given in Table 1. The weight of ground leaves of Aegle marmelos is found to be 500.3 grams.

2.3 Collection of test organism

Three micro-organisms (Escherichia coli, Staphylococcus aureus and Candidus albicans) were used for the study. All the tested strains are reference stains and were collected from the Microbiology Laboratory of Georgetown Public Hospital Corporation, Georgetown (GHPC). All cultures are maintained in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar (Himedia MM012) slants at 4º C.

2.4 Extraction and preparation of test solutions

The grounded leaves of Aegle marmelos was extracted in each acetone, ethanol, and methanol solvents. At a time 20 g of dried pulverized leaves were soaked with 200 mL of solvent for 48 h. Solvent is decanted each time and residue again soaked with same solvent for 24 h. The total extract is combined and filtered. The evaporation of solvent was done on rotavapour (Buchi). The respective solvent was added to viscous semi solid liquid extract to make up the desired volume of extract solution.

*2.5 Anti-Microbial Assay***:**

2.5.1 Materials

Mueller Hinton Agar, Agar plates and microbial discs were purchased from the Caribbean Medical, Parika in Guyana. Solvents acetone, ethanol, and methanol obtained from Aldrich. Scitilation vials 20 mL were obtained from Meditron Scientific Sales, Georgetown, Guyana.

2.5.2 *Aseptic chamber*

Aseptic chamber consists of a wooden box of L = 1 meter, B = 1 meter and D = 0.5-meter area. Chamber is cleaned with 70 % ethanol twice and irradiated with short wave UV light for 1 h.

2.5.3 Potato Dextrose *Agar (PDA) medium*

Potato Dextrose Agar (PDA) medium prepared according to method reported by Talaro [36]. This is the medium on which cultured bacteria Escherichia coli, and Staphylococcus aureus were grown. The 200 g potato was peeled finely chopped and boiled to a mash in distilled water.Each 12.5 g dextrose and 12.5 g Agar was placed in a 1 L measuring cylinder. Distilled water was added to make the solution to 500 m L. The content was stirred until the consistency of solution mixture. The stirred mixture poured into conical flasks, plugged with cotton wool and tightly wrapped by aluminum foil. The flasks were autoclaved at 121 ºC, 15 psi, for 15 minutes

2.5.4 *Mother plates*

Mother plates were prepared by pouring Potato Dextrose Agar mixture into Petri dishes and to cool at room temperature, in the aseptic chamber.

2.5.5 *Antimicrobial assay*

Antimicrobial assay was done by well diffusion method, poison plate method, paper disc method and streak plate method. Which are as follows:

2.5.5.1 Well diffusion method:

In this method well are made using a sterilized the cork borer on the seeded nutrient agar in a petri dish to which the test compound (leaves extract) of different concentration (25, 50, 75, 100, 125 µL s) are added. The treated petri discs are incubated at room temperature for 24 hrs. The inhibition zone formed around each well indicates the antibacterial activity. The procedure was repeated in duplicate and inhibitory zone was measured by ruler in mm [37].

2.5.5.2 Poison plate method:

The test organism (S. aureus, E. coli, C. albicans) seeded into nutrient medium were poured into petri discs and allow to cool and solidify. A 9.0 cm sterile cork borer was used to make a disk on pathogen plate. Pathogen disc was taken from pathogen plate and kept at the center of test compound (leaves extract of 25, 50, 75, 100, 125 µL concentrations) seeded plate with the help of a sterile inoculum needle and was incubated for 2 to 3 days. The inoculum needle was sterilized with the alcohol and flame before each application. The experiment was done in duplicate and zone of inhibition was measured [38].

2.5.5.*3 Paper disc plate method:*

The circular discs of 6 mm diameter were prepared from whatman no.1 filter paper and sterilized in a autoclave. These paper discs were impregnated with test compounds (leaves extract 25, 50, 75, 100, 125 µ L s) in respective solvent (acetone, ethanol, methanol) for overnight and placed on nutrient agar plates seeded with test organism (S. aureus, E. coli, C. albinos) . The plates are incubated at room temperature for 12 hr. After 12 hr zone of inhibition around each disc was measured by horizontal and vertical method and the diameter was recorded. A reference control was prepared using only the several (acetone, ethanol, methanol) and kept for comparison. The test done in duplicate to ensure the reliability of the results [39].

2.5.5. 4 Streak plate method:

The molten agar medium (20 m L) and each leaves extract and each leave extract (25, 50, 75, 100, 125 µL) was poured into a sterile petri dish under aseptic condition. It was cooled at room temperature. After cooling each bacterial culture was taken at 12, 24, 36 hour intervals and using the surface of agar medium in the form of parallel strokes (streaks). The test repeated in duplicates. The plates were incubated at room temperature for 24 hours and inhibitory zone was measured. Control plates without the plant extract were also maintained for the reference [40].

2.6 Phytochemical analysis of the plant extracts

2.6.1 Materials

Glacial acetic acid, thyonil chloride, dichloromethane, copper sulfate, lead acetate, diethyl ether, ferric chloride, acetic anhydride, antimony chloride, amyl chloride etc. obtained from Aldrich.

2.6.2 Method

Phytochemical analysis of all the aqueous plant extracts was carried out by suitable methodologies in search of active ingredient responsible for antimicrobial toxicity. The phytochemicals include under study were saponins, terpenoids, alkaloid, cardiac glycoside, phenol, tannins, phlobatannins, steroid phytosteroid and flavonoids the analysis was carried out according to the methodologies of Edeoga et al. [41].

Table 1. Percentage moisture content for Aegle marmelos (bael leaves)

Sample Number	Weight of green leaves (gram)	Weight of dry leaves at 10 am(grams)	Weight of dry leaves at 4 pm (grams)	Percentage moisture content %
1	140	55	55	60.71
2	149	56.7	56.7	61.95
3	117.5	46	46	60.85
4	155.8	55.7	55.7	64.25
5	121	44.5	44.5	63.22
6	124	43	43	65.32
7	112	41	41	63.39
8	141	52	52	63.12
9	156	54	54	65.38
10	150	52.4	52.4	65.06

$$\text{Percentage moisture contents} = \frac{\text{Weight of green leaves} - \text{weight of dry leaves}}{\text{weight of green leaves}} \times 100$$

3.0 RESULTS AND DISCUSSION

3.1 Well diffusion method

Antimicrobial activity of Aegle marmelos leaves extract against E. coli, S. aureus and C. albicans are summarizes in **Tables 2-4** by well diffusion methods.

Table 2. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in different solvent against E. coli

Compared with control by well diffusion method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.20	0.22	0.25
	50	0.28	0.26	0.28
	75	0.34	0.30	0.30
	100	0.38	0.33	0.38
	125	0.40	0.36	0.42

*Duplicate

Table 3. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various

Solvent against S. aureus

Compared with control by well diffusion method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle Marmelos (Bael)	25 control	0	0	0
	25	0.26	0.24	0.24
	50	0.28	0.28	0.26
	75	0.32	0.32	0.31
	100	0.36	0.35	0.34
	125	0.39	0.37	0.39

*duplicate

Table 4. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against C. albicans.

Compared with control by well diffusion method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle Marmelos (Bael)	25 control	0	0	0
	25	0.20	0.22	0.29
	50	0.24	0.26	0.30
	75	0.28	0.28	0.35
	100	0.32	0.30	0.39
	125	0.34	0.34	0.47

*duplicate

3.2 Poison Plate methods

Antimicrobial activity of Aegle marmelos leaves extract against E. coli, S. aureus and C. albicans are summarizes in **Tables 5-7** by poison plate methods

Table 5. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against E. coli

Compared with control by poison plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.31	0.23	0.26
	50	0.32	0.26	0.29
	75	0.37	0.28	0.30
	100	0.41	0.30	0.32
	125	0.49	0.32	0.34

*duplicate

Table 6. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against S. aureus

Compared with control by poison plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.22	0.31	0.20
	50	0.26	0.33	0.23
	75	0.28	0.38	0.34
	100	0.29	0.42	0.35
	125	0.31	0.50	0.36

*duplicate

Table 7. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against C. albicans

Compared with control by poison plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.19	0.23	0.34
	50	0.20	0.25	0.34
	75	0.22	0.27	0.39
	100	0.24	0.28	0.43
	125	0.26	0.29	0.51

*duplicate

3.3 Paper disc method:

Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against E. coli, S. aureus and C. albicans are summarized in **Tables 8-10** by paper disc methods.

Table 8. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvent against E. Coli
Compared with control by paper disc plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.31	0.21	0.22
	50	0.32	0.23	0.24
	75	0.37	0.34	0.26
	100	0.41	0.36	0.28
	125	0.49	0.38	0.30

Table 9. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against S. aureus
Compared with control by paper disc plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.17	0.30	0.24
	50	0.18	0.29	0.26
	75	0.19	0.34	0.28
	100	0.20	0.38	0.30
	125	0.21	0.46	0.31

*duplicate

Table 10. Antifungal activity of crude of leaves extracts of Aegle marmelos in various

solvents against C. albicans

Compared with control by paper disc plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.25	0.26	0.31
	50	0.32	0.30	0.32
	75	0.39	0.32	0.37
	100	0.42	0.36	0.41
	125	0.45	0.39	0.47

*duplicate

3. 4 Streak plate method:

Antimicrobial activity of Aegle marmelos leaves extract against E. Coli, S. aureus and C. albicans are summarized in **Tables 11-13** by streak plate methods.

Table 11. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against E. coli

Compared with control by streak plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.19	0.20	0.24
	50	0.21	0.22	0.25
	75	0.23	0.23	0.26
	100	0.24	0.25	0.27
	125	0.26	0.28	0.29

*duplicate

Table 12. Antimicrobial activity of crude of leaves extracts of Aegle marmelos in various solvents against S. aureus
compared with control by streak plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.17	0.19	0.21
	50	0.19	0.21	0.23
	75	0.23	0.23	0.25
	100	0.25	0.25	0.29
	125	0.28	0.27	0.31

*duplicate

Table 13. Antifungal activity of crude of leaves extracts of Aegle marmelos in various

solvents against C. albicans

Compared with control by streak plate method

Plant	Leaves extract solvent (µL)	Diameter of the inhibitory zone (mm)*		
		Acetone	Ethanol	Methanol
Aegle marmelos (Bael)	25 control	0	0	0
	25	0.21	0.24	0.27
	50	0.23	0.25	0.31
	75	0.25	0.29	0.34
	100	0.29	0.32	0.35
	125	0.32	0.35	0.37

*duplicate

3.5 Phytochemical analysis:

Phytochemical analysis **Table 14** of the Aegle marmelos (Bael) methanol leaves extract revealed the presence of tannins, saponins, flavonoids, alkaloids, cardiac glycosides, terpenoids, phenol, steroids and phytosteroids and phlobatannins.

Table 14. Phytochemical analysis of Bael (Aegle marmelos) methanolic leaves extract

S. No.	Phyto constituents	
1	Tannins	Present
2	Saponins	Absent
3	Flaronoids	Present
4	Alkaloids	Present
5	Cardiac glycosides	Absent
6	Terpenoids	Present
7	Phenol	Present
8	Steriods and Phytosteroids	Present
9	Phlobatannins	Absent

It is observed from **Tables 1-3** that in acetone solvent maximum (0.40 mm: 125 µL) inhibitory zone was observed for E. coli and equal (0.20 mm: 25 µL) inhibitory zone was observed for E. coli and C. albicans. In ethanol solvent maximum (0.37 mm: 125 µL) inhibitory zone was observed for S. aureus and equal (0.22 mm: 25 µL) inhibitory zone was observed for both E. coli and C. albicans. In methanol solvent maximum (0.47 mm: 125 µL) inhibitory zone was observed for C. albicans and minimum (0.24 mm: 25 µL) inhibitory zone was observed for both S. aureus.

It is observed from **Tables 4 – 6** that in acetone solvent maximum (0.49 mm: 125 µL) inhibitory zone was observed for E. coli and minimum (0.19 mm: 25 µL) inhibitory zone was observed for C. albicans. In ethanol solvent maximum (0.50 mm: 125 µL) inhibitory zone was observed for S. aureus and equal (0.23 mm: 25 µL) inhibitory zone was observed for E. coli and C. albicans. In methanol solvent maximum (0.51 mm: 125 µL) and minimum (0.24 mm: 25 µL) inhibitory zone was observed for C. albicans and S. aureus, respectively.

It is observed from Tables **7-10** that in acetone solvent maximum (0.49 mm: 125 µL) inhibitory zone was observed for E. coli and minimum (0.17 mm: 25 µL) inhibitory zone was observed for E. coli and S. aureus, respectively. In ethanol solvent maximum (0.46 mm: 125 µL) and minimum (0.21 mm: 25 µL) inhibitory zone was observed for S. aureus and E. coli, respectively. In methanol solvent maximum (0.42 mm: 125 µL) and minimum (0.22 mm: 25 µL) inhibitory zone was observed for C. albicans and E. coli, respectively.

It is observed from **Tables 11-13** that in acetone solvent maximum (0.32 mm: 125 µL) and minimum (0.17 mm: 25 µL) inhibitory zone was observed for C. albicans and S. aureus, respectively. In ethanol solvent maximum (0.35 mm: 125 µL) and minimum (0.19 mm: 25 µL) inhibitory zone was observed for C. albicans and S. aureus, respectively. In methanol solvent maximum (0.37 mm: 125 µL) and minimum (0.21 mm: 25 µL) inhibitory zone was observed for C. albicans and S. aureus, respectively.

In general, it is observed from **Tables 2-13** that inhibitory zone follows the order methanol > ethanol > acetone in most of the essay this may due to high polarity of methanol solvent. Inhibitory zone on antimicrobial potential of leaves extract increases as the amount of leaves extract increases from 25 µL to 125 µL. this may be due to increase in amount of extracts. The order of inhibitory zone among from methods use for assay studies are as follow:

Well diffusion method > Poison plate method > paper disc method > streak plate method.

The result from Tables 2-13 indicated that all plant extracts showed antimicrobial activity toward the gram positive bacteria S. aureus, gram negative bacteria E. coli and fungus C. albicans. The diameter of inhibitory zone is the measure of antifungal potential of the leaves extract. The maximum and minimum inhibitory zone represent higher and lesser antimicrobial potential, respectively. It is observed from Table 14 that methanol leaves extract of Aegle marmelos tested positive for tannins, flavonoids, alkaloids, terpenoids, phenol, steroids and phytosteroids and negative for saponins cardiac glycosides, phlobatannins phytoconstituents. Aegle marmelos plant with leaves and fruits is shown in Figure 1.

The ten wonder benefits of bael are the followings:

(i) Bael for Tuberculosis: In Ayurveda, it is used for the treatment of tuberculosis.

(ii) Bael for Gynecological disorders**:** The regular consumption of Bael helps to prevent gynecological related issues.

(iii) Bael for Urinary diseases: Use of bael leads you to overcome the problems of urinary diseases.

(iv) Bael for diabetes prevention: it has a bitter pungent, full of antioxidants and helps to stimulate the pancreas to secrete insulin, which leads to lowering of blood sugar. The leaves can be used against diabetes.

(v) Bael for digestive disorder: It supports intestinal formulations and protects the digestive system from ulceration, reduces the frequency of irritable Bowel syndrome (IBS), intestinal spasm thus beneficial in treating of diarrhea, dysentery and other infections of Elementary canal.

(vi) Bael for fever prevention: The leaf juice with honey is helpful in the prevention of fever.

(vii) Bael for epilepsy: Flowers are use as epilepsy tonic.

(viii) Bael Nutritional facts: It is rich in alkaloids, polysaccharides, antioxidants, beta carotene, vitamin C, vitamin B, and many other bio-chemical substances. It also contains tannins, calcium, phosphorous, iron, protein and fiber. The 100 gram of Bael contains the following nutrients: calorific value (137 Kcal), moisture (61.5 g), protein (1.8 g), fat (.3 g), minerals (1.7 g), fiber (2.9 g), carb (31.8 mg), calcium (85 mg), phosphorous (50 mg), Iron (7 mg), Beta carotene (55 UG), thiamine (.13 mg), niacin (,13 mg), vitamin C (8 mg), potassium (600 mg) and copper (21 mg).

(ix) Bael for piles treatment: the extract of unripe Bael fruit is helpful in curing piles and hemorrhoids.

(x) Bael fights ulcer: Due to its smoothing effects it is useful in combating ulcers like gastric ulcers, gastroduodenal ulcers etc.

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The parallel studies in the phytochemical analysis, antifungal and antibacterial screening of Aegle marmelos has been reported in the chemical literature. Pandey and Pande [42] has investigated phytochemicals, anti-inflammatory and antioxidant potential of Aegle marmelos L leaves. The scientific data on the nutritional and bioactive composition of the Aegle marmelos fruit alongwith its pharmacological activities has evaluated by Khanal et al. [43]. Ajay et al. [44] has described phytochemical analysis of bael fruit extract by GC-MS. The GC-MS analysis revealed the presence of six major compounds viz. Heraclenin, Imperatorin, Methoxsalen, Germacrene B, Alpha-Guaiene and Caryophyllene. An updated review on current state of research on A. marmelos elucidating it constituents and their most relevant biological activity is presented by Monika et al. [45]. Ahmad et al [46] has examined phytochemical analysis, cytotoxicity, in vitro antioxidant and antidiabetic activities of A marmelos leaf extract, Phytoconstituents were analyzed using GC-MS and HPLC. Jain et al. [47] has examined that Aegle marmelos hydroalcoholic leaf extract is inferred to possess anxiolytic and antidepressant therapeutic responses and can serve as a potential agent against the available synthetic marketed preparation. Screening and evaluation of potential antifungal plant extracts against skin infecting fungus Trichophyton rubrum has been determined by Margret and Caroline [48]. Sukadee [49] has studied antifungal activities of five plants Aegle marmelos L. Eupatorium odoratum L. Phyllanthus acidos L Skeels, Houttuynia cordata thumb and Clausena excavate against C. capsici. The scientific progress on the fruits of A. marmelos related to nutritional and phytochemical composition and pharmacological activities with its potential in the nutraceutical market discussed by Sathasivampillai et al. [50]. Aodah et al. [51] has investigated anticarcinoma, antioxidant and anti- carciogenic effects of A,

marmelos leaf essential oil on human oral epidermal health. Antidiabetic and anti-inflammatory potential of coumarins enriched extract derived from Aegle marmelos L. correa fruit pulp has been described by Tiwari et al. [52].

4.0 CONCLUSIONS

1. In most of the antimicrobial essay studies maximum and minimum antimicrobial potential was observed for methanol and acetone solvent, respectively.
2. It is found from present studies that diameter of inhibitory zone increases as the amount of leaves extract increases from 25 µL to 125 µL.
3. The C albicans and E. coli were found to have highest and lowest antimicrobial potential essay studies.
4. Among four methods used for antimicrobial essay studies, well diffusion method and streak plate methods were found to have highest and lowest inhibitory zone, respectively.
5. The phytoconstituents tannins, flavonoids, alkaloids, terpenoids, phenol, steroids and phytosteroids were found to present in methanol leaves extract of Aegle marmelos.
6. It can also be concluded from present studies that leaves extract of Aegle marmelos has antimicrobial property and its potential increases as their amount increases. Aegle marmelos extracts can be used for the treatment of tuberculosis, urinary disease, diabetes, digestive disorder, epilepsy, ulcer etc. Present research work is very useful for the researchers of similar research interest.

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Figure 1. Aegle marmelos (Bael) plant with leaves and fruits