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RESEARCH ARTICLE

ISOLATION AND CHEMICAL STANDARDIZATION OF 50% ETHANOLIC EXTRACT OF UNRIPE FRUIT OF BAEL {AEGLE MARMELOS (L) CORR.}

***Singh Madhu[1] and Prasad Kamleshwar[2]**

[1]Department of Pharmacy, Aligarh College of Pharmacy, Aligarh
[2]Department of Roga nidan, J.D. Ayurvedic Medical College & Hospital Bhankari, Aligarh

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ABSTRACT

In the present study, the chemical standardization of unripe fruit of bael {*Aegle mormelos (L)* Corr.} was assessed preliminary phytochemical screening of bael fruits show the positive test for alkaloids, flavonrides, glycosides, tannins, saponins, steroids and triterpenoids. Major constituents of fruit is mucilage and minerals a coumarin. The preliminary HPTLC studies revealed that the solvent system ethyacetate : methanol: formic acid (7:2:1) was ideal for the 50% ethanolic extract and gave well resolved peaks of crude extract of bael. The numbers of peaks and the Rf values along with the area under the curve are summarized along with the HPTLC profile of 50% ethanolic extract of bael.

The 50% ethanolic extract of bael is used for preparation, identification and isolation of active fraction from beutenol fraction of unripe fruit. On the basis of % yields and isolation of review on phytoconstituents, n-butenol fraction was loaded. Preparative TLC was performed in silica gel 60 F_{254} preparative TLC plates. Individual band were scrapped from the preperative TLC plates and extracted with methanol and dried under reduced pressure. Compound was subjected for spectral analysis and characterization .1HNMR spectroscopy, Mass spectroscopy and IR spectroscopy of spectral analysis shows that active constituent is -sitosterol.

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INTRODUCTION

Bael is a sacred fruit bearing tree and used as a medicines since ancient era but [1]ancient system of medicine has been narrated details of medicinal uses in different ailments like diarrhoea, constipation, haemorrhoids and gastric ulcers etc., it also have great values of antimicrobial, anti inflammatory and ulcer healing properties,[2] that is why it was used in various ailment in ancient era. The pulp of unripe fruit is mucilaginous in nature and it is a good source of proteins and minerals as phosphorous, potassium, calcium, magnesium and iron.[3]

In this research work, the air dried fruits was powdered and subjected to extraction with increasing order of polarity of different solvent. Among various fractions, butanol fraction of fruit is fractionated with silica gel chromatography (column chromatography) and then subjected to preparative TLC. The isolated compound was colorless powder, which was further subjected to IR, 1HNMR and mass spectroscopy for proper characterization and elucidation. Spectral analysis shows that active constituent is -sitosterol. Preliminary phytochemical screening of 50% ethanolic extract of bael fruit gives the positive test of phytosterol, alkaloids, carbohydrates, glycosides, proteins and saponins.[4]

MATERIAL AND METHOD

Collection of the fruits

The whole unripe fruit of *bael* was collected from the Botanical Garden of National Botanical Research Institute (NBRI), Lucknow. The plant materials were authenticated and the voucher specimens were deposited in the Pharmacognosy and Ethnopharmacology Division, NBRI, Lucknow for future reference.

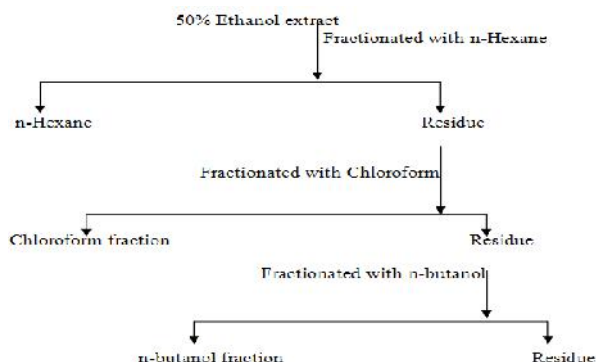
Preparation of 50% ethanol extract

The freshly collected fruits were washed with distilled water and all parts are air-dried at 30 ± 2^0C, and then dried in tray drier under the control conditions and powdered. The powdered fruit materials was percolated with petroleum ether to remove fatty substances, the marc was further exhaustively extracted with of 50% ethanol for 3 days. The extract was separated by filtration and concentrated on rotavapour and then dried in lyophilizer under reduced pressure and low temperature obtain solid residue.

Corresponding author:* **Singh Madhu
Department of Pharmacy, Aligarh College of Pharmacy, Aligarh

Preparation of extract and identification of active fraction

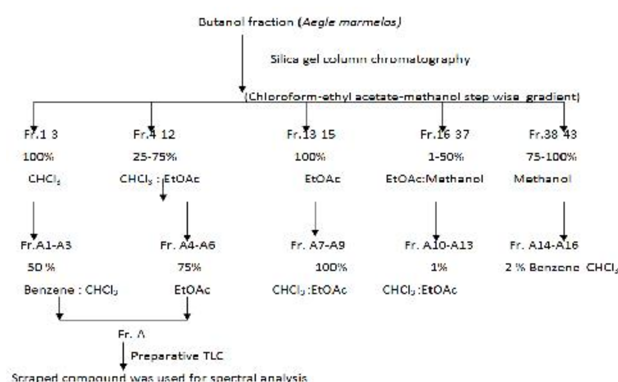
The extract was dissolved in methanol: water (10:90) and fractionated successively with *n*-hexane, chloroform and *n*-butanol with the help of separating funnel for 4 time each, to obtain n-hexane fraction, chloroform faction, n-butanol faction and aqueous portion. The flow diagram for fractionation is described bellow-



Isolation procedure of compounds from n-butanol fraction

On the basis of % yield and review on phytoconstituents, n-butanaol fraction 25gm was loaded on silica gel column (silica gel 0.5 kg) and eluted with 1.5L each of 100% chloroform, 25% ethyl acetate in chloroform, 50% ethyl acetate in chloroform, 75% ethyl acetate in chloroform, 100% ethyl acetate, 1% methanol in ethyl acetate, 2% methanol in ethyl acetate, 5% methanol in ethyl acetate, 10% methanol in ethyl acetate, 20% methanol in ethyl acetate, 30% methanol in ethyl acetate, 40% methanol in ethyl acetate, 50% methanol in ethyl acetate, 100% methanol. Each solvent run 2 times therefore we get 30 fractions[5]. Preparative TLC was performed in silica gel 60 F_{254} preparative TLC plates (20 × 20 cm with 4 × 20 cm concentration zone, 0.5 mm layer thickness and fluorescence at 254 nm were pre-heated at 105 ± 5 ºC for 30 min for the separation of compounds. A relatively large amount of fraction (50 mg/ml) was applied on the plates with a TLC sample applicator. The plates (×10) were developed with the solvent ethyl acetate: Methanol: formic acid (7: 3: 1). Individual bands showed at R_f 0.49 and 0.32 were scraped from the preparative TLC plates and extracted with methanol and dried under reduced pressure.[6]

Compound with R_f 0.49 was subjected for spectral analysis. IR spectra were taken on a Perkin Elmer FT-IR spectrometer, ^{1}H



NMR spectra were recorded with a JEOL AL300 FTNMR spectrometer and mass spectra on a JEOL JMSDX30 spectrometer. The Fractionation pattern of butanol fraction are mentioned bellow-

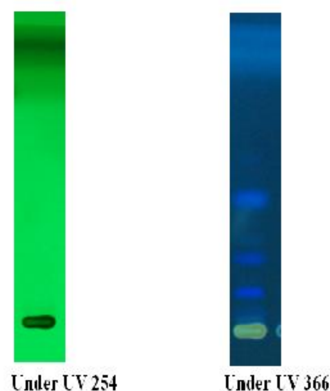
High Performance Thin Layer Chromatography (HPTLC) Analysis

Procedure:- Reflux 5 g of the finely powdered drug with 25ml of methanol on a water bath for 25 min consecutively three times, filter and remove the solvent under reduced pressure. Dissolve 25 mg of the extractive in 20 ml of methanol. Apply 10 µl of extracts on precoated Silica gel 60 F254 plates with 0.2mm thickness (Merck) using automatic applicator (CAMAG Linomat IV). The plates were then run using different solvent system Ethyl acetate : Methanol : Formic acid in the ratio of 7 : 3 : 0.5, respectively in a CAMAG twin through chamber up to a distance of about 9 cm, dry it and scan.

Visualization and scanning

The plates were visualized under UV 254 nm, 366 nm and visible. If required spray the plate with anisaldehyde -sulfuric acid and heat at 110°C for 10 min. Record the Rf values and color of the resolved bands and video documented using Desaga video documentation unit. The plates were scanned densitometrically using CAMAG TLC scanner at suitable wavelength.

Solvent system: Ethyl acetate : Methanol (80:20)
Developing solvent: Anisaldehyde sulphuric acid



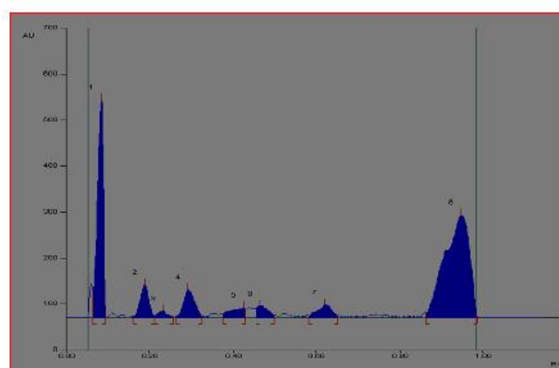


Figure showing the HPTLC finger print profile of *Aegle marmelos* at 330nm

Characterization of compound by different spectroscopy

1HNMR Spectroscopy

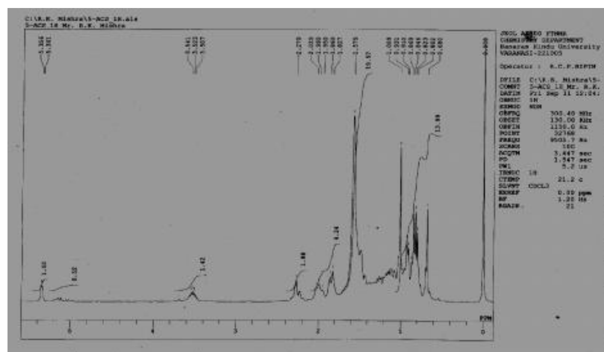
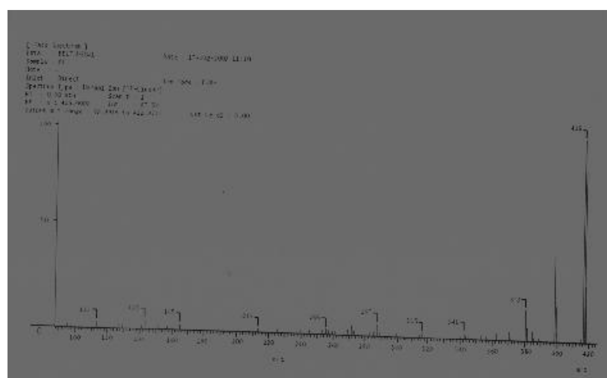


Figure showing the HPTLC finger print profile of *Aegle marmelos* at 330nm

1HNMR ($CDcl_3$ 300MHz) has given signals at 5.356 (1H, m H-6), 3.523 (2H), 2.270-2.020 (m, 2H), 1.990-1.827 (m,5H),1.673-1.575 (m, 20H), 1.009-0.993 (m,5H), 0.910-0.681 (m,14H) ppm. Other peaks are observed at 1.67, 3.507, 2.27 ppm.

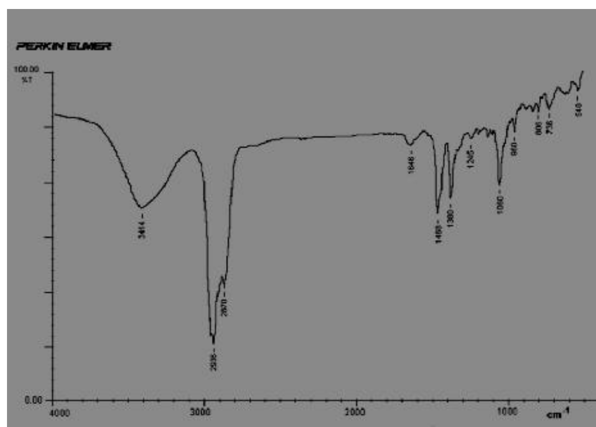
Mass spectroscopy



m/z 415 m+1, 396, 379, 255, 213, 287, 143,113.

IR spectroscopy

The IR absorption spectrum showed absorption peak at 3414 cm^{-1} (O-H stretching), 2936 cm^{-1} (aliphatic C-H stretching),1646 cm^{-1} (C=C absorption peak), other absorption peak include 1060cm^{-1} (cycloakane).



Preliminary Phytochemical Screening:- The qualitative Chemical tests for various phytoconstituents were carried out using the 50% ethanolic extract of fruits.[7] Which are tabulated following as-

S.No.	Constituents	Test	Result
1.	Carbohydrate	Molish's test	+ve
		Fehling's test	+ve
2.	Glycoside	Legal's test	+ve
		Borntrager's test	+ve
		Baljet test	+ve
3.	Fixed oil and fats	Spot test	+ve
		Saponification test	-ve
4.	Proteins and amino acids	Million's test	+ve
		Ninhydrin test	+ve
		Biuret test	+ve
5.	Saponins	Foam test	+ve
6.	Phenolics and tannins	$FeCl_3$ test	+ve
		Gelatin test	+ve
		Lead acetate test	-ve
7.	Phytosterol	Salkowiski test	+ve
		Libermannburchard test	+ve
8.	Alkaloids	Dragendroff's test	+ve
		Mayer's test	+ve
		Wagner's test	+ve
		Hager's test	+ve
9.	Flavonoids	Aqueous NaOH test	+ve
		Aqueous NaOH test	+ve
		Shinoda's test	-ve

RESULT AND DISCUSSION

The 1HNMR spectra of compound shows the presence of six methyl signals that appeared as 2- methyl singlet at 0.68 and 1.009; three methyl doublets that appeared at 0.802, 0.23 and 0.931; and a methyl triplet at 0.846. The 1HNMR spectra also showed one olifinic proton at 5.356. The 1HNMR spectra also showed a proton connected to the C-3 hydroxy group which presented as a triplet of doublet at 3.53. The typical 6H of the steroidal skeleton was evident as a multiplet at 5.356 that integrated for one pattern. The 1HNMR spectrum showed two doublets centered at 0.910 and 0.869 which could artibuted to two methyl groups at C-26and C-27 respectively. The compound shows positive Leibermann-Burchard reaction indicated its sterol nature.[8]

In mass spectroscopy, the weak molecules ions were given at m/z 415 characteristic peak this suggest that the sample contains molecular weight 415 ($C_{29}H_{50}O$; molecular weight, 414.71) and compound having characteristic fragments observed at m/z 415, 396, 381,287,255,213, and 145. The molecular weight and fragmentation pattern indicate that the presenting compound is -sitosterol.[9]

IR spectroscopic analysis the observed absorption bands are 3414 cm^{-1} that is characteristic of O-H stretching. Absorption at 2936 cm^{-1} is due to aliphatic C-H stretching. Other absorption frequencies include 1646 cm^{-1} as a result C=C stretching. At 1468cm^{-1} is a bending frequency for cyclic (CH_2). The absorption frequency at 1060 cm^{-1} signifies cycloalkane.[10]

CONCLUSION

The number of peaks and the R_f values along with the area under the curve are summarised along with the HPTLC profile of 50% ethanolic extract of *A.marmelos*. Preliminary

qualitative phytochemical screening of *A.marmelos* fruits extracts showed the presence of alkaloids, flavonoids, glycosides, tannins, saponins, steroids and triterpenoids. The structure of the isolated new compound was identified as - sitosterol on the basis of spectroscopic chemical test also proved the sterol nature of the compound.

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