Nature of Pharmaceutical Technology **ISSN: 2231 – 0436 (Print)**

Investigation of Phytochemical and antimicrobial activity of *Aegle marmelos* (L.) Correa (Bael) fruits and fruit pulps

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Received: 11 June 2018 / Accepted: 30 June 2018/ Published Online:15 September 2018
http://www.gtrpcompany.com/npt.htm

Citation: Maridass M, Raju G. Investigation of Phytochemical and antimicrobial activity of *Aegle marmelos* (L.) Correa (Bael) fruits and fruit pulps. Nature of Pharmacetical Technology,2018,8(3),1-3.

Abstract

The aim of this paper was investigation of phytochemicals constituents and antimicrobial activity of the petroleum ether extract of *Aegle marmelos* (L.) Correa (Bael) fruit and fruit pulps. Materials and Methods: 25gms of air dried fruits pulps of *Aegle marmelos* (L.) Correa (Bael) was extracted with 750 ml of Petroleum ether for 12 hrs in Soxhlet apparatus. The collected extract was concentrated distillation and vacuo. The extract of *Aegle marmelos* was investigated by preliminary phytochemicals methods and antimicrobial activity tested for 24hr. The antimicrobial activity was tested organisms of *Bacillus subtilis, Escherchia coli, Stphaylocuccus aureus, Pseudomonas fluorescence, Aspergillus niger* and *Candida albicans* for disk diffusion method. Results and Discussion: The results of investigation of phytochemicals constituents of *Aegle marmelos* were reveals that alkaloids, flavonoids, terpenoids were present int fruits extract and except in tannins and saponins. The best results of Aegle marmelos (L.) Correa (Bael) showed significant activity against all tested bacteria and moderate anti fungal activity against *Aspergillus niger* and *Candida albicans*.

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Keywords: Rutaceae, *Aegle marmelos* (L.), fruits; phytochemicals,antimicrobial activity

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1 INTRODUCTION

The medicinal plants of Aegle *marmelos* (L.) Correa (Bael) is belongs to the family Rutaceae. The vernacular name known as different name such as Wood apple, Kaitha, Maredu Pandu, Vilam Palam, Belada Hannu, Koovalam, Kothu, Koth Bel, etc. The medicinal values of leaves, bark, roots, fruits and seeds are used by Indian traditional medicine, the Ayurveda and in various folk medicine. Literature review of this plants, earlier studies on phytochemical examination of fruit pulps was identified in tannins, flavonoids, alkaloids, saponins, phenols and steroids [1]. Roots were containing coumarins such as scoparone, scopoletin, umbelliferone, marmesin and skimmianine, alkaloids, halopine and terpenes reported in earlier authors [2]. Contituent of Umbelliferone, skimmianine, marmin, β- sitosterol, lupeol, and ɣ-sitosterol, fagarine, furoquinoline and alkaloids [2]. Leaves are present in the active contituents such as marmenol, a new 7-geranyloxycoumarin [7-(2, 6-dihydroxy-7- methoxy-7-methyl-3-octaenyloxy) coumarin], rutacine, ɣ-sitosterol, aeglenine, aegeline, marmeline, fragrine, dictamine, cinnamide and different derivatives of cinnamides. lupeol, rutin, marmesin, β-sitosterol, flavone, glycoside, O-isopentenyl halfordiol, cuminaldehyde, eugenol, cineol, citral, citronellal and phenylethyl cinnamides [2]. Leaf Oil α-Phellandrene, p-cymene, p-Menth-1-en-3; 5-diol, Limonene [2]. Leaf Oil α-Phellandrene, p-cymene, p-Menth-1-en-3; 5-diol, Limonene. Fruit Rind umbelliferone, dictamine, xanthotoxol, xanthotoxin, scaparone, isopimpinellin, isoimperatorin, N-2 methoxy-2-[4 methoxyphenyl] ethylenamid e[2]. Fruit Pulp steroids, terpenoids, phenolics compounds, mucilage, pectin, lignin, fat and volatile oil, insulin, proteins, carbohydrates, alkaloids, cardiac glycosides and bitter principles [2]. Seed oil Seeds have essential oil which is bitter in taste and contains D- limonene, α-D-phellandrene, cineol, citronellal, citral, P-cyrnene,

cuminaldehyde palmitic acid, stearic acid, linoleic and linolenic acid while seed residue contains about 70% protein [2]. Therefore, investigation of phytochemicals and evaluated for antimicrobial activity of the petroleum extracts of *Aegle marmelos* fruits and fruit pulps.

2 MATERIALS AND METHODS

2.1 Collection of Plant Materials

Fresh fruits of *Aegle marmelos* were collected from Tamiraparani River side, Tirunelveli, Tamil nadu. The plant materials ware authenticated identified by senior author.

2.2 Extraction of active compounds

The fruits were cleaned, air-dried for one weak and ground into a powder. A portion of the powder was subjected to successive extraction with methanol, 12h using Soxhlet apparatus. The extract was concentrated by solvent evaporation, concentrated, weighed and preserved at room temperature until further use.

2.3 Preliminary Phytochemical Screening

Air-dried and powdered plant materials were extracted and were subjected to preliminary phytochemical testing to detect for the presence of different chemical groups of compounds. for constituent of alkaloids, flavonoids, triterpenoids, anthraquinones, saponins and tannins were identified as standard methods.

2.4 Antimicrobial activity

2.4.1 Test Microorganisms and Growth Media

The tested microbial organism of *Bacillus subtilis, Escherchia coli, Stphaylocuccus aureus, pseudomonas fluorescence, Aspergillus niger* and *Candida albicans* were selected for this study. The bacterial and fungal stock cultures were maintained and incubated for 24 hours at 37°C on nutrient agar and potato dextrose agar (PDA) respectively, following refrigeration storage at 4°C. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

2.4.2 Investigation of Antimicrobial Activity

In vitro antibacterial and antifungal activities were examined for petroleum ether extract of *Aegle marmelos* in disk diffusion Method. The antimicrobial activities of fruits of *Aegle marmelos* against tested bacteria and fungi in *Bacillus subtilis, Escherchia coli, Stphaylocuccus aureus, Klebsiella pneumoniae, Salmonella typhi, Pseudomonas fluorescence, P. aeruginosa, Protius valgaris, Vibrio cholerae, Aspergillus niger* and *Candida albicans*. Tested extract of *A. marmelos* fruits were dissolved in dimethyl sulfoxide, sterilized by filtration using sintered glass filter, and stored at 4°C. The sets of five dilutions (5, 25, 50, 100, and 250µg/ml) of *Aegle marmelos* extract and standard drugs were prepared in double-distilled water using nutrient agar tubes. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains (10^8cfu) and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of in incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the tested organisms to the fruits of *Aegle marmelos* extract were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

3 RESULTS AND DISCUSSION

The phytochemical investigation of medicinal plants of *A. marmelos* showed active compounds of alkaloids, phenolic compounds and flavonoids, and except tannin and saponins respectively (Table-1). These secondary metabolites are reported to have many biological and therapeutic properties [3-5], so this species is expected to have many medicinal uses. Earlier studies, various solvent fractions revealed that carbohydrate, tannins, saponins, glycosides and flavonoids were present in the extract [6]. Fresh fruits and pulps yield in distillation yellowish-green oil with a peculiar aromatic odour, marmelosin reported in the marmesinine, βsitosterol-β-D-glucoside and rutin in the leaves. Ali et al.15 isolated Marmenol, a new 7-geranyloxycoumarin [7-(2,6-dihydroxy-7- methoxy-7-methyl-3-octaenyloxy) coumarin] from the leaves of methanolic extract of A. marmelos.

Table -1: Investigation of phytochemicals constituents of petroleum ether extract of A. marmelos.

Sl. No	Active Constituents	Present/ Absent
1	Alkaloids	+++
2	Phenolic Compounds	++
3	Flavonoids	+
4	Essential Oils/Terpenoids	+++
5	Tannins	-
6	Saponins	-

"+" Active compound

The results of the antimicrobial activity of petroleum ether extract of *A. marmelos* fruits were investigated by using disc diffusion method (Tables -2). The best results of *A. marmelos* fruits were active against *Bacillus subtilis, Stphaylocuccus aureus, Salmonella typhi, Pseudomonas fluorescence, P.aeruginosa* and *Vibrio cholerae.* Antifungal activity of *A. marmelos* fruits were observed in moderately active against *Aspergillus niger*, and *Candida albicans*. Previously, According to Poonkothai and Saravanan,(2008) reported that several extracts of leaf, bark and fruits of *A. marmelos* were found to be more effective against the bacteria tested[7]. Previous studies, best results of antimicrobial activity of

several plants such as *Hibiscus sabdariffa, Lysiloma acapulcensis, Loeselia mexicana* (Lam) and *Hibiscus sabdariffa* were against *Staphylococcus aureus, Streptococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi*, and *Candida albicans* [8]. The strongest antibacterial activity was found in the water extract of *Hibiscus sabdariffa, Lysiloma acapulcensis* and *Loeselia mexicana* showed the best antifungal activity against dermatophyte [8]. According to Erturk [9] who assessed the antibacterial activities of several plant extracts of *J. officinale* active against *B. subtilis, E. coli, S.aureus*, and *P. aeruginosa*. Previous studies, pytochemical identification of *A. marmelos* are isolated and reported that eighteen chemical components are ledene oxide-(II) (18.16%); menthol, 12 - (butyn-3-one-1-yl), (1R, 2S, 5R) (7.04%); (-)-caryophyllene oxide (7.10%); and himachalol (6.15%)[10]. Several studies were confirmed the phytochemicals constituents contribute the medicinal properties. In conclusion, the phytochemical study revealed the presence of tannins, saponins, glycosides and flavonoids which are compounds capable of causing varied physiochemical and pharmacological effects

Table -2: Investigation of antimicrobial activity of petroleum ether extract of A. marmelos.

Sl. No.	Microorganisms	Active	Moderate active	Inactive
1.	*Bacillus subtilis*	+++		
2.	*Escherchia coli*		++	
3.	*Stphaylocuccus aureus*	+++		
4.	*Klebsiella pneumoniae*			-
5.	*Salmonella typhi*	+++		
6.	*Pseudomonas fluorescence*	+++		
7.	*P.aeruginosa*	+++		
8.	*Protius valgaris*		++	
9.	*Vibrio cholerae*	+++		
10.	*Aspergillus niger*		++	
11.	*Candida albicans*		++	

12- 16 mm Best activity; 8-11 moderate activity

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4 ACKNOWLEDGMENTS

The authors are thankful to University Grants Commission, New Delhi, India for financial assistance for this work.

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