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Extraction, Characterization and Pharmacological Evaluation of *Aegle marmelos* Leaves

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Abstract

In the present day, antibiotic drugs are gradually becoming obsolete due to the development of antimicrobial resistance. As a result, the scientific community is in search of new antibiotic drugs which can be safely administered to the patents. Natural products are generally known for their nontoxic nature and many of them are known to produce a variety of pharmacological activities. The aim of this work is to extract the leaves of Aegle marmelos, phytochemical characterization of the extract, identification of phytoconstituents by thin layer chromatography, ATR-FTIR Spectroscopy of extract and evaluation of its antimicrobial activity. Extraction of the leaves of Aegle marmelos has been conducted using a Soxhlet apparatus. About 10.32% yield of extract was obtained. Phytochemical screening of the ethanolic leaf extract by standard methods showed the presence of secondary metabolites such as alkaloids, carbohydrates, phenolic compounds, flavonoids, saponins and triterpenoids which were confirmed by TLC. ATR-FTIR Spectroscopic study was conducted to determine the type of functional groups present in extract. The ethanolic leaf extract also produced antibacterial activity against gram positive bacteria Staphylococcus aureus and gram-negative bacteria Escherichia coli and zone of inhibition was 14 mm and 16 mm respectively when compared to standard antibiotic tetracycline. From this research it can be inferred that ethanolic leaf extract of Aegle marmelos has antimicrobial activity because of the presence of secondary metabolites in it. Further investigation will hereby be conducted in future regarding the route of administration of the extract and the type of dosage form.

Keywords: Aegle marmelos, natural products, secondary metabolites, TLC, functional groups, antimicrobial

Introduction:

Antimicrobial agents are substances that are produced by various species of microorganisms like bacteria, fungi, actinomycetes, etc. They can be referred to as substances which suppress or inhibit the growth of other microorganisms eventually causing their destruction. [1] Antibiotics were a great discovery of the 20th century which resulted in the reduction of morbidity and mortality due to infectious diseases to a huge extent. But the inappropriate and irrational use of the same by the common population gave rise to antibacterial resistance. The mechanisms employed by the pathogenic bacteria to develop intrinsic resistance to antibiotics are alterations of target sites, active efflux of drugs, enzymatic degradations, etc. Antibiotics, besides suppressing the bacterial activity also produces many adverse reactions.[2] All these factors led the modern scientists to

search for new antibiotics from plant sources as herbal drugs are known to produce lesser side effects than synthetic medicine. [3]

Aegle marmelos is an essential medicinal plant in Ayurveda, often known as bael. It is the member of the family Rutaceae. It grows profusely in India, Burma, Bangladesh, Ceylon, Indo-China, and Thailand. It is a medium to large-sized, armed, deciduous tree with axillary alternate trifoliate leaves that are 2.5 cm long. The tree bears short flowers and globular fruits. In the indigenous systems of medicine, the different plant parts of bael tree have been used for various ailments. [4] The aim of this work is to identify the secondary metabolites present in *Aegle marmelos* leaf extract, determine the functional groups present in it and also to evaluate the antimicrobial activity of the extract against gram positive bacteria *Staphylococcus aureus* and gram-negative bacteria, *Escherichia coli*.

Material and Method:

Materials

Plant: The plant sample was collected from Bankura District, West Bengal in the month of March, 2023 and was authenticated as *Aegle marmelos* at Central National Herbarium (CNH), Botanical Survey of India, Ministry of Environment, Howrah, West Bengal.
Solvents: Ethanol, Chloroform, Distilled water, Petroleum ether
Other Reagents: HCl, Mayer's reagent, Wanger's reagent, Dragendroff's reagent, Alpha-naphtol, conc. H_2SO_4, Sodium hydroxide, Ammonia solution, Ferric Chloride, Ethyl acetate, Fehling's A & B Solution, Lead acetate, Ninhydrin, Silica gel 60 F254, nutrient agar, nutrient broth, Potassium Bromide, Tetracycline.
All the reagents and chemicals utilized in this research work were of analytical grade.

Methods

Extraction of leaf powder and calculation of yield

The collected fresh leaves of *Aegle marmelos* plant were rinsed with water and dried under a shade, after which the dried leaves were grounded into a powder using a mixer grinder. About 5 gm of powdered sample was successively extracted with petroleum ether, chloroform, and ethanol in a Soxhlet extractor for about 10 hours. After completion of extraction, the solvents were filtered and using a Rotary evaporator (Superfit), condensed masses of extracts were obtained. [5] The yield of extract was calculated by the formula:
%Yield = Weight of Extract / Weight of Leaf Powder ×100%

Phytochemical screening of leaf extract

Phytochemical screening of *Aegle marmelos leaf* extracts was performed using standard protocols for identification of alkaloids, carbohydrates, flavonoids, phenolic compounds, saponins and triterpenoids. [6]

Identification of phytoconstituents by thin layer chromatography

Thin layer chromatographic study of ethanolic extract was conducted for the presence of phenolic compounds and alkaloids. TLC was carried out on glass slides containing coating of silica gel 60 F254. For TLC of alkaloids solvent system used was Chloroform: Methanol in the ratio 12:2. For phenolic compounds solvent system was Chloroform: Methanol (27:0.3). [7] Rf value was calculated by the formula:
Rf = Distance travelled by solute/Distance travelled by solvent.

ATR-FTIR Spectroscopy of leaf extract

Attenuated total reflectance-FTIR Spectroscopy (Bruker, Apha II) of *Aegle marmelos* ethanolic leaf extract was conducted to determine the type of functional group present in it. The dried extract was mixed with KBr, pressed into a pallet, and analyzed at frequency range of 400 to 4000 cm^{-1}. [8]

Determination of antimicrobial activity of leaf extract

Study of antimicrobial activity of the ethanolic extract was conducted by disc diffusion assay, where, spread plate technique was used to distribute the bacterial culture evenly in the nutrient agar plates. 6 mm Whatman filter paper discs were punched, 300 mg/ml leaf extract was made to absorb in them and discs were air-dried for 48 h at 37 °C. The discs were then placed on the inoculated agar plates and incubated for 24-48 h at 37 °C to observe the zone of inhibition around the discs. Antimicrobial activity of extract was studied against gram-positive bacteria *Staphylococcus aureus* and gram-negative bacteria *Escherichia coli*. The results were compared with standard antibiotic tetracycline. DMSO was used as negative control. [9]

Result and Discussion:

Many scientists have carried out research on herbal medicine due to their low cost, various pharmacological activities, and less side effects compared to synthetic drugs. [10] In the present study, phytochemical screening of ethanolic leaf extract of *Aegle marmelos* was found to contain maximum no. of phytoconstituents compared to petroleum ether or chloroform extract as in Table 1. Ethanolic extract produced a yield of 10.32%. TLC of ethanolic extract was conducted for identification of alkaloids as well as phenolic compounds and the Rf values were calculated (Table 2). HPTLC or HPLC studies are required to be conducted further for detection of the specific alkaloids and phenolic compounds present in the extract. ATR-FTIR Spectroscopy was performed to determine the functional groups which is represented in Table 3 and Fig. 1. Antimicrobial study of leaf extract at concentration of 300 mg/ ml against the bacteria *Staphylococcus aureus* and *Escherichia coli* showed zone of inhibition of 14 mm and 16 mm respectively as in Table 4 and Fig. 2. Tetracycline was taken as standard antibiotic for comparison.

Table 1: Phytochemical study of different leaf extracts of *Aegle marmelos*

SL NO.	PHYTO-CONSTITUENTS	PETROLEUM ETHER EXTRACT	CHLOROFORM EXTRACT	ETHANOL EXTRACT
1.	Alkaloids	_	++	++
2.	Carbohydrates	_	+	++
3.	Flavonoids	_	+	++
4.	Phenolic Compounds	_	_	++
5.	Saponins	+	_	+
6.	Triterpenoids	_	_	+

Table 2: TLC of *Aegle marmelos* leaf extract (ethanolic)

Chemical Constituents	Distance travelled by solvent	Distance travelled by solute	Rf value
Phenolic Compounds	7	6.3	0.9
Alkaloids	7	6.4	0.91

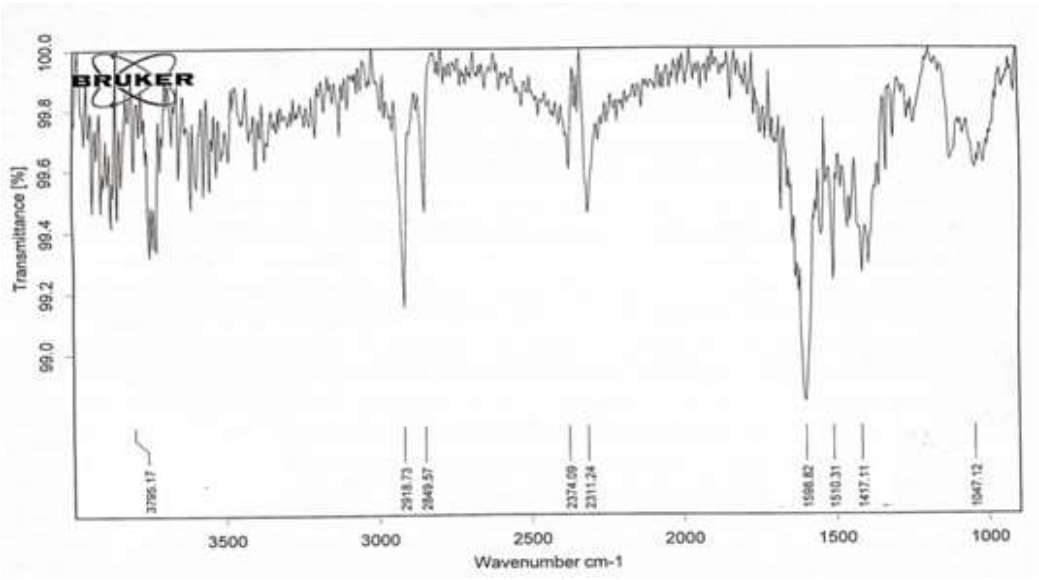

Fig. 1: ATR-FTIR Spectroscopy of *Aegle marmelos (*ethanolic*)* leaf extract

Table 3: Functional groups in *Aegle marmelos* ethanolic extract by ATR-FTIR Spectroscopy

Peak (cm^{-1})	**Functional group**
3795.17	Phenolic (O-H)
2918.73	Alkane (C-H)
2849.57	Alkane (C-H)
2374.09	C≡N
2311.24	C≡N
1598.82	N-H
1510.31	N-H
1417.11	Phenols (C-O)
1047.12	Alcohols (O-H)

2A **2B**

2C **2D**

Fig. 2: Zone of inhibition in the agar plates by 2A: Standard antibiotic tetracycline against *S. aureus*, 2B: *Aegle marmelos* leaf extract against *S. aureus*, 2C: Standard antibiotic tetracycline against *E. coli* and 2D: *Aegle marmelos* leaf extract against *E. coli*

Table 4: Antimicrobial activity of *Aegle marmelos ethanolic* leaf extract and zone of inhibition against *Staphylococcus aureus* and *Escherichia coli bacteria*

Bacteria	Zone of Inhibition (mm)	
	Leaf extract (300 mg/ml) conc	Antibiotic Tetracycline (300mg/ml) conc
Staphylococcus aureus	14	20
Escherichia coli	16	22

Conclusion:

This study concludes that *Aegle marmelos* ethanolic leaf extract possess antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* bacteria due to the presence of different functional groups and secondary metabolites in it. This extract can thus be utilized for the formulation of an appropriate antibiotic dosage form which can be safely administered in the human body.

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