IJIRR

International Journal of Information Research and Review
Vol. 04, Issue, 08, pp.4453-4458, August, 2017

RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL AND *INVITRO*- ANTIMICROBIAL ANALYSIS OF *AEGLEMARMELOS* (L.) CORREA LEAF EXTRACT

***Samidha M. Pawaskar and K. C. Sasangan**

Department of Biochemistry, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai – 400,077, Maharashtra, India

ARTICLE INFO

Article History:

Received 14th May, 2017
Received in revised form
29th June, 2017
Accepted 10th July, 2017
Published online 30th August, 2017

Keywords:

*Aeglemarmelos (*L.) Correa,
Preliminary phytochemical analysis,
Invitro-antimicrobial activity.

ABSTRACT

The present study was undertaken to evaluate the preliminary phytochemical constitution and *invitro*-antimicrobial activity of *Aeglemarmelos* (L.) Correa leaf extract. Preliminary phytochemical screening of the freshly prepared plant leaf extract showed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids. The *invitro*-antimicrobial activity of the successive leaf extracts of *Aeglemarmelos* (L.) Correa was studied in petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water, against various gram positive and gram negative bacterial strains using zone of inhibition. Both Agar well diffusion method and Agar disc diffusion method were used to evaluate the antibacterial efficacy. The Minimum inhibitory concentration (MIC) of all these solvent extracts of said plant was determined by Agar well diffusion method. The reference antibiotics Chloramphenicol and Ampicillin (Antibacterial); Nystatinand Clotrimazole (Antifungal) were also tested against these standard microorganisms used in the assay and the results were compared with that of the plant extracts. The *invitro*-antimicrobial activity study showed that all the seven successive extracts of the leaf powder of *Aeglemarmelos* (L.) Correa, exhibited prominent antimicrobial and antifungal activity against all microorganisms used in the study. Highly polar solvents ie.ethanol, methanol and water showed the most significant antibacterial and antifungal activity against all tested organisms. The results of the study revealed that the leaf powder of *Aeglemarmelos* (L.) Correa can be considered as a possible source of various phytochemical constituents having an *in-vitro* antimicrobial potential.

INTRODUCTION

Phytochemicals (Secondary Metabolites) are naturally occurring, non-nutritive chemicals that have protective or disease preventive properties because of which they find a great application in herbal medicine and food. Pronounced "fight-o-chemicals," phytochemicals fight to protect the health. Although phytochemicals are not yet classified as nutrients, substances necessary for sustaining life, they have been identified as containing properties for aiding in disease prevention (Polk *et al.,* 1996). Medicinal plants serve as an important source of this phytochemicals (secondary metabolites) which have been recently proved to have protective or disease preventive properties including - antibacterial, anticancer, antifungal, and antioxidant (Krishnaraju *et al*., 2005). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo *et al.,* 1996; Iwu *et al.,* 1999). Natural antimicrobials can be derived from barks, stems, leaves, flowers and fruits of plants, various animal tissues or from microorganism (Gordon *et al*., 2001). Although some therapeutic benefits can be traced to specific plant compounds, many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value. The Bael tree, which is the only species in the genus *Aegle*, is slow-growing tree, of medium size, grows up to 40 or 50 ft (12-15 meters) tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping.Bael occurs in dry forests on hills and plains of northern, central and southern India, Burma, southern Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Vietnam, Laos, Cambodia and Thailand. Also in mixed deciduous and dry dipterocarp forests. *A. marmelos* is a subtropical species. The *Aeglemarmelos* tree is one of the most useful medicinal plants of India. Its medicinal properties have been described in the ancient medical treatise in Sanskrit, *CharakaSamhita* (www.ecosensorium.org/2011).

****Corresponding author: Samidha M. Pawaskar***
Department of Biochemistry, K. J. Somaiya College of Science and Commerce, Vidyavihar, Mumbai – 400,077, Maharashtra, India

Several chemical constituents have been isolated from various parts of the bael tree. These include alkaloids, coumarins and steroids. The leaves contain skimianin, sterol and aegelin. Considering the aforesaid, it is believed that the need of the hour is to search for new antimicrobials. With this in mind, in the present work, the leaf extracts of *Aeglemarmelos* (L.) Correaare screened for their potential phytochemical constituents and antimicrobial activity.

MATERIALS AND METHODS

Collection of plant material: Leaves of *Aegle marmelos (L.) Correa.*were collected from Mumbai and Talegaon – Dabhade (district - Maval, Pune). The plant samples *Aegle marmelos (L.) Correa.* (Acc. no.-08649,08652) was authenticated by the expert taxonomist of St. Xavier's College, Mumbai.

Preliminary Phytochemical Study: (Pawaskar, *et al*., 2017 e, f, g, h, i, j, k) For preliminary qualitative screening of various phytochemicals about 5g of the *Aegle marmelos (L.) Correa*.plant leaf powder was extracted separately with 100 ml of methanol and water by continuous shaking with the help of rotary shaker for 8 hours. The extract was filtered, concentrated by evaporation and was used for checking the presence of alkaloids, tannins, saponins, flavonoids, glycosides, phenolic compounds, terpenoids and steroids using known qualitative assays as followed.

Test for Terpenoids: A volume of 5 ml of the plant extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added to form a layer. A reddish brown coloration of the interface was formed to show the presence of terpenoids (Pawaskar and Kale, 2007; Sivaraj, Balakrishnan, Thenmozhi and Venckatesh, 2011).

Test for Steroids and Phytosterols: 2 ml of acetic anhydride was added to 0.5 ml of the plant extract of each sample with 2 ml of H_2SO_4. The colour change from violet to blue green in the sample indicated the presence of steroids and sterols (Pawaskar and Kale, 2007; Sivaraj *et al*., 2011).

Test for Tannins: 0.5 ml of the plant extract was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish green or a blue-black coloration (Trease and Evans, 1989). Blue colour indicated the presence of Gallic tannins and green black colour indicated presence of Catecholic tannins (Pawaskar and Kale, 2007; Sivaraj *et al.,* 2011).

Test for Alkaloids: To 2 ml of plant extract, 1.5 ml of 1% HCl was added. After heating the solution in water bath, 6 drops of Mayors reagents/ Wagner's reagent/ Dragendroff reagent was added. Formation of Orange precipitate indicates the presence of alkaloids (Oguyemi, 1979; Pawaskar and Kale, 2007; Venkatesan, Karrunakarn, Selva and Palani, 2009).

Test for Cardiac Glycosides (Keller-Killani Test): To 5 ml of the plant extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. Then it was underplayed with 1 ml concentrated sulphuric acid. A brown ring of the interface indicates a deoxy sugar characteristic of cardio glycosides. A violet ring may appear below the ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer (Finar, 1983; Pawaskar and Kale, 2007; Sivaraj *et al.*, 2011).

Test for Saponins: 5ml of the plant extract was boiled in 5ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion (Pawaskar and Kale, 2007; Sivaraj *et al*., 2011).

Test for Phenols: To 2 ml of the plant extract, 1 ml of 1% ferric chloride solution was added. Blue or green color indicates phenols (Martinez and Valencia, 2003; Pawaskar and Kale, 2007; Sivaraj *et al*., 2011).

Test for Flavonoids: A portion of the plant extract was separately heated with 10ml of ethyl acetate in a water bath for 3min. The mixture was filtered and 4ml of each filtrate were shaken with 1ml of dilute ammonia solution. A yellow colour observation indicates the presence of flavonoids (Harborne, 1973; Pawaskar and Kale, 2007).

Test for Reducing sugars: To 2 ml of crude plant extract, 5 ml of Distilled water was added and filtered. The filtrate was boiled with 3-4 drops of Fehlings solution A and Fehlings B solution in excess (1-2 ml) for 2 minutes. Formation of the orange red precipitate indicated presence of the reducing sugars (Pawaskar and Kale, 2007; Venkatesan *et al*., 2009)

Invitro - Antimicrobial Study

Plant Material: Matured leaves were selected for the study. The plant material was thoroughly washed, 3-4 times with tap water and once with distilled water, so as to remove all the impurities and foreign organic matter. These samples were then placed in between filter paper pads to remove maximum moisture and then shade dried in the beginning and further dried in an oven at 50-60^{0}C for 25 minutes. The dried material was powdered to obtain a fine powder (mesh size 2 mm) and then sieved. This was then stored in plastic containers at 4°C until use.

Preparation of plant extracts for the assay: The dried and finely ground leaf powder of *Aegle marmelos (L.) Correa*., (20g each) was successively extracted with petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and water by using Soxhlet apparatus for about 8-12 hrs, at a temperature not exceeding the boiling point of the solvents. The resulting extracts (details as shown in Table 1) were concentrated, residues were weighed and reconstituted in methanol and were further used for the assay (Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

Table 1. Percentage of extraction in various solvents for the leaf powder of *Aegle marmelos* (L.) Correa

Sr. No.	Solvent	% Extracted
1.	Water	*31.48 ± 1.72*
2.	Methanol	*27.59 ± 0.98*
3.	Ethanol	*23.06 ± 1.26*
4.	Acetone	*14.82 ± 2.59*
5.	Ethyl acetate	*16.37 ± 2.06*
6.	Chloroform	*11.65 ± 0.99*
7.	Petroleum ether	*9.81 ± 1.34*

*All values are expressed as mean ± SD for three determinations

Preparation of standard antibiotics solution: Two standard broad spectrum antibacterial antibiotics (for both gram positive and gram negative bacterial strains) viz. Ampicillin (Bacteriocidal) – from Beta Lactum medicines, Chloramphenicol (Bacteriostatic) – Other antibacterials and two commonly used antifungal antibiotics viz. Nystatin and Clotrimazol (used for fungal infections; especially for mold and yeast infections - most notably Candida) were used for the assay for comparative analysis of the leaf extract of *Aegle marmelos (L.) Correa*. (According to WHO Model List of Essential medicines, 1977; 18th edition; Pawaskar andSasangan, 2015). Commercially available powdered forms of the antibiotics were dissolved in distilled water to make up standard antibiotic solutions of concentration 0.5mg/ml, (Potency specifications of antibiotics, WHO, 1997; Pawaskar and Sasangan, 2015) which were further used for the assay.

Preparation of culture (inoculum): The bacterial cultures were isolated on nutrient agar slants and incubated at 37^0C for 24 hrs and the fungal cultures were isolated on Sabouraud agar and incubated at 30^0C for 48 hrs and the fully grown cultures were then stored in the refrigerator and used whenever required (Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

Preparation of culture suspension: Loopful of cultures from the slants was suspended in small amount of nutrient broth or saline as required (Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

Agar well diffusion method: All the extracts obtained after 8 to12 hrsSoxhlet extraction were weighed and reconstituted in methanol. Each microorganism was suspended in sterile saline and diluted to approximately 10^6 colony forming units (cfu/ml) or approximately 0.1 OD (Optical density) reading. These culture suspensions were then spread (flood) inoculated onto the surface of sterile Mueller Hinton Agar (MHA) plates. The wells 10 mm in diameter were cut into these seeded agar plates using a sterile cork-borer. 0.05 ml (50 µl) of each extract was then introduced into each well (four wells in four imaginary quadrants on each plate) using a micro-pipette. After refrigeration of these plates at 4°C for 2 hrs, the plates were incubated at 37°C for 24 hrs (Antibacterial) and at 30ºC for 48 hrs (Antifungal) and were then examined for any zones of growth inhibition. The diameters of these zones of inhibition were measured in millimetres (Bauer, Kirby and Sherris, 1966; Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

Agar disc diffusion method: The agar disc diffusion method was also employed for the determination of antimicrobal activities of the above mentioned leaf extracts of *Aegle marmelos (L.) Correa*. In short, a suspension of the microorganisms to be tested, as mentioned above, was spread (0.1 ml of 10^6 cells/ml) on the solid media plates ie. on the surface of sterile Mueller Hinton agar plates, poured to 3-4 mm in depth. Sterile filter paper discs, 6 mm in diameter, were soaked with the above mentioned plant extracts and were placed on the inoculated plates. These plates were refrigerated at 4ºC for 2 hrs for pre-diffusion of the extracts and then were incubated at 37°C for 24 hrs (Antibacterial) and at 30ºC for 48 hrs (Antifungal). All the plates were examined for any zones of growth inhibition and the diameters of these inhibition zones were measured in millimeters (Bauer *et al*., 1966; Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

Minimum Inhibitory Concentration: Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. The MIC of leaf extracts of *Aegle marmelos (L.) Correa*.was determined against all the microorganisms by agar well diffusion method by subjecting each organism to different concentrations of each plant extract. Each microorganism was suspended in sterile saline and diluted at ~10^6 colony forming units (cfu / ml) or ~ 0.1 OD reading. These microorganisms were then flood inoculated onto the surface of MHA. The wells, 10 mm in diameter, were cut from the agar and were filled with 0.1 ml of the plant extract of increasing concentrations into the respective wells. The plates were refrigerated at 4ºC for 2 hrs and then were incubated at 37ºC for 24 hrs (Antibacterial) and at 30ºC for 48 hrs (Antifungal). All the plates were examined for zones of growth inhibition and the diameters of these zones were measured in millimeters. The agar well containing least concentration of the plant extract; showing inhibitory zone was considered as MIC for the respective micro-organism (Bauer *et al*., 1966; Pawaskar and Kale, 2006; Pawaskar and Sasangan, 2015).

RESULTS AND DISCUSSION

Preliminary Phytochemical Study

Table 2 has the results ofpreliminary qualitative screening of various phytochemicals from the leaf extract of *Aeglemarmelos*(L.) Correa.showing the presence of terpenoids, steroids andphytosterols, tannins, alkaloids, glycosides, saponins, reducing sugar, phenols and flavonoids. The extraction of various phytochemicals was seen to be more effectively done in polar solvents (acetone, ethanol, methanol and water) than the nonpolar solvents. Especially, ethanolic leaf extract of the plant under study showed presence of all of the tested phytochemicals. Hence, it can be reported that alcoholic extract was the best one for extracting the active principle than others.

Table 2. Results of Preliminary qualitative Screening of various Phytochemicals from the leaf extract of *Aegle marmelos* (L.) Correa.in different solvents

Sr. No.	Phytochemicals	PE	CL	EA	AC	ET	ME	WT
1	Terpenoids	-	+	-	+	+	+	-
2	Steroids and Phytosterols	+	+	-	+	+	+	-
3	Tannins	+	+	-	+	+	-	+
4	Alkaloids	+	+	+	+	+	+	+
5	Glycosides	-	+	+	+	+	+	+
6	Saponins	-	-	-	+	+	-	+
7	Phenols	-	-	-	-	+	-	+
8	Flavonoids	-	-	-	-	+	+	-
9	Reducing Sugars	-	-	-	-	-	+	+

Ulahannan, *et al*., (2008), in their study have indicated presence of phenol and alkaloids in acetone and methanol extracts. Flavonoids were detected by them only in methanol extract of the plant and terpenes not detected in any of the tested extracts. However, in contrast, our study showed the presence of phenols in ethanol and aqueous extracts of the leaves of *Aeglemarmelos* (L.) Correa. We found, alkaloids to be present in all the tested extracts viz. petroleum ether, chloroform, ethyl acetate, acetone, ethanol, methanol and

water. Flavonoids were detected in both ethanolic and methanolic extracts and terpenoids in chloroform, acetone, ethanolic and methanolic leaf extracts of the plant.

Phytochemical analysis of the petroleum ether, benzene, chloroform, ethanol and water extracts of *Aeglemarmelos,* was also done by Sivaraj, *et al*. (2011) and the absence of terpenoids in all the extracts was reported by them.

Table 3. The zones of inhibition for organisms with successive extracts of the leaf powder of *Aegle marmelos* (L.) Correa. (By Agar well diffusion Method)

Micro-organisms	PE	CL	EA	AC	ET	ME	WT	AMP	CLP	NYT	CLZ
E.coli	11	10	11	10	14	13	12	_	13	_	_
Proteus vulgaris	10	11	11	10	13	12	12	_	15	_	_
Staph. aureus	11	11	11	10	15	13	12	22	_	_	_
Klebsiella pneumoniae	12	10	11	10	16	14	13	_	19	_	_
Pseudomonas aeruginosa	11	12	11	12	15	14	13	_	16	_	_
Shigella flexneri	10	12	11	10	17	15	13	_	19	_	_
S.typhi	11	12	12	11	15	13	12	_	20	_	_
S. paratyphi A	12	12	13	12	16	14	13	_	21	_	_
S. paratyphi B	11	12	10	13	15	13	11	_	12	_	_
Bacillus subtilis	12	13	14	12	15	13	12	_	15	_	_
Strep. pyogenes	11	13	12	13	15	14	12	_	18	_	_
Vibrio cholerae	10	13	16	12	17	15	14	_	17	_	_
Enterobacteraerogenes	09	10	09	11	14	12	13	_	18	_	_
Candida albicans	13	16	17	13	23	20	14	_	_	26	30
S. cerevicae	13	14	13	11	19	17	13	_	_	26	30

(Note: "-" means - ZOI was not seen.)
Note: PE: Pet ether extract; CL: Chloroform extract; EA: Ethyl acetate extract; AC: Acetone extract; ET: Ethanol extract; ME: Methanol extract; WT: Water extract; AMP: Ampicillin; CLP: Chloramphenicol; NYT: Nystatin and CLZ: Clotrimazol.

Table 4. The zones of inhibition for organisms with successive extracts of the leaf powder of *Aegle marmelos* (L.) Correa (By Agar disc diffusion Method)

Micro-organisms	PE	CL	EA	AC	ET	ME	WT	AMP	CLP	NYT	CLZ
E.coli	10	09	10	09	12	11	10	_	11	_	_
Proteus vulgaris	09	10	10	09	12	11	11	_	15	_	_
Staph. aureus	09	09	09	10	13	12	11	13	_	_	_
Klebsiella pneumoniae	11	09	10	09	14	12	12	_	19	_	_
Pseudomonas aeruginosa	09	10	09	10	13	11	10	_	14	_	_
Shigella flexneri	09	11	10	09	14	13	12	_	18	_	_
S. typhi	12	11	12	08	14	13	12	_	17	_	_
S. paratyphi A	11	11	09	10	15	14	13	_	14	_	_
S. paratyphi B	10	11	09	09	13	12	10	_	12	_	_
Bacillus subtilis	11	09	11	09	13	11	10	_	14	_	_
Strep. pyogenes	09	11	10	11	14	13	11	_	16	_	_
Vibrio cholerae	09	09	10	09	12	11	09	_	14	_	_
Enterobacteraerogenes	09	10	09	10	13	11	10	_	15	_	_
Candida albicans	11	12	11	10	15	13	12	_	_	18	23
S. cerevicae	10	12	10	09	14	13	11	_	_	19	25

(Note: "-" means - ZOI was not seen.)
Note: PE: Pet ether extract; CL: Chloroform extract; EA: Ethyl acetate extract; AC: Acetone extract; ET: Ethanol extract; ME: Methanol extract; WT: Water extract; AMP: Ampicillin; CLP: Chloramphenicol; NYT: Nystatin and CLZ: Clotrimazol.

Table 5. The minimum inhibitory concentrations (mg/ml) of the leaf extract of *Aegle marmelos* (L.) Correa in different solvents

Micro-organisms	PE	CL	EA	AC	ET	ME	WT
E.coli	31	41.5	31	41.5	23.5	25	23.5
Proteus vulgaris	32.5	39	39.5	32.5	22	25	25
Staph. aureus	38.5	36	36.5	38.5	26.5	31.5	33
Klebsiella pneumoniae	32.5	38.5	35	36.5	23	25.5	28.5
Pseudomonas aeruginosa	38.5	33.5	38.5	33.5	22.5	25	25.5
Shigella flexneri	38.5	32.5	35.5	38	23.5	25.5	28.5
S. typhi	30.5	26.5	26.5	36.5	23.5	25.5	26.5
S. paratyphi A	26.5	29	30.5	28.5	21	25	29
S. paratyphi B	35.5	31.5	38	26.5	23.5	25.5	29
Bacillus subtilis	33.5	31.5	26.5	33.5	23.5	28.5	31.5
Strep. pyogenes	38	33	35	33	25	28.5	31
Vibrio cholerae	35.5	31.5	24	33.5	23	26.5	28.5
Enterobacteraerogenes	33.5	31	33.5	30.5	22	28.5	25.5
Candida albicans	35	29	27.5	37	21.5	23.5	25.5
S. cerevicae	35.5	31.5	35.5	32.5	22.5	25	30.5

(Note: "-" means - ZOI was not seen.)
Note: PE: Pet ether extract; CL: Chloroform extract; EA: Ethyl acetate extract; AC: Acetone extract; ET: Ethanol extract; ME: Methanol extract; WT: Water extract; AMP: Ampicillin; CLP: Chloramphenicol; NYT: Nystatin and CLZ: Clotrimazol.

The result of the study has revealed that ethanol and aqueous extracts of the leaves of *Aeglemarmelos* (L.) Correa. showed considerably high amounts of most of the phytochemicals. This may possibly be one of the reasons for highest antibacterial activity shown by the ethanolic leaf extracts of the *Aeglemarmelos* (L.) Correa. plants.

Invitro - Antimicrobial Study

The zones of inhibition for the selected organisms using successive extracts of the leaf powders of *Aeglemarmelos* (L.) Correa. by Agar well diffusion method and Agar disc diffusion method are presented in Table-3 and Table-4 respectively and the minimum inhibitory concentration(mg/ml) of the leaf extracts of *Aeglemarmelos* (L.) Correain different solvents is presented in Table-5. All the seven successive extracts of the leaf powder of *Aeglemarmelos* (L.) Correa.exhibited prominent antimicrobial and antifungal activity against all microorganisms used in the study.In Agar well diffusion method, highly polar solvents ie.ethanol, methanol and water showed the most significant antibacterial and antifungal activity against all tested organisms for the leaf extracts of *Aeglemarmelos* (L.) Correa.; with the ethanol extract showing maximum inhibition in the range of 13 mm - 23 mm for the leaf extract of *Aeglemarmelos* (L.) Correa.

Similar pattern of results was also observed in Agar discdiffusion method. Highly polar solvents ie.ethanol, methanol and water showed the most significant antibacterial and antifungal activity against all tested organisms for the leaf extracts of *Aeglemarmelos* (L.) Correa.; with the ethanol extract showing maximum inhibition in the range of 12 mm - 15 mm for the leaf extract of *Aeglemarmelos* (L.) Correa. The minimum inhibitory concentration results of the leaf extracts of *Aeglemarmelos* (L.) Correainall the different solvents, indicated that – *Staphylococcus aureus and Streptococcuspyogenes* were the least susceptible among the organisms tested for *Aegle marmelos* (L.) Correa. The results of the entire study reveal that the leaf extracts of *Aeglemarmelos* (L.) Correa*,* inall the different solvents used for extraction, possesses potential antimicrobial activity against the pathogens used for screening. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world. Plants have been reported to possess antimicrobial, antifungal and other activities. This has been elucidated by various workers (Pawaskar S.M., *et. al, 2006*; Pawaskar S.M., *et. al, 2015;* Sasidharan., *et. al* 1998; Sudharameshwari*., et. al*, 2007; Ramya, *et al.,* 2008).

In the present study ethanolic and methanolic leaf extracts of *Aeglemarmelos* (L.) Correa., showed maximum inhibition against both Gram-positive and Gram-negative bacteria. In aqueous extract, moderate activity was observed followed by ethyl acetate and Chloroform extract. Acetone and Petroleum ether extracts were comparatively less effective against any of the organisms tested. Among the different microorganisms tested, maximum inhibition was found in *Vibrio cholera, Shigellaflexneri, Klebsiella pneumonia* and*S.paratyphi.*Similar antibacterial activity of the leaf extracts of *Aeglemarmelos* (L.) Correa.has been reported previously (Balakrishnan., *et. al,* 2006; Rajasekaran., *et. Al* ,2008; Rana., *et. al,*1997). Rana, *et al*., (1997) have reported the antifungal activity of essential oils isolated from the leaves of *Aeglemarmelos* (L.) Correa.in which, fungus, *Fusariumudum*was reported to exhibit maximum resistance. However, in our study, both the tested fungal strains viz. *Candida albicans*and*S.cerevicae* showed very high susceptibility towards the leaf extracts of *Aeglemarmelos* (L.) Correa. The results of the present study have provided supportive scientific evidence that the leaf extracts of *Aegle marmelos* (L.) Correa.possessa potential and broad spectrum of activity against a panel of bacteria. These promissory results form a primary platform for further phytochemical and pharmacological studies that may open the possibility of finding new clinically effective antibacterial compounds.

Conclusion

Based on these results, further chemical and pharmacological investigations to isolate and identify the chemical constituents in the leaf extracts of *Aeglemarmelos* (L.) Correaand to screen other potential bioactivities may be recommended.

Acknowledgement

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

Financial Support and Sponsorship: Nil

Conflicts of Interest: The author declares no competing interests.

REFERENCES

Balakrishnan, N., Bhaskar, V.H., Jayakar, B. and Sangameswaran, B. 2006. Antibacterial activity of *Mimosa pudica*, *Aegle marmelos* and *Sidacordifolia*. *Pharmacognosy Magazine,* 2 (7):198-199.

Bauer, A.W., Kirby, W.M.M. and Sherris, J.C. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.,* 45:493-496.

Colombo, M.L., Bosisio, E. Pharmacological activities of Chelidoniummajus L. 1996. (Papaveraceae). *Pharmacol., Res.,* 33:127-134.

Finar, L.L. 1983. Organic chemistry: Vol 25th Edition, Longman, London., 696-765.

Gordon, M.C., David, J.N. 2001. Natural product drug discovery in the next millennium.Pharm. Biol., 39:8-17.

Harbone, J.B.Phytochemical Methods.1stedition.Champanand Hall, London, UK. 1973.

http://www.ecosensorium.org/2011/01/aegle-marmelos-tree-with-immense.html

Iwu, M.W., Duncan, A.R., Okunji, C.O. 1999. New antimicrobials of plant origin. In: Janick J. ed. Perspectives on New Crops and New Uses. Alexandria, VA: ASHS Press. 457-462.

Krishnaraju, A.V., Rao, TVN., Sundararaju, D. 2005. Assessment of bioactivity of Indian medicinal plants using Brine shrimp (Artemiasalina) lethality assay. *Int. J. Appl. Sci. Eng.,* 2:125-134.

Martinez, A. and Valencia, G. Manual de practicas de Farmacognosia y Fitoquimia: 1999.1. Medellin: Universidad de Antiquia; Marchafotiquimica, 2003; 59-65.

Oguyemi, A.O.In: Sofowora A. ed., Proceedings of a Conference on African Medicinal Plants. Ife-Ife: Univ Ife: 1979;20-22.

Pawaskar S. M. and Sasangan K. C. In vitro antimicrobial activity of *Cynodondactylon* (l.) Pers. Leaf extracts. Antimicrobial activity by zone of inhibition estimation. *Indian Drugs.* 2015; 52(04):37-41.

Pawaskar S. M. and Sasangan K. C. Pharmacognostical and physicochemical evaluation of Aegle marmelos (L.) Correa. leaf extract. *Int J of Pharmaand Biosciences.* 2017. 8(2): (P) 356-363.

Pawaskar S.M., *et. al.* Antibacterial activity of successive extracts of *Mimosa pudica*. *Indian drugs,* 2006; 43(6):476-480.

Pawaskar S.M., *et. al.* Evaluation of phytochemical and anti-oxidative potential of aqueous whole plant extract of Mimosa pudica, *Indian Journal of Clinical Biochemistry,* 2007; Vol. 22.

Polk, Melanie. Feast on Phytochemicals. AICR newsletter. 1996; Issue 51.

Rajasekaran, C., Meignanam, E., Premkumar, N., Kalaivani, T., Siva, R., Vijayakumar, V., R amya, S. and Jayakumararaj, R. In Vitro Evaluation of antibacterial activity of phytochemical extracts from leaves of *Aegle marmelos* (L.) Corr. (Rutaceae), Ethnobotanical Leaflets, 2008; 12:1124-28.

Ramya, S., Govindaraji, V., Kannan, N.K. and Jayakumararaj, R. In Vitro evaluation of antibacterial activity using crude extracts of *Catharanthusroseus* L. (G.) Don. Ethnobotanical Leaflets, 2008; 12:1013-1018.

Rana, B.K., Singh,U.P., Taneja, V. Antifungal activity and kinetics of inhibition by essential oil isolated from leaves of *Aegle marmelos*, *J Ethnopharmacol.,* 1997; 57:29-34.

Samidha M Pawaskar, Sasangan K C. In vitro - antioxidant and preliminary phytochemical analysis of Aegle marmelos (l.) correa. leaf extract. *Asian J Pharm Clin Res*. 2017.Vol 10, Issue 6, 1-6.

Samidha M. Pawaskar And K. C. Sasangan. "Pharmacognostical investigation and physicochemical parameters of *Annonasquamosa* Linn leaves". *J. Pharm. Sci. and Res.* 2017.Vol. 9(5), 618-623.

Samidha M. Pawaskar and K. C. Sasangan. Invitro-Antioxidant and Preliminary phytochemical analysis of Cynodondactylon (L.) Pers. leaf extract. *International Journal of ChemTech Research.* 2017.In Press.

Samidha M. Pawaskar and K. C. Sasangan. Pharmacognostical and physicochemical evaluation of *Moringaoleifera* Lam. leaves. *International Journal of ChemTech Research.* 2017.10(4): 132-142.

Samidha M. Pawaskar and K. C. Sasangan. Pharmacognostical and physicochemical evaluation of *Cynodondactylonlinn*. Pers. Leaf extract. *Int J of Pharmaceutical Sciences and Research (IJPSR)*.2017.Vol. 8(9): 1000-08.

Samidha M. Pawaskar and K. C. Sasangan. Physico-chemical and preliminary phytochemical investigation of *Cynodondactylon* (L.) Pers. leaf extract. *Bionano Frontier* .2017. Vol. 10 (2). In Press.

Samidha M. Pawaskar and K. C. Sasangan. Preliminary Phytochemical and In vitro - Antimicrobial analysis of *annonasquamosa linn*. leaf extract. *J. Pharm. Sci. and Res.* 2017.Vol. 9(5), 618-623.

Samidha M. Pawaskar and K. C. Sasangan. Preliminary phytochemical and in vitro antioxidant analysis of *Annonasquamosalinn*. leaf extract. *Int. J. Res. Ayurveda Pharm.* 2017.8 Suppl 2. 241-247

Samidha M. Pawaskar, K. C. Sasangan. Physico-Chemical and Phytochemical Investigation of Aegle marmelos (L.) Correa. Leaf Extracts. *Int. J. Pharm. Sci. Rev. Res.,* 2017. 44(1), Article No. 35, Pages: 149-155.

Sasidharan, V.K., Krishnakumar, T. and Manjula, C.B. Antimicrobial Activity of Nine Common Plants in Kerala, India. PJS, 1998; 127(1):59-67.

SivarajRajeshwari, Balakrishnan, A., Thenmozhi, M. and Sivaraj, Venckatesh R. Preliminary phytochemical analysis of *Aegel marmelos*, *Rutagraveolens*, *Opuntiadellini*, *Euphorbia royleana* and *Euphorbia antiquorum*. *International Journal of Pharmaceutical Sciences and Research,* 2011; 2(1):146-150.

Sudharameshwari, K. and Radhika, J. Antibacterial screening of *Aegle marmelos*, *Lawsoniainermis* and *Albizzialibbeck. African Journal of Traditional, Comple-mentary and Alternative Medicines,* 2007; 4(2):205-210.

Trease, G.E., Evans, W.C. Pharmacognsy. 11th edition, BrailliarTiridel Can. Macmillan Publishers, London. 1989.

Ulahannan, Rijamol K., Thomas Toji and Sadasivan, C. Antibacterial action of leaves of *Aegle marmelos*. nt. *Journal (Sciences),* 2008; 2(2):134-138.

Venkatesan D., Karrunakarn C.M., Selva Kumar S. and PalaniSwamy P.T.Identification of Phytochemical Constituents of *Aegle marmelos* Responsible for Antimicrobial Activity against Selected Pathogenic Organisms. *Ethnobotanical Leaflets,* 2009; 13:1362-72.

****