UK Journal of Pharmaceutical and Biosciences
Available at www.ukjpb.com

ISSN: 2347-9442

Physicochemical, Phytochemical Screening and Antimicrobial Activity of *Aegle marmelos*

Asha Gupta[1]*, Tessy Thomas[1], Shagufta Khan[2]

[1]Department of Botany, Govt. P.G. College, Sehore (MP)- 466001, India
[2]GrowTips Biotech, Saket Nagar, Bhopal (MP)- 462024, India

Article Information
Received 1 March 2018
Received in revised form 29 May 2018
Accepted 30 May 2018

Keywords:
Aegle marmelos,
Physicochemical,
Phytochemical,
Antimicrobial

Corresponding Author:
E-mail : growtips_200254@yahoo.com
Mob.: +919826016389

Abstract

The present study was aimed to evaluate the physicochemical, phytochemical screening and antimicrobial assessment of *Aegle marmelos* fruits extract. The powder was evaluated for loss on drying, alcohol extractive value and aqueous extractive value. The petroleum ether, ethanol and aqueous extract were prepared by soxhlet extraction process. The phytochemical analysis of extracts was performed, and antimicrobial activity of ethanol extracts against various strains was done. The loss on drying, alcohol and aqueous extractive values were 3.9%, 14.2% and 17.6%, respectively. Phytochemical analysis showed the presence of carbohydrates, alkaloids, glycoside, flavonoids, saponins, tannins phenols in ethanol extracts. The ethanol extracts demonstrated moderate to strong antimicrobial activity against *S. epidermis, S. aureus, E. coli, P. aeruginosa* and *C. albicans*. Thus in future, extract of *Aegle marmelos* may be beneficial for another several species of microbes.

1 Introduction

Plants are indispensable to man for his life. All phyta of plants viz. Thallophyta, Bryophyta, Pteriodophyta and Spermatophyta, contain species that yield official and unofficial products of medicinal importance. Medicinal plants are the local heritage with global importance. World is endowed with a rich wealth of medicinal plants. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as “Medicinal plants”[1].

Medicinal plants are generally used in different ways, as complex mixture made of single plant extract containing a broad range of constituents or multi component mixture comprised of several closely related biologically active components. These components in medicinal plants are known as chemical compounds that act directly or in directly to prevent disease.

In the developing countries medicinal plants are an important source of life saving drugs for human kind. In many regions of the world which supply the bulk of global demand for 'natural medicines' with increasing threat to their population in the world and its genetic diversity.

Aegle marmelos is an important medicinal plant of India and are reported to have various medicinal properties in traditional medicinal systems. The medicinal properties of *Aegle marmelos* plant have been described in the Ayurveda which translates as "knowledge of life," 5000 years back to the ancient Sanskrit text, the Vedas[2]. It is as fresh and useful to humans today as it was in the ancient times yet more relevant and applicable in these modern times.

The protective role of *Aegle marmelos* is due to the presence of antioxidative constituents like phenolics, flavonoids, tannins, etc. which are able to delay or inhibit the oxidative stress. The chemical constituents present in *Aegle marmelos* are Skimmianine, Aegeline, Lupeol, Cineol, Citral, Citronella, Cuminaldehyde, Eugenol, Marmesinine, Marmelosin, Luvangetin, Aurapten, Psoralen and Marmelide. *Aegle marmelos* is mildly astringent and used to cure dysentery, diarrhoea, hepatitis, tuberculosis, dyspepsia and good for heart and brain. Roots have antidiarrhoetic, antidote to snake venom, anti-inflammatory and wound healing properties[2]. Hence, we planned to evaluate the physicochemical, phytochemical screening and antimicrobial activity of *Aegle marmelos*.

2 Materials and Methods

Preparation of Extract

2.1 Plant collection

The fruits of *Aegle marmelos* were collected from the local market, Bhopal (M.P.) India. Collected material were shade dried in an open air and grinded into powder for further use.

2.2 Physiochemical study of fruit powder

2.2.1 Extractive Values

2.2.1.1 Determination of alcohol soluble extractive value

5gms of the air-dried coarse powder was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Out of that filtrate, 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of ethanol soluble extractive value was calculated with reference to the air-dried drug.

2.2.1.2 Determination of water soluble extractive value

Weigh accurately the 5 gm of coarsely powdered drug and macerate it with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowing standing for 18 hours. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. Then 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

2.2.1.3 Loss on drying

About 1.5 gm. of powdered drug was weighed accurately in a tarred porcelain dish which was previously dried at 105°C in hot air oven to constant weight and then weighed. From the difference in weight, the percentage loss of drying with reference to the air dried substance was calculated.

2.3 Preparation of the crude extracts

The powder of the *Aegle marmelos* fruits**,** were packed in the Soxhlet apparatus and extracted with petroleum ether, ethanol and distilled water until the completion of extraction. The extract was filtered while hot and the resultant extract was distilled in vacuum under reduced pressure in order to remove the solvent completely, and later dried in a desiccator. After that extracts of petroleum ether, ethanol and aqueous were kept in air tight container for further study.

2.4 Preliminary Phytochemical analysis

The extracts were analyzed by the following procedures. To test for the presence of the alkaloids, saponins, tannins, terpenoids, flavonoids, glycosides, volatile oils and reducing sugars.

2.4.1 Test for alkaloids

(a) Dragendorff's test: To 1 ml of the extract, add 1 ml of Dragendorff's reagent (Potassium Bismuth iodide solution). An orange-red precipitate indicates the presence of alkaloids.

(b) Mayer's test: To 1 ml of the extract, add 1 ml of Mayer's reagent (Potassium mercuric iodide solution). Whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

(c) Hager's test: To 1 ml of the extract, add 3ml of Hager's reagent (Saturated aqueous solution of picric acid), yellow coloured precipitate indicates the presence of alkaloids.

(d) Wagner's test: To 1 ml of the extract, add 2 ml of Wagner's reagent (Iodine in Potassium Iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

2.4.2 Test for saponins

Take small quantity of alcoholic and aqueous extract separately and add 20 ml of distilled water and shake in a graduated cylinder for 15 minutes lengthwise. A 1cm layer of foam indicates the presence of saponins.

2.4.3 Test for Glycosides

(a) Legal test: Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaline. The formation of pink red to red colour shows the presence of glycosides.

(b) Baljet test: To 1ml of the test extract, add 1ml of sodium picrate solution and the yellow to orange colour reveals the presence of glycosides.

(c) Keller-Killiani test: 1gm of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filtrate is shaken with5ml of chloroform. The chloroform layer is separated in a porcelein dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acid containing 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

(d) Borntrager's test: Add a few ml of dilute Sulphuric acid to1ml of the extract solution. Boil, filter and extract the filtrate with chloroform. The chloroform layer was treated with 1ml of ammonia. The formation of red colour of the ammonical layer shows the presence of anthraquinones glycosides.

2.4.4 Test for carbohydrates and sugars

(a) Molisch's test: To 2 ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of Carbohydrates.

(b) Fehling's test: To 1 ml of the extract, add equal quantities of Fehling solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

(c) Benedict's test: To 5 ml of Benedict's reagent, add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

2.4.5 Test for tannins and phenolic compounds

(a) Take the little quantity of test solution and mixed with basic lead acetate solution. Formation of white precipitates indicates the presence of tannins.

(b) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish black colour product shows the presence of tannins.

(c) The little quantity of test extract is treated with Potassium ferric cyanide and ammonia solution. A deep red colour indicates the presence of tannins.

2.4.6 Test for flavonoids

(a) The drug in alcoholic and aqueous solution with few ml of ammonia is seen in U.V. and visible light, formation of fluorescence indicates the presence of flavonoids.

(b) Little quantity of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow colour solution formed, disappears on addition of an acid indicates the presence of flavonoids.

(c) Shinoda's test: The alcoholic extract of powder treated with magnesium foil and concentrated HCl give intense cherry red colour indicates the presence of flavonones or orange red colour indicates the presence of flavonols.

(d) The extract is treated with sodium hydroxide, formation of yellow colour indicates the presence of flavones.

(e) The extract is treated with concentrated H_2SO_4, formation of yellow or orange colour indicates flavones.

2.4.7 Test for steroids

(a) Libermann-Burchard test: 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the tap and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green colour shows the presence of sterols.

(b) Salkowski test: Dissolve the extract in chloroform and add equal volume of conc. H_2SO_4. Formation of bluish red to cherry colour in chloroform layer and green fluorescence in the acid layer represents the steroidal components in the tested extract.

2.4.8 Test for triterpenoids

Noller's test: Dissolve two or three granules or tin metal in 2ml thionyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink colour indicates the presence of triterpenoids[3-7].

2.5 Antimicrobial activity

2.5.1 Preparation of media

The antimicrobial activity of *Aegle marmelos* was evaluated against bacterial strains in ethanol, and aqueous extracts by using agar well diffusion method[8]. Nutrient agar plates were prepared for all extracts, 50µl inoculums of each selected bacterium (*S. epidermis, S. aureus, E. coli, P. aeruginosa* and *C. albicans*) was uniformly spreaded on agar plates with the help of glass spreader, after five minutes three wells approximately 5mm diameter was bored with the help of borer. The plant extract were poured into the wells. The plates were incubated at 37°C for 24 hrs. Petri plates containing 20 ml of agar medium were seeded with a 24 hours culture of the bacterial strains. In each plate, hole of 6-mm diameter was made using a sterile borer. The discs (6mm in diameter) were impregnated into plant extracts at various concentration (100 to 200 µg/ml) separately and placed on the inoculated agar. The inoculums size was adjusted so as to deliver a final inoculums of approximately 108 colony-forming units (CFU)/ml. Incubation was performed for bacteria and fungus at 37 ºC for 24 hrs and 37 ºC for 72 hrs respectively.

The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well. A standard Ciprofloxacin was used as a positive control. All assays were carried out in triplicate. The nutrient agar was used as a growth medium for microbes. 38g of the agar was dissolved in 1000ml of distilled water in a conical flask with swirling to homogenize. The flask was cotton plugged and sterilized by means of autoclave at 121°C for about 15 minutes. 20 ml of the agar solution was dispensed into sterilized petridishes near a gas flame in order to prevent contamination after which plates were covered and allowed to gel.

2.5.2 Determination of minimum inhibitory concentration (MIC)

On the day before experiment sterile culture tubes were taken to laminar air flow chamber. 20ul of the bacterial strain namely *S. epidermis, S. aureus, E. coli, P. aeruginosa* and *C. albicans* from the glycerol stock was inoculated in to culture tubes containing autoclaved LB broth and left in orbital shaker at 150rpm over night at temperature 37 °C for bacterial growth. Next day morning sub culturing of the bacterial strain was done in 100 ml LB broth by inoculating overnight culture (1.5ml of

culture was transferred into flasks containing fresh broth) in laminar air flow chamber. Next the inoculated fresh broth with bacteria strain was left in orbital shaker at 150 rpm, temperature 37 °C for 1 to 2 hours to get an absorbance of 600 nm. After the incubation period the turbidity was observed. These bacterial cell suspension cultures were used later for the antimicrobial studies. The LB broth (4.5ml) was added in test tubes followed be adding extract of different concentration (50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml) in test tube. It was done in triplicates. After that the increasing concentrations of ethanol extracts were added in triplicates. All these activity were done inside laminar air flow chamber. MIC of all samples was determined by broth dilution method. A two fold serial dilution of the ethanolic extracts were prepared and optical density (absorbance) of the samples was measured at 600 nm with the help of UV-Visible spectrophotometer. Further the test was done for standard drug (Ciprofloxacin) at 50 µg/ml.

3 Results and Discussion

Plants play an important role in having beneficial therapeutic effects in traditional Indian system of medicine. Studies on ethnomedicinal plants are gaining consensus in recent years in India and Abroad. Thus, in the present study, three different extracts (aqueous, ethanol, petroleum ether) of fruit pulp of *Aegle marmelos* were subjected to both qualitative and quantitative phytochemical analysis to explore its antimicrobial activity for its medicinal applications.

3.1 Physicochemical parameters

The loss on drying of the *Aegle marmelos* powder was 3.9%. The alcohol and aqueous extractive values of *Aegle marmelos* were 14.2% and 17.6%, respectively (Table 1). The findings of extractive values indicate that both plants contains higher amount of highly water soluble phytoconstituents.

Table 1: Physico-chemical parameters of *Aegle marmelos* fruit powder

Physicochemical Parameters	Values
Loss on drying	3.9% w/w
Alcohol extractive value	14.2% w/w
Water extractive value	17.6% w/w

Extractive values of crude drugs are useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

3.2 Phytochemical screening

Qualitative analysis of plant crude extract of *Aegle marmelos*, indicated the presence of alkaloids, carbohydrates, glycosides, flavonoids, phenols and saponins present in the fruits pulp (Table 2). These results are similar to Chetna *et al.,* (2014)[9] were it was reported that the phytochemical screening of water extract from the dried ripe fruits and Farina Mujeeb *et al.,* (2014)[10] from leaves of *Aegle marmelos* revealed the presence of major bioactive compounds including phyto-sterols, carbohydrate, protein, alkaloids, glycosides, polyphenols, flavonoids and saponins, which may retain a wide range of pharmacological actions.

Table 2: Phytochemicals present in fruits of *Aegle marmelos* extracts

Test	Petroleum ether extract	Ethanol extract	Aqueous extract
Alkaloids	-	+	-
Carbohydrates	-	+	-
Glycosides	-	+	+
Flavonoid	-	+	+
Polyphenols	-	+	+
Saponins	-	+	+
Steroids	+	-	-
Triterpeoids	-	-	-

+ Presence of constituent, - Absence of constituent

From the above table it is observed that ethanol extract shows all positive tests for the presence of different phytochemicals except streroids and triterpeoids which was followed by aqueous extract where most of tests were detective positive while as in petroleum ether extracts there were hardly any positive test for any phytochemical compound which shows there inertness related these solvents. As these compounds were soluble in methanol and aqueous solvent and is therefore not surprising that these plant extracts can be testified and could be responsible for the antimicrobial property. Phytochemical analysis showed the presence of reducing sugars, alkaloids, glycoside, flavonoids, saponins, tannins phenols in ethanol extracts and these biochemical's were absent in petroleum ether extracts. These results were in consonance with the studies carried out by various researchers[11-17].

In the present experiment it was observed that the amount of phytochemicals were found more in ethanol extracts than aqueous, shows a similar correlation observed by the studies of Rajan *et al.,* (2011)[15] and Bahera *et al.,* (2014)[16].

The result highlighted the significance of wild fruit as a cheap source of nutrient for the rural and tribal people. It brings into focus the rich nutritional composition of the fruit and the scope for their use as an alternative source of bio nutrition. There is a

need to explore more wild fruits that will add new dimensions toward traditional management and conservation of plant wealth.

3.3 Antimicrobial activity

In the past, many researchers has evaluated the pharmaceutical importance of different parts of *Aegle marmelos* plant and found that the plant possesses natural healing capacity in curing many ailments along with antiinflammatory, antipyretic, anti-diarrhoeal, anti-diabetic, analgesic, antimicrobial, radio protective, anticancer and antiviral properties.

The *Aegle marmelos* fruit pulp contains many functional, biological and pharmacological active compounds such as alkaloids, coumarins, flavonoids, carotenoids, terpenoids, phenolics and antioxidants which helps us in protecting against various chronic diseases.

Thus the antimicrobial activity against pathogenic microbes was also evaluated. The crude extract was screened for antimicrobial activity using the agar well diffusion method developed. The present investigation shows the efficacy of all extracts against the selected pathogenic microbes (**Table 3).** Antimicrobial activity of ethanol extracts were investigated against *S. epidermis, S. aureus, E. coli, P. aeruginosa* and *C. albicans*. The zones of inhibition of ethanol extract of *Aegle marmelos* against all the microorganisms are displayed in table 3.

Table 3: Antibacterial activity of ethanol extract of *Aegle marmelos* fruit extracts

Concentration of extract	Zone of inhibition (mm)				
	S. epidermis	***S. aureus***	***E. coli***	***P. aeruginosa***	***C. albicans***
100 µg/ml	4.2±0.05	3.5±0.37	5.1±0.73	8.2±0.47	2.3±0.56
200 µg/ml	12.6±0.35	10.3±0.41	14.6±0.52	16.1±0.86	8.6±0.24
Standard	17.3±0.18	19.3±0.92	22.1±0.29	18.7±0.14	19.8±0.32

Our findings are similar to the results of leaf extracts of *A. marmelos* which shows varying degree of antibacterial activity has reported by Rastogi R.P. and Mehrotra N. (1993)[18]. It is reported that the presence of compounds Cuminaldehyde and Eugenol present in the leaves may be responsible for antibacterial activity[19]. It was reported earlier by Mohan V *et al.*, (2005)[20] that aqueous, acetone and petroleum ether extracts of *A. marmelos* were more effective against *B. coagulans, B. subtilis, B. thuringiensis, P. aeruginosa and S. aureus.* Dried fruit pulp extract of same plant showed great potential antibacterial activity against *P. aerigonosa, S. flexneri, E.coli, S.subtilis, S.epidermidis and S. aureus*[21].

3.4 MIC study

In this investigation the antimicrobial activity was also studies by measuring Minimum inhibitory concentration (MIC) of ethanol extracts at different concentrations level against all microbes.

Through the studies it is observed that all microbes show minimum inhibition in the ethanol extract of fruit pulp. (Table 4 - 8). The extract carried out using all bacterial strain revealed that all concentrations specially at 300-400 ug/ml, has the potential to inhibit maximum growth. The antibacterial compound mainly found in *Aegle marmelos* were tannins, phlobatannins, saponins, terpenoids, alkaloids and poly phenols.

Besides, the crude extract was tested for antimicrobial activity against two gram positive strains of *Staphylococcus aureus* at different concentrations of 100, 200, 300 and 400ug/ml at different time span of 3hrs. It was found that a concentration range 100-200ug/ml of the ethanol extracts was effective in inhibiting the growth of bacterial strain. 200ug/ml was effective for *S. epidermis* in inhibiting the growth of the above strain. When the similar study was carried out using other strain *E. coli*, it was found that 100ug/ml of ethanol extract, was effective in inhibiting the growth of bacteria. The broad spectrum antimicrobial activity of fruit pulp extracts of *Aegle marmelos* was indicated by the inhibition of growth of the bacteria, which in turn in future, might lead to further study for the development of novel bioactive antimicrobial agents.

The results achieved were in accordance with similar studies carried out by Lambole *et al.*, (2010)[22] and Behera *et al.*, (2014)[16]. There are few reports have also mentioned significant *in vitro* antimicrobial activity by the methanol extracts of *Aegle marmelos* leaves and flowers[18,23].

Rajasekaran C (2008)[24] used Chloroform, Ethanol, Petroleum ether, Dichloromethane and aqueous extract for the study on selected gram negative and gram positive bacteria. He found that ethanol and chloroform extracts were more effective than other of solvents, more or less similar to the present study. A study by Venkatesan D (2009)[25] using only when treated with crude thanolic extracts on bacteria such as S. *aeruginosa, Bacillus subtilis, Escherichia coli* and *Pseudomonas* revealed that *Bacillus subtilis* showed maximum zone of inhibition when

treated with crude ethanolic extracts which also were observed in the present study, which also were observed in the present study that concentration of 50-100 ug/ml is effective in inhibiting the growth of bacterial strain ATCC29213(*Staphylococcus aureus*).

Table 4: Antibacterial activity of ethanol extract of *Aegle marmelos* fruit extracts against *S. epidermis*

Treatment	***S. epidermis***		
	Absorbance (0 hr)	**Absorbance (3 hr)**	**Absorbance (6 hr)**
Control	0.28±0.25	1.26±0.18	3.14±0.39
Extract 100 µg/ml	0.23±0.14	0.95±0.64	0.72±0.47
Extract 200 µg/ml	0.26±0.36	0.79±0.72	0.82±0.82
Extract 300 µg/ml	0.24±0.72	0.65±0.39	0.57±0.53
Extract 400 µg/ml	0.27±0.41	0.48±0.42	0.29±0.75
Standard (50 µg/ml)	0.26±0.35	0.31±0.21	0.21±0.92

Values are mean ± SEM of triplicate determinations

Table 5: Antibacterial activity of ethanol extract of *Aegle marmelos* fruit extracts against *S. aureus*

Treatment	***S. aureus***		
	Absorbance (0 hr)	**Absorbance (3 hr)**	**Absorbance (6 hr)**
Control	0.21±0.72	1.85±0.15	3.24±0.84
Extract 100 µg/ml	0.30±0.93	1.23±0.61	0.91±0.49
Extract 200 µg/ml	0.26±0.25	0.96±0.57	0.83±0.62
Extract 300 µg/ml	0.32±0.81	0.91±0.19	0.72±0.17
Extract 400 µg/ml	0.28±0.49	0.73±0.34	0.59±0.34
Standard (50 µg/ml)	0.25±0.73	0.46±0.68	0.28±0.56

Values are mean ± SEM of triplicate determinations

Table 6: Antibacterial activity of ethanol extract of *Aegle marmelos* fruit extracts against *E. coli*

Treatment	***E. coli***		
	Absorbance (0 hr)	**Absorbance (3 hr)**	**Absorbance (6 hr)**
Control	0.32±0.68	1.18±0.53	2.92±0.43
Extract 100 µg/ml	0.28±0.14	0.86±0.82	0.76±0.28
Extract 200 µg/ml	0.23±0.37	0.71±0.59	0.65±0.94
Extract 300 µg/ml	0.26±0.49	0.58±0.47	0.49±0.78
Extract 400 µg/ml	0.24±0.42	0.39±0.19	0.29±0.49
Standard (50 µg/ml)	0.22±0.25	0.28±0.29	0.19±0.17

Values are mean ± SEM of triplicate determinations.

Sudharameshwari *et al.,* (2007)[22] found out that *Aegle marmelos* showed maximum zone of inhibition against *P.aeruginosa and S.aureus* in etanolic extracts which is in consonance with the present study. Using Ethanolic, Methanolic, Ethyl acetate and Hot water extract from leaves, fruits and peels of Aegle marmelos, MIC value was determined by broth dilution method by Pandey (2011)[13] and Behera *et al.*, (2014)[16] was done in the present study. Hence from the study demonstrates that the ethanol extraction of the fruit pulp can be use as compound in future studies.

Antimicrobials derived from plants possess vast curative properties since they have fewer side effects as compared to synthetic antimicrobials drugs. *Aegle marmelos* is of utmost importance for ethnobotanical purposes, and it has been placed in the priority list of thirty two medicinal plants by The National Medicinal Plants Board of Govt. of India[26].

Table 7: Antibacterial activity of ethanol extract of *Aegle marmelos* fruit extracts against *P. aeruginosa*

Treatment	***P. aeruginosa***		
	Absorbance (0 hr)	**Absorbance (3 hr)**	**Absorbance (6 hr)**
Control	0.25±1.02	1.12±0.18	2.61±0.42
Extract 100 µg/ml	0.29±0.85	0.81±0.34	0.72±0.61
Extract 200 µg/ml	0.21±0.41	0.65±0.79	0.52±0.73
Extract 300 µg/ml	0.23±0.97	0.51±0.51	0.39±0.58
Extract 400 µg/ml	0.28±0.64	0.32±0.41	0.21±0.91
Standard (50 µg/ml)	0.21±0.51	0.28±0.63	0.22±0.24

Values are mean ± SEM of triplicate determinations

Table 8: Antifungal activity of ethanol extract of *Aegle marmelos* fruit extracts against *C. albicans*

Treatment	***C. albicans***		
	Absorbance (0 hr)	**Absorbance (3 hr)**	**Absorbance (6 hr)**
Control	0.24±0.63	1.61±0.52	3.05±0.47
Extract 100 µg/ml	0.21±0.19	1.19±0.76	0.93±0.82
Extract 200 µg/ml	0.29±0.48	0.92±0.81	0.79±0.25
Extract 300 µg/ml	0.25±0.62	0.84±0.36	0.68±0.63
Extract 400 µg/ml	0.23±0.31	0.69±0.49	0.49±0.27
Standard (50 µg/ml)	0.26±0.95	0.31±0.19	0.21±0.18

Values are mean ± SEM of triplicate determinations

4 Conclusion

The present study contributes to the current knowledge of presence of various phytochemicals active compounds of *Aegle marmelos* possessing significant broad spectrum antibacterial efficacy. Further fractionation and purification will elucidate the potential compound, which is a pressing need because of the upcoming resistance of the currently available antibiotics.

5 Conflict of interests

None

6 Authors contributions

AG and TT design and carried out research work and SK drafted the manuscript.

7 References

1. Sandhya B, Thomas S, Isabel W, Shenbagarathai R. Ethanomedicinal plants used by the valaiyan community of piramalai hills (Reserved forest), Tamil Nadu, India –A pilot study, African ethnomedicines. African journal of Traditional, Complementary and Alternative Medicines. 2006; 3: 101-114.
2. Anonymous. The wealth of India. Raw materials. CSIR, New Delhi; 5:275.
3. Singh B, Ahamad A, Pal V. Evaluation of Antibacterial Activity and Phytochemical Screening of *Azadirachta indica* Leaves Extracts Against *Staphylococcus aureus*. UK Journal of Pharmaceutical and Biosciences. 2015; 3(4): 43-47.
4. Gupta AK, Ahirwar NK, Shinde N, Choudhary M, Rajput YS, Singh A. Phytochemical Screening and Antimicrobial Assessment of Leaves of *Adhatoda vasica, Azadirachta indica* and *Datura stramonium.* UK Journal of Pharmaceutical and Biosciences. 2013; 1(1): 42-47.
5. Purohit P, Bais RT, Singh P, Khan S. Assessment of antibacterial activity and phytochemical screening of

Hemidesmus indicus root extracts. UK Journal of Pharmaceutical and Biosciences. 2014; 2(6): 67-72.

6. Kenwat R, Prasad P, Sahu RK, Roy A, Saraf S. Preliminary phytochemical screening and in vitro antioxidant efficacy of fruit oil of *Martynia annua*. UK Journal of Pharmaceutical and Biosciences. 2014; 2(1): 16-22.
7. Nelofar Gulam Nabi, Mukta Shrivastava. Phytochemical Screening and Antioxidant Activity of Ethanol Extract of *Psoralea corylifolia* seeds. UK Journal of Pharmaceutical and Biosciences. 2017; 5(2): 01-07.
8. Ahmad I, Beg AJ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens. J. Ethnopharmacol. 2001;74: 113-23.
9. Laddha CS, Kunjalwar SG, Itankar PR, Tauqeer M. Nutritional and phytochemical assesment of wild edible fruit of *aegle marmelos* (linn.) Used by the tribes of Bhiwapur tahsil Nagpur district. India Asian J. Pharm. Clin Res. 2015; 8: 76-78.
10. Mujeeb F, Bajpai P, Pathak N. Phytochemical evaluation, antimicrobial activity and determination of bioactive components from leaves of *Aegle marmelos.* Bio Med Res Int. 2014; 1-11.
11. Baliga MS, Bhat PH, Joseph N, Fazal F. Phytochemistry and medicinal uses of the bael fruit (Aegle marmelos Correa):Aconcise review. Food Research International. 2011; 44(7): 1768-1775.
12. Dhanaraj TS, Murugaiah K, Jagadeesan M. Herbal Tech Industry. 2011; 7-10.
13. Pandey A, Mishra R. Journal of Pharmaceutical and Biomedical sciences. 2011; 13:1-6.
14. Kaur P, Kaur S, Walia A, Kumar S. Antigenotoxic activity of polyphenolic rich extracts from *Aegle marmelos* (L.) Correa in Human Blood Lymphocytes and E. coli PQ 37”. Rec. Natural Product. 2009; 3(1): 68-75.
15. Rajan S, Gokila M, Jency P, Brindha P, Sujatha RK. Antioxidant and phytochemical properties of *Aegle marmelos* fruit pulp. International Journal of Current Pharmaceutical Research. 2011; 3(2): 65-70.
16. Behera P, Raj VJ, Basavaraju R. Phytochemical and antimicrobial activity of fruit pulp of *Aegle marmelos.* Journal of Chemical and Pharmaceutical Research. 2014; 6(8): 319-326.
17. Sudharameshwari K, Radhika J. Antibacterial Screening of *Aegle Marmelos, Lawsonia Inermis and Albizzia Libbeck.* Afr J Tradit Complement Altern Med. 2007; 4(2): 199–204.
18. Rastogi RP, Mehrotra BN. Ancient modern concordance in Ayurvedic plants. Compendium of Indian medicinal plants. R.P. Rastogi, Ed., vol. 3, Central Drug Research Institute, Lucknow, India. Information Directorate, New Delhi, India. 1993.
19. Duke JA. Hand book of biologically active phytochemicals and their activities. *CRC Press.* 1992.
20. Mohan V, Molly AG, Eldo N. *Aegle marmelos* extract as a potent bactericide. Asian Journal of microbiology, biotechnology and environmental science. 2005; 7: 639-644.
21. Supriya D, Ashish S, Ankit S, Nirmala G, Agrawal RC. Evaluation of *in vitro* antibacterial potential of ripe fruits of *Aegle marmelos*. International Journal of Pharmacy and Pharmaceutical Sciences. 2012; vol. 4(3): 179–181.
22. Lambole VB, Murti K, Bhatt VK, Kumar SP, Gajera V. International Journal of Pharmaceutical Sciences Review and Research. 2010*;* 5(2): 67-72.
23. Kothari S, Mishra R, Bharat S, Tonpay SD. Antimicrobial activity and phytochemical screening of serial extracts from leaves of *Aegle marmelos* (Linn.). Acta Poloniae Pharmaceutica. 2011; 68: 687–692.
24. Rajashekaran C, Meignanam E. *In vitro* evaluation of antibacterial activity of phytochemical extracts from leaves of *Aegle marmelos* corr. (Rutaceae). Ethano. Leaflets. 2008; 2: 1124-1128.
25. Venkatesan D, Karrunakarn MC, Selva Kumar S, Palani Swamy PT. Identification of phytochemical constituents of *Aegle marmelos* responsible for antimicrobial activity against selected pathogenic organisms. Ethnobotanical Leaflets. 2009; 13: 1362–1372.
26. Kala KP. Ethnobotany and ethno conservation of *Aegle marmelos* (L.) Correa. Indian Journal of Traditional Knowledge. 2006; 5(4): 537–540.