

Phytochemical Investigation and Evaluation of Antimicrobial Activity of *Aegle Marmelos* Linn Thorns

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Abstract: *Aegle marmelos*, is a Traditional medicinal plant commonly known as 'Bael', with Ethnomedicinal application and has a great mythological significance for Hindus Traditional system of medicines like Ayurveda, Siddha and Unani have been highlighted the use of *A. marmelos* parts (bark, leaves, fruits, flowers etc.) for the treatments of various diseases. Present study aims at Phytochemical and biological evaluation of thorn extract of *Aegle marmelos* Linn. Standardization of powder is done by determination of ash value, extractive value and moisture content of *Aegle marmelos*. Pharmacognostic study was carried out in which macroscopic and microscopic characteristics were studied. For characterization plant parts used are Thorns of *Aegle marmelos*. The extraction was done by successive method in different solvents ranging from Non polar to polar solvents. The percentage yield of thorn is found to be in the range 0.4 %, 2.4933 %, 1.6 %, 0.2 %, 1.57 and 5.13% respectively. Phytochemical screening, conclude that the given plant material shows the presence of alkaloid, glycoside, flavonoids, tannin, phenolic, carbohydrates, proteins and amino acids. Transverse section of *Aegle marmelos* L. plant Thorn shows the presence of cork cambium, cortex, stone cells, starch grains, parenchymatous cells and Trichomes. The antimicrobial activity was checked using different test organisms.

Keywords: Ethnomedicinal, *Aegle marmelos*, mythological, Traditional

I. INTRODUCTION

Natural products provided the encouragement for most of the active ingredients in medicines. Their high chemical and the effects of evolutionary pressure to develop biologically active molecules could be recognised to the success in drug discovery. Plants are rich sources of fine chemicals, largely unknown and explored, they still make an significant contribution to health care in spite of the great advances in the field of modern medicine. The chemistry of natural products comprises biosynthesis, extraction, identification, quantification, structural elucidation, physicochemical properties and reaction. The medicinal plants are helpful for healing as well as for treating of human ailments because of presence of phytochemical constituents.^[1]

India is a rich source of medicinal herbs and species, which comprises about more than 2000 species and has an massive geographical area with high possible abilities for Ayurvedic, Unani, Siddha traditional medicines. Natural product chemistry has progressed into an interdisciplinary area of science deals with the isolation, characterization and determination of biological activity of pure phytochemical, as well as extracts or enriched fractions

In present time W.H.O is also encouraging the use of herbal medicines which are prefer traditionally from last many years. In recent times, W.H.O conducted a survey in which they found that around 20,000 medicinal plants are used either in pharmaceutical industry or in traditional medicine system. Number of peoples using these herbal medicines because they have faith in that natural medicines are more effective and are safer to use.^[2]

1.1 Importance of Natural Products

Natural products played an important role on this earth, so human existence has been made possible. The outstanding wonder of nature always stands as golden mark for realising the herbal drug discovery. Nature held to be a therapeutic abundance to treat number of ailments ranging from common cold to various type of illness since the dawn of civilization.

Natural products are playing vital role in drug discovery program of the pharmaceutical industry and various research organisations. Plants have been used for medicinal purpose through history and cultures and even across species a majority of the world still trusts heavily on natural products as herbal remedies for their primary health care system

1.2 *Aegle marmelos* Linn.

Aegle marmelos belongs to family Rutaceae and it is considered to have many medicinal as well as nutraceutical properties. It is commonly known as Bael or wood apple plant and is used in various traditional medicines. It is cultivated as temple garden plant and the leaves of *Aegle marmelos* L. are used during the worship of Lord Shiva. This is an important medicinal plant having traditional and folk medicines and ethnomedicinal applications.

The medicinal properties of bael indicate that it is a treasured source of variety of bioactive compounds. Plant and the medicines derived from plant are used by humans to treat and to relieve physical and mental illness. Researchers are now work on identification and validation the substances from plants to treat various health ailments. It has been found that different parts of plant like leaves, fruits, seeds etc, are helpful to human body as they provide health and nutritio nal promoting compounds².



Fig 1.1: Bael plant³

1.3 Vernacular Names of *Aegle marmelos* L.

- Bengali: Bel, Shreefal
- English: Wood/Stone apple, Bengal Quince, Indian Quince
- Latin: *Aegle marmelos*
- Marathi: Kaveeth
- Old Hindi: Sir Phal
- Sanskrit: Shreephal, Bilva, Bilwa
- Tamil: Vilva, Maram, Vilva Pazham
- Telugu: Maredu
- Urdu: Bel

Bael is a medium sized tree (25-30 feet) slender, aromatic tree, and it grows slowly. Plant is having short stem, flaking soft bark and branches are sometimes spiny. Older plants have straight and stiff spines and sharp spikes of about one inch length. The leaflets are 4-10 cm long and 2-5 cm in width and are oval or lancet in shape. Leaves are further divided into 3-5 leaflets and mature leaves are having particular fragrance. Fruit is of diameter 2-4 inches and are spherical or oval in shape. Shell of fruit is woody and hard.

1.4 Chemical Constituents Present in Plant

Bael gets its medicinal values on basis of the various bioactive compound present in it like alkaloids, coumarin, polysaccharides, essential oils etc. The other nutritional constituents present in Bael fruits are water, sugar, protein, fiber, fat, calcium, phosphorus, potassium, Iron and vitamins (Vit A, Vit B, Vit C and Riboflavin). The major Alkaloids present

in Bael are aegelin, aegelinine, fragine, o-methyl halforodinine, oiso pentanyl halfordinol, ethyl cinnamide, ethyl cinnamide. It contains 9% tannin in the pulp of wild fruits and its percentage is less in cultivated type.

Tannins are also present in leaves as skimmianine. The essential oil of the leaves contains d-limonene, 56% α -diphellandrene, cineol, citronellal, citral; 17% pcyrene, 5% cumin aldehyde. The coumarins present in Bael fruit include marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methyl ether xanthotoxol, scoparone, scopoletin, umbelliferone, marmalades and marmenol. Ascorbic acid, sitosterol, crude fibers, α -amyrin, crude proteins are other minor constituent. The various polysaccharides present in Bael are Galactose, arabinose, uronic acid, L-rhamnose. Carotenoids are principle pigment responsible for imparting pale yellow colour to fruit.³

1.4.1 Active Constituents Present in Plant:

1. **Alkaloid:** Aegelin, aegelinine, marmeline, dictamine, fragrine, O-methylhalfordinine, Oisopentanylhalfordinol, N-4-methoxy styryl cinnamide.
2. **Coumarin:** Marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methylether, xanthotoxol, scoparone, scopoletin, umbelliferone, psoralen and marmelide.
3. **Polysaccharide:** Galactose, arabinose, uronic acid and L-rhamnose was obtained on hydrolysis.
4. **Tannin:** Tannin was also present in leaves and fruit as skimmianine. Carotenoids were also reported, which impart pale colour to fruit.

1.6 Extraction

Extraction is the most crucial step in the analysis of medicinal plants, because it is necessary to remove the desired chemical components from the plant materials for further separation and characterization. The basic operation included steps, such as pre-washing, drying of plant materials or freeze drying, grinding to obtain a homogenous sample and often improving the kinetics of analytic extraction and also increasing the contact of sample surface with the solvent system.

Potential active constituents are not lost, distorted or destroyed during the preparation of the extract from plant samples are possible if proper actions must be taken. If the plant was selected on the basis of traditional uses, then it is needed to prepare the extract as described by the traditional healer in order to mimic as closely as possible the traditional 'herbal' drug. The solvent selection for system largely depends on the specific nature of the bioactive compound being targeted. Different solvent systems are available to extract the bioactive compound from natural products.

The extraction of hydrophilic compounds uses polar solvents such as methanol, ethanol or ethyl-acetate. For extraction of more lipophilic compounds, dichloromethane or a mixture of dichloromethane/methanol in ratio of 1:1 are used. In some instances, extraction with hexane is used to remove chlorophyll. As the target compounds may be non-polar to polar and thermally labile, the suitability of the methods of extraction must be considered. Various methods, such as sonification, heating under reflux, Soxhlet extraction and others are commonly used for the plant samples extraction. In addition, plant extracts are also prepared by maceration or percolation of fresh green plants or dried powdered plant material in water and/or organic solvent systems⁵

Bael (*Aegle marmelos* Corr.) is an indigenous fruit of India belongs to family Rutaceae. *Aegle marmelos* is extensively distributed all over India and is acknowledged by various names at various places



Fig: Plant of *Aegle marmelos*L.

1.7 Vernacular Names of *Aegle marmelos* L.:⁵

- Bengali: Bel, Shreefal
- English: Wood/Stone apple, Bengal Quince, Indian Quince
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- Telugu: Maredu
- Urdu: Bel

1.8 Taxonomy

Kingdom	Plantae
Order	Spindales
Family	Rutaceae
Subfamily	Aurantioideae
Genus	<i>Aegle</i>
Species	<i>A. marmelos</i>

Table 1.1 - Taxonomical classification ⁷

1.8.1 Leaves:

The deciduous, alternate leaves, borne singly or in 2's or 3's, are composed of 3 to 5 oval, pointed, shallowly toothed leaflets, 4-10cm long, 2-5cm wide, the terminal one with a long petiole. Mature leaves produce a disagreeable odour when bruised. Leaf is trifoliate, alternate, each leaflet 5- 14 X 2-6 cm, ovate with tapering or pointed tip and rounded base, untoothed or with shallow rounded teeth. Young leaves are pale green or pinkish, finely hairy while mature leaves are dark green and completely smooth.



Fig 5.2: Leaf of *Aegle marmelos*

1.8.2 Flowers

Flowering occurs in April and May soon after the new leave. Fragrant flowers, in clusters of 4 to 7 along the young branch lets, have 4 recurved, fleshy petals, green outside, yellowish inside, and 50 or more greenish yellow stamens. Greenish white, sweetly scented, bisexual, actinomorphic, ebracted. Hypogynous, stalked: stalk, 8 mm long, diameter of a fully open flower, 1.8 cal: borne in aternal panicles of about 10 flowers, arising from the leaf axil: calyx, gamosepalous, five-lobed, pubescent, light green, very small in comparison with petals: corolla polypetalous, with 5 petals, imbricate,

leathery, pale yellow from above and green from beneath, length 4mm: androecium, polyandrous, numerous, basifixed, 4mm long, dehiscent longitudinally: gynoecium, light green, 7mm long, having capitate stigma and terminal style. Extract of distilled flower is used as tonic for stomach, intestine, anti-dysenteric, anti-diabetic, diaphoretic and local anaesthetic.



Fig: flower of *Aegle marmelos*

1.8.3 Fruits

The fruit, round, pyriform, oval, or oblong, 5-20cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, grey-green until the fruit is fully ripe, when it ripe turns yellowish. It is dotted with aromatic, minute oil glands. Inside, there is a hard central core and 8 to 20 faintly defined triangular segments, with thin, dark-orange walls, filled with aromatic, pale orange, pasty, sweet, resinous, more or less astringent pulp. Fruit ripens in 10 to 11 months from bloom—March to June. Fruits contain 61.5g water, 1.8g protein, 0.39g fat, 1.7g minerals, 31.8g carbohydrates, 55mg carotene, 0.13mg thiamine, 1.19mg riboflavin, 1.1mg niacin, and 8mg per 100g of edible portion vitamin C.

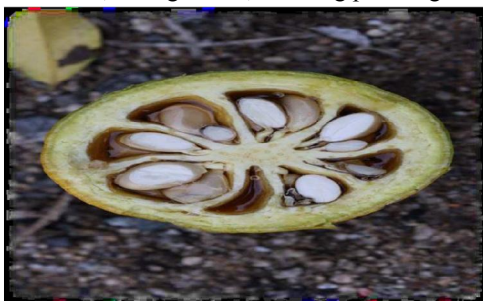


Fig: Fruit of *Aegle marmelos*

1.8.4 Seeds:

In the pulp 10 to 15 flattened-oblong seeds are present. The seeds are about 1cm long, bearing woolly hairs and each enclosed in a sack of adhesive, transparent mucilage that solidifies on drying. Seed oil exhibit antibacterial effect against vibrio cholera, E.coli. Essential oils also exhibit antifungal activity against physallospora tucumanensis



Fig: Seed of *Aegle marmelos*
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1.8.5 Thorn:

Bael tree armed with straight, sharp, axillary thorns, 2-5 cm long. Considerable variation in thorn, its number, size, shape is tunic in different genotypes. In some of the genotypes, thorn is small and short. It can also be observed that the leaf convert into spine in pair in very few genotypes. Generally, two thorns at a node are common. Goma Yashi is thornless under rained dryland conditions of eastern India. In some of the genotypes, thorn may be seen in primary branches, but not at secondary or tertiary branches render dry land condition. However, it may vary in different agro climatic conditions.⁸



Fig: Thorn of *Aegle marmelos*

1.9 Physicochemical parameters of *Aegle marmelos*:

Sr No.	Plant parts	Chemical constituents
1	Leaf	Skimmianine, Aegelin, Rutin, α -sitosterol, β -sitosterol, Flavone, Lupeol, Cineol, Citral, Glycoside, O- isopentenyl, Hallordiol, marmeline, Citronellal, Cuminaldehyde, Phenylethyle cinnamamides, Eugenol, Marmesinin, Aegelin
2	fruit	Marmelosin, Luvangetine, Auraptin, Psoralen, Marmelide, Tannin, Phenol
3	Seeds	Essential oils,-D-limonene, A-D-phellandrene, Cineol, Citronellal, Citral, P-Cymene, Cumin aldehyde
4	Root	Alkaloid, Halopine, Coumarins, Terpinens
5	Bark	Fagarine, Marmin, Furoquinoline, Alkaloids

Table 2: Chemical constituents present in different parts of *Aegle marmelos*

1.10 Nutritional value of *Aegle marmelos*

Nutrients	Values per 100
Energy	137 k. cal
Carbohydrate	31.8 g
Protein	1.8 g
Fat	0.3 g
Water	55 g
Fibre	2.9 g
Vitamins	Value per 100 g
Vitamin A	55 mg
Vitamin C	60 mg
Vitamin B1	0.13 mg
Vitamin B2	1.19 mg

Vitamin B3	1.1 mg
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Table 3: Nutritional value of *Aegle marmelos*

1.11 Medicinal Uses

- Bael's fruit serves as stool binding. In fact it is used in condition like diarrhoea, dysentery etc.
- Bael leaves powder has an anti-diabetic effect.
- Juice of bael leaves with black pepper i.e. kali marich taken three times a day is helpful in jaundice.
- Syrup prepared from bael fruit pulp, with tamarind used to treat burning sensation on skin, diarrhoea, yellow coloration of skin, nausea etc.
- Poultice of bael leaves applied on eyes gives good result for pain and redness in eyes

II. MATERIAL AND METHOD

2.1 Experimental Work

A. Collection

The whole plant of *Aegle marmelos* Linn. is collected from suitable geographical location samarth college area, belhe in the state of Maharashtra, in the month of Feb-March 2022

B. Chemicals Required

Sr. No	Chemicals	Make
1	Acetone	Research-Lab Fine Chem industries
2	Chloroform	RANKEM
3	Distilled water	Lab prepared
4	Ethanol	Research-Lab Fine Chem industries
5	Ethyl acetate	Research-Lab Fine Chem industries
6	Fehlings solution A	Moly Chem
7	Fehlings solution B	Moly Chem
8	Ferric chloride	Thermo Fisher Scientific
9	Glacial acetic acid	Research Lab Fine Chem
10	Hydrochloric Acid	LOBA
11	Magnesium turning	Research Lab
12	Petroleum ether	Avra
13	Pyridine	Research Lab
14	Sodium Hydroxide	LOBA
15	Sodium Nitroprusside	Loba cheime
16	Sulphuric Acid	LOBA
17	Toluene	Research Lab
18	Zinc Dust	Loba cheime

Table 4 : Chemical used in project

C. Preparation of Reagents⁸

The following Reagents were freshly prepared in the laboratory are as follows.

D. Mayer's Reagent:

Weighed about mercuric chloride (1.36 g) and potassium iodide (5 g) and dissolve it in 100 ml distilled water.

E. Dragendorff's Reagent

Weighed bismuth nitrate (0.5 g) into empty beaker and add concentrated HCl (10 ml). Weighed potassium iodide (4 g) into another beaker and add little water until it dissolves, mix both solutions.

F. Wagners Reagent

Dissolve iodine (2.5 g) and potassium iodide (12.5 g) in 250 ml of water to prepare the reagent.

G. Hager's Reagent

Dissolve picric acid (1 g) in water (100ml) to prepare the reagent.

2.2 Preparation of *Aegle marmelos L.* Plant Extract.

Hot Continuous Extraction (Soxhlation):

After collection of thorns of *Aegle marmelos*, they are washed with running tap water and shade dried at room temperature. The dried thorns are grinded to powder by using grinder to make a coarse powder



Fig 6.1: *Aegle marmelos* thorn extraction

Hot continuous Soxhlet extraction is used for the extraction of powder. Successive method is employed for the extraction and different solvents are used as per polarity. Continuous extraction is started from non-polar to polar solvent (Petroleum ether, chloroform, acetone, ethyl acetate, ethanol and water). 100 g of thorn powdered thorns of *Aegle marmelos* with 500 ml of different solvents are subjected to successive extraction using Soxhlet extractor for 24 hrs after completion of extraction process evaporation of solvent is carried out at room temperature then stored in a refrigerator in tight closed container and used for further experimental work.

$$\text{Percentage} = \frac{\text{Weight of dried extract}}{\text{Weight of Plant material take}} \times 100$$



Fig 6.3: Crude extracts of *Aegle marmelos* thorns

2.3 Preliminary Phytochemical Screening

Phytochemical investigation is carried out for all the extracts as per standard procedure. The crude extracts of various solvents are tested for the presence of alkaloid, glycoside, tannins, flavonoids, carbohydrates, saponins and proteins.⁹

A. Detection of Alkaloid

- **Hager's test:** 2mg of thorn extract taken in a test tube and add few drops of Hager's reagent, yellow color indicate presence of alkaloid.
- **Wagner's test:** 2 mg of thorn extract in a test tube and add iodine solution in potassium iodide (Wagner's reagent), formation of brown color ppt indicate presence of alkaloid.

B. Detection of Glycoside

- **Legal test:** To thorns extract of *aegle marmelos* add pyridine and alkaline solution of sodium Nitroprusside solution. An appearance of blood red color indicate presence of cardiac glycoside.
- **Killer-Killani test:** To extract add glacial acetic acid, add 1 drop of Fecl3 solution and conc. H2SO4. Reddish brown colour appear at the junction of two liquid and upper layer appear bluish green

C. Carbohydrate

- **Fehling's test:** Two ml of thorn extract mixed with equal volumes of Fehling A and Fehling B in different tubes and boiled for few minutes. Both the contents were mixed as they attain nearly the boiling point. The appearance of brownish-red precipitate formation indicated the presence of carbohydrates.
- **Benedict's test:** To 0.5 ml of thorn extract of *Aegle marmelos* add 5 ml of Benedict's reagent and boiled for few min. formation of red, yellow or green color depending on amount of reducing sugar present in test solution.
- **Molish test:** Extract is treated with 2 drops of alcoholic solution of α -naphthol in a test tube. Formation of violet ring at the junction indicate the presence of carbohydrate.

D. Flavonoids

- **Shinoda test:** To extract add 5 ml of 95% ethanol /t-butyl alcohol, few drops of HCl and 0.5 g magnesium turnings. Orange, red pink to purple colour appear (flavonols, dihydro derivatives, and xanthenes).
- **Sulphuric acid test :** Add sulphuric acid to the thorn extract. Flavones, and flavonols dissolve into it and give a deep yellow solution. Chalcones and aurons give red or red bluish solution. Flavones give orange to red colour.

E. Saponin

- **Froth formation test:** Two ml of thorn extract was taken in a test tube and shaken until a stable froth or foam was formed for 5 minutes (in presence of saponin), however, no foam was formed for 5 minutes indicating the absence of saponin in thorn extract.

F. Tannin

- **Lead acetate test:** To the thorn extract add lead acetate solution, formation of white precipitate indicate presence of tannin.
- **Ferric chloride test:** Thorn extract is treated with few drops of 5% Ferric chloride solution. Formation of brownish black solution or greenish black solution indicate presence of tannin.

2.4 Antibacterial activity against different bacteria by Well plate diffusion Method:

The antibacterial activity of the plant extracts was tested against gram positive and gram-negative bacteria, bacillus subtilis (MTCC 441) and Escherichia Coli (ATCC 11229).

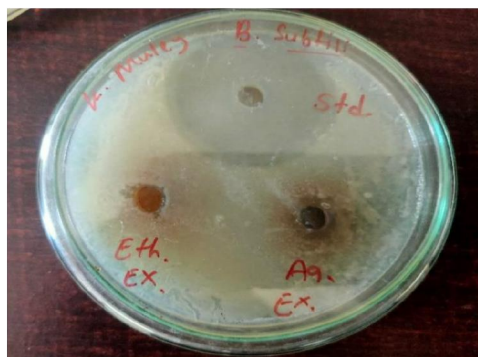
A. Well Diffusion Method

The inoculum of the microorganism was prepared from the bacterial cultures. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify. 100 μ l of broth of bacterial strain was pipette out and spread over the medium evenly with a spreading rod till it dried properly. Wells of 6mm in diameter were bored using a sterile cork borer.

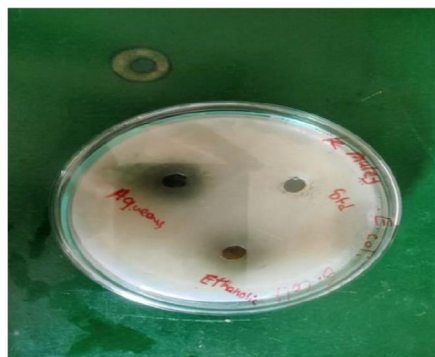
Solutions of both the extracts (5000 μ g/ml) in DMSO were prepared. 100 μ l of plant extracts solutions was added to the wells. The petri plates incubated at 37 0C for 24 h. streptomycin (1mg/ml) was prepared as a positive control DMSO was

taken as negative control. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZOI) all the determination were performed in triplicates ¹¹

2.5 Observation



A) *E. coli*



B) *Bacillus subtilis*

Fig: Zone of inhibition against bacterial Plate images

III. RESULT

Sr No	Chemical test	Pet ether extract	Chloroform extract	Acetone extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1	Alkaloid	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
2	Glycoside	- Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
3	Tannin & phenolics	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
4	Flavonoids	- Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
5	saponins	- Ve	+ Ve	+ Ve	+ Ve	+ Ve	+ Ve
6	Carbohydrat	- Ve	+ Ve	- Ve	- Ve	+ Ve	+ Ve

Table 8: Phytochemical screening of extracts of *Aegle marmelos* thorns

Sr No	Samples	Conc. (mg/ml)	Zone in diameter against <i>E. Coli</i>	Zone in diameter against <i>Bacillus subtilis</i>
1	Control	-	00	00
2	Standard	1	16 mm	16 mm
3	Aqueous extract	5	21 mm	10 mm
4	Ethanollic Extract	5	22 mm	----

Table 9. Antimicrobial Activity of aqueous and ethanolic extract against *Bacillus Subtilis* and *E. coli*

IV. DISCUSSION

In the present work plant selection, collection is done at the Samarth institute of pharmacy and it is identify as twig of plant *Aegle marmelos linn* belonging to family **Rutaceae**. This plant is used for further analysis.

Successive extraction method is used for extraction of given plant material and different fraction are made using various solvent as per polarity of solvent from non-polar to polar. Prepared fractions of given plant material is characterised by Thin layered chromatography.

Morphology of *Aegle marmelos* L. is studied. Standardisation of powder is done by the determination The percentage yield of thorn is found to be in the range 0.4 %, 2.4933 %, 1.6 %, 0.2 %, 1.57 and 5.13% respectively. Phytochemical

screening conclude that the given plant material shows the presence of alkaloid, glycoside, flavonoids, tannin, phenolic, carbohydrates, proteins and amino acids.

Transverse section of *Aegle marmelos* L. plant Thorn shows the presence of cork cambium, cortex, stone cells, starch grains, parenchymatous cells and Trichomes. The presence of yellowish brown appearance is due to the presence of high tannin content in thorns. Starch grain of variable sizes were detected.

Antibacterial Study

In present study the in vitro antimicrobial activity was performed by well diffusion assay. Experiment was performed on aqueous and ethanolic extract of *Aegle marmelos* thorns, in concentration of 5 mg/ml. The result of study showed that Thorn extract of *Aegle marmelos* validated diverse antimicrobial activities against tested organisms.

On evaluation it was observed that thorn extract has shown highest zone of inhibition against E coli, in contrast the extract exhibit moderate zone of inhibition against bacillus subtilis. The aqueous extract of *Aegle marmelos* thorn extract shows moderate zone of inhibition against Bacillus subtilis while ethanol shows poor antibacterial activity.

Against bacillus subtilis. Similarly ethanolic thorn extract of aegle marmelos exhibit highest zone of inhibition against Escherichia coli about 22mm diameter of zone, while aqueous extract produce zone of inhibition about 21 mm. In previous antibacterial study it was reported that both ethanolic and aqueous extract of *Aegle marmelos* leaves showed considerably more activity than aqueous extract.

The activity against candida albicans were found to be lesser as compared to antibacterial activity. When compared with standard drug sample the extract exhibit powerful antimicrobial activity and zone of inhibition was found to be acceptable. However, extract showed satisfactory result against E. coli and bacillus subtilis. The thorn extract exhibit higher activity in gram negative bacteria as compared to gram positive bacteria the growth of E. coli, a gram negative is inhibited better than gram positive mesophile Bacillus subtilis.

The efficacy of *Aegle marmelos* thorn extracts against Escherichia coli might be due to the existence of the phytoconstituents. It was reported in a study that the antibacterial activity of the plant extract may be due to the phytochemical content. Previous studies revealed that higher concentrations of phenolic compounds in plant extracts might be the reason for the higher antibacterial activity. Secondary metabolite like flavonoids were found to reveal antibacterial activity by the mechanisms like inhibiting nucleic acid synthesis, cytoplasmic membrane functions and energy metabolism.

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