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Isolation, characterization and antibacterial activity of leaves extract of bael (*Aegle Marmelos*)

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Abstract

Bael tree, (A*egle marmelos*, Family- Rutaceae), is a sacred tree used for medicinal purpose. Some of the medicinal properties are astringent, antidiarreal, antidystentric, demulcent, stromachic, fever curing, insulin-promoter, anti-inflammatory, cardio tonic and cures ophthalmic, urinary trouble, palpitation and many more. Most of these properties are believed to be due to presence of bioactive alkaloids in Bael, thus in present work antibacterial phytoconstituent has been isolated from A*egle marmelos* leaves which displayed potent antibacterial activity against *Staphylococcus aureus, Solmonella typhi, Bacillus subtilis* and *Escherichia coli*.

Key-Words: Alkaloid, Antibacterial agent, Phytomedicine, A*egle marmelos*.

Introduction

A*egle marmelos* (Bael) is a sacred tree from India, of Rutaceae family, related to citrus. It is a beautiful medium size tree (average is 8.5 m tall), with spines on its branches and very aromatic. Leaves are pale green and trifoliate. Flowers are greenish white, sweetly scented, fruits are yellowish green. It is a good source of vitamin C and protein (1). There are several medicinal uses of Bael in curing diarrhea, fever, poor absorption, and bleeding, vomiting, nausea with blood, bronchitis, and gingivitis. Decoction of leaves is febrifuge, expectorant, asthmatic complaints (1). The leaves contain many constituents like alkaloids, aegeline, alkaloid coumarine, and marmine, sterol sitosterol, and essential oils d-limonene (1). Aegeline has recently attracted the interests of several researches (2-8). Review of literature suggested that most of the pharmacological properties are due to the presence of alkaloids in Bael. The study involves the isolation, and characterization of the bioactive constituents in the plant leaves and evaluation of antibacterial activity against some pathogenic bacteria for possible development of new drugs for the prevention and treatment of infections.

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Material and methods

General experimental procedure

The IR spectra were determined on a Thermo Nicolet 470 FT – IR spectrometer. The 1H NMR and 13C NMR spectra were recorded on a Bruker Avance 400 FT spectrometer for 1H NMR and 75 FT spectrometer for 13C NMR, using TMS as internal standard. Chemical shifts are expressed in parts per million (ppm). Column chromatography was carried out using silica gel (200-300 mesh) and to monitor the preparative separations, analytical thin layer chromatography (TLC) was performed at room temperature on pre-coated 0.25 mm thick silica gel 60 F254 aluminum plates 20 x 20 cm Merck, Darmstadt, Germany. Reagents and solvents were all of analytical grades and procured from Merck.

Plant materials

The plant material required for the study was fresh. Tender leaves were harvested as it has 0.15-0.2% yields from the month of November to March. These leaves were kept in sunlight and the dry leaves were powdered and stored.

Extraction and isolation of plant materials

The leaves (2 kg) were dried on the laboratory bench for 10 days. The dry sample was milled and ground into powder (1.3 kg). The powdered plant sample (1 kg) was packed into a Soxhlet apparatus and extracted exhaustively with ethanol for 24 h. The ethanolic extract was concentrated using a rotary evaporator at 40°C and then left on the bench to get crude extract

(48.5 g). The crude extract was partitioned between chloroform and water. A chloroform soluble fraction was obtained. Chloroform fractions was subjected to column chromatography over silica gel and eluted gradually with petroleum ether, petroleum ether-chloroform (90:10; 80:20; 70:30)

The yield of final solid was re-crystallized from hexane then subjected to thin layer chromatography (Chloroform: methanol 7:3) which confirm presence of one band Rf (0.71). The isolated compound coded as AL1 & subjected to qualitative test of alkaloids.

The *in vitro* antibacterial activity of isolated compound (AL1) was carried out for 24 h culture of selected bacteria. The bacteria used were Gram-negative organisms: *Salmonella typhi, Escherichia coli* and two Gram-positive strains comprising *Staphylococcus aureus* and *Bacillus subtilis*. Cultures were brought to laboratory conditions by resuscitating the organism in buffered peptone broth and thereafter nutrient agar (peptone 5 g/l and meat extract 3 g/ml) and incubated at 37°C for 24 h. The antibacterial activity was performed by a filter paper disc diffusion technique. The medium (7 g nutrient agar in 250 ml distilled water, autoclaved at 115°C for 15 min.) was cooled to 50°C. The medium (20 ml) was poured into a sterile Petri dish and allowed to solidify, set for 8 h then observed for contamination. The sterility of the medium was tested using autoclave 121°C 15 psi for 15 min. Compound AL1 (1 g) was dissolved in 1 ml of absolute ethanol and made up to 10 ml with distilled water to give a concentration of 100mg/ml (10% dilution). A colony of each test organism was sub-cultured on nutrient broth which contains peptone (5 g/l and meat extract 3 g/l) and incubated aerobically at 37°C for 8 h. 30 ml of the nutrient broth was used to flood the agar plates. A sterilized Whatman No. 1 filter paper disc soaked in AL1 was used to test for the sensitivity or antimicrobial effect of compound isolated from A*egle marmelos.* The plates were incubated at 37°C for 24 h. After incubation, plates were observed for zones of inhibition (in mm diameter). The minimum inhibitory concentration was determined. The sensitivity susceptibility of the test bacteria to standard drug was tested using incubated agar plate and Streptomycin. The zones of inhibition of Streptomycin on the test organisms were measured and compared with those of isolated compound of the same concentration.

Statistical analysis

All measurements were replicated three times and standard deviations determined. The student t-test at $P < 0.05$ was applied to assess the difference between the means (9).

Results and Discussion

The ^{1}H NMR spectrum of isolated compound AL1 revealed the presence of secondary methyl. The presence of the aromatic ring is easily established and identified by the IR characteristics signal Vmax 1623 Cm^{-1} and ^{1}H and ^{13}C spectra. The ^{1}H spectrum gives the aromatic proton at d H 6.66482. The IR spectrum showed peaks at Vmax 3420 cm^{-1} (OH), 2926 cm^{-1} (CH), 2853 cm^{-1} (aliphatic C-H stretching) and 1059 cm^{-1} (C-O) stretching (Table 1). The isolated compound also exhibited significant anti-bacterial activity *in vitro* against a wide range of pathogenic microorganisms (Table 2). The compound successfully inhibited *B. subtilis, S. typhi, S. aureus* and *E. coli*. *B. subtilis* was found to be more sensitive to the isolated compound. Many of these organisms are natural flora of the skin and also known etiologic agents of several skin and mucous membranes infections of man (10).

The inhibition effect of these pathogenic organisms may be the reason behind the use of *plant* in herbal medicine for the treatment of asthma, cough, diarrhea, fever, dystentric, urinary trouble. The level of inhibitory activity of isolated compound was found to be good at concentration 5-10 mg/ml. The minimum inhibitory concentration (MIC) of the compound was found between 2-6 mg/ml for different pathogenic microorganisms (Table 3). *S. aureus* is the common cause of urinary tract infections and traveler's diarrhea (11); isolated compound shows varying degrees on inhibition of the growth of these pathogens. This finding supported the use of the leaves of A*egle marmelos* (Bael) in the treatment of diarrhea and urinary tract.

This study demonstrates that A*egle marmelos* possess significant antibacterial activities which justify the traditional use of *plant* in phytomedicine. The isolated compound from A*egle marmelos* need to be further evaluated to explore its therapeutics importance in drug development process.

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Table 1: Spectral analysis of isolated compound (AL1)

Comp. code	IR spectra (cm^{-1})	^1H NMR Spectra (δ) in ppm
AL1	3420 (OH), 2920 (CH_2), 2853 (CH_2), 1623 (C=C aromatic), 1456 (NH), 1059 (CO),	7.40 (r, 4H, aromatic ring), 6.15-6.78 (s, 4H, aromatic ring), 4.58 (r, 2H, OH), 3.64 (m, 1H), 1.25-2.59 (r, 2H, CH_2 in ring).

Table 2: Antibacterial activity of isolated compound (AL1)

Pathogens	Zone of inhibition (mm) (Mean ± SD)	
	Isolated compound (AL1)	Streptomycin
Escherichia coli	11 ± 0.2	15 ± 0.4
Solmonella typhi	11 ± 1.1	15 ± 0.5
Staplococc aureus	14 ± 0.2	18 ± 1.2
Bacill subtilis	12 ± 0.0	17 ± 1.5

Table 3: The Minimum Inhibitory Concentration (MIC) regimes of isolated compound (AL1)

Pathogens	MIC (mg/ml^{-1})	
	Isolated compound (AL1)	Streptomycin
Escherichia coli	5.00	0.25
Solmonella typhi	6.25	0.5
Staplococc aureus	5.00	0.065
Bacill subtilis	2.50	0.065