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Isolation and characterization of phytoconstituents from petroleum ether extract of *Momordica cochinchinensis* fruits

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Abstract
The purpose of the study is to isolate and characterize the chemical constituents from fruits of *Momordica cochinchinensis.* The principal theme of the study is to develop applied chromatographic techniques for the separation, isolation and detection of the compounds. The petroleum ether extract of fruits of *Momordica cochinchinensis* was saponified and unsaponifiable matter was subjected to column chromatography and elution of column was carried out by Petroleum ether (100%) with increasing concentration of ethyl acetate for the separation of phytoconstituents. The isolated compounds were characterized and analyzed by physical characteristics, IR, NMR and Mass spectroscopy. Three known compounds lupeol, β-amyrin and β-sitosterol were determined for the first time from fruits of *Momordica cochinchinensis*. From the present study, it is concluded that the plant consists of phytosterols which can be isolated and characterized by chromatographical and spectroscopical method.

Keywords: *Momordica cochinchinensis,* phytosterols, lupeol**,** β-amyrin and β-sitosterol

Introduction
From thousands of years Natural products have been used by human societies. Natural sources have provided considerable value to the pharmaceutical industry over the past half century. Research studies leading to extraction, isolation and biological study of plant constituents have formed the major field of study. Various leads from plant sources were taken for discovering the new active therapeutic agents. Hence herbal medicine has played important role in managing the health conditions like diabetes, hypertension, inflammation, obesity, cancer, etc [1].
The plant *Momordica cochinchinensis* (Gac) is a Southeast Asian fruit found throughout the region from Southern China to Northeastern Australia, mostly Vietnam and throughout India. It grows on dioecious vines and usually collected from fence climbers or from wild plants [2]. It is reported that *Momordica cochinchinensis* Spreng, is one of the special fruits containing extraordinarily high levels of carotenoids, especially β -carotene (more than 16 mg/100 g) and lycopene (more than 50 mg/100 g), mainly in the red aril [3]. It is also reported to contain a protein that may inhibit the proliferation of cancer cells and also β-carotene with several phytonutrients, Vit-E, fatty acids, carbohydrates, flavonoidal glycosides [4].
Traditionally, Gac has been used as both food and medicine and promotes healthy vision by relief of dry eyes. It also possesses antioxidant, anti-microbial and antidiabetic properties. The seeds are considered to be good for cough and pains in the chest [5, 6].
But these studies are not enough for identifying and characterizing the bioactive compounds in the plant. The purpose of the study is to identify and characterize the bioactive principles from fruits of *Momordica cochinchinensis.*

Materials and Methods
Plant material: The fruits of *Momordica cochinchinensis* was collected from rural area of Kolkata. The herbarium of the *Momordica cochinchinensis* was authenticated by Botanical Survey of India, Pune Voucher specimen (MA 01) was deposited in library.

Preparation of Extracts: Fruits of *Momordica cochinchinensis* was extracted by Soxhlet extractor with Pet ether and macerated with ethanol and water successively.

Storage of Extracts: All the extracts were stored in tightly closed glass bottles in refrigerator at 2-8 ^{0}C.

Preliminary Phytochemical analysis of extracts: All the extracts were tested for the presence of various chemical constituents [7, 8].

Isolation of Phytoconstituents from Pet ether extract of *M. cochinchinensis* fruits

A) **Separation of Unsaponifiable Fraction from Pet Ether (PE) extract:** PE extract was taken in a flask and to it 0.5 N methanolic KOH was added and kept overnight at room temperature. Next day mixture was refluxed for 6 h and cooled. Twice of its volume distill water was added, extracted with diethyl ether and allowed to stand for few minutes. Ethereal layer was separated, the procedure was repeated twice. Ethereal layer was combined and washed with distill water till neutral to litmus paper. Ether was evaporated to obtained unsaponifiable fraction [9, 10].

B) **Isolation of Phytoconstituents from Unsaponifiable Fraction of PE Extract:** The unsaponifiable fraction was loaded on Silica gel (60-120 mesh) column. Elution of column was carried out by PE (100%) with increasing concentration of ethyl acetate (EA). Elution of the column with PE (100%) was carried out to afford 70-165 fractions (each fraction 250 ml). All fractions were monitored by TLC. All fractions were collected, mixed and concentrated to obtained white solid powder (Compound 1). Elution of the column with 3% EA was carried out to afford fractions 195-260. All fractions were collected, mixed and concentrated to obtained white solid powder (Compound 2). Fraction 305-375 eluted with 5 % EA afforded white crystals (Compound 3).

Spectroscopical Characterization: UV-visible spectrum was measured on a JASCO V-530 UV-Visible spectrophotometer using methanol as solvent. IR spectra of isolated compounds were taken on a FT-IR spectrophotometer (Perkin Elmer) at room temperature. ^{1}H and ^{13}C NMR spectra were taken on a Mercury Plus 300MHz NMR SPECTROMETER (VARIAN, USA). ^{1}H and ^{13}C chemical shifts (δ, ppm) are relative to the solvent signals used as references. Mass spectrum was recorded on Accu TOF mass spectrometer (JEOL).

Result and Discussion

Results

The results of preliminary phytochemical screening of various extracts of fruits of *Momordica cochinchinensis* revealed the presence of glycosides, flavonoids, steroids, phenolic compounds, saponins, carotenoids and carbohydrates (Table 1).

Table 1: Preliminary Phytochemical Screening of *M. cochinchinensis*

S. No	Constituents	*Momordica cochinchinensis*		
		Pet Ether Extract (PEMC)	Ethanolic Extract (AlcMC)	Aqueous Extract (AqMC)
1	Phytosterols	+	–	–
2	Glycosides	–	+	–
3	Carbohydrates	–	–	+
4	Flavonoids	–	+	–
5	Alkaloids	–	–	–
6	Tannins	–	–	–
7	Proteins	–	–	–
8	Saponins	–	+	+
9	Carotenoids	+	–	–
10	Phenolic compounds	–	+	–

+Presence, – Absence

Compound 1

Elution of the column with PE (100%) furnished white coloured compound 1. It gave pink colour with Libermann-Burchard reagent indicating the triterpenoid nature of the molecule. It was soluble in PE and $CHCl_3$ indicating non polar nature of compound. Melting point of compound was found to be in the range of 210-219° (Table 2). IR spectrum showed characteristic absorption bands for hydroxyl groups (3386 cm^{-1}), absorption band of C-O stretching (1595 cm^{-1}), C-H stretching (2945 cm^{-1}) and C=C bending (1460 cm^{-1}). The mass spectrum of compound 1 exhibited a molecular ion peak at *m/z* 426 consistent with the molecular formula $C_{30}H_{50}O$.

The ^{1}H NMR spectrum of 1 showed two one-proton multiplets at δ value 0.78 to 1.07 ppm assign to methyl protons. One multiplet proton peak at δ 4.68 ppm, 4.57 ppm attributes to alkene protons. ^{13}C NMR spectrum exhibited signals between δ value 14.08 - 55.2 ppm indicating the presence of several methyl and methylene proton in the structure. The ^{13}C NMR spectrum of 1 exhibited signals for isopropene group attached to cyclic ring carbon at δ value 151.1 ppm (C-20), 109.5 ppm (C-29); Signal at δ 79.1 ppm (C-3) indicates the presence of electronegative group like OH. On the basis of physical characteristics ^{1}H NMR, ^{13}C NMR, Mass, and IR spectral evidence, the structure of compound 1 as elucidated as lupeol (Fig 1).

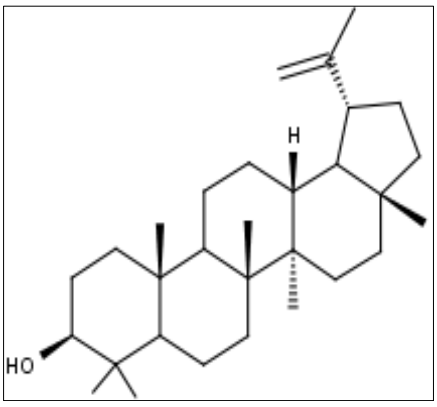

Fig 1: Chemical structure of compound 1(Lupeol)

Compound 2

Elution of the column with PE: EA (97: 03) furnished white coloured compound 2. It gave bluish colour with AS reagent indicating the triterpenoid nature of the molecule. It was soluble in PE indicating non polar nature of compound. Melting point of compound was found to be in the range of 192-200º (Table 2). IR spectrum showed characteristic absorption bands for hydroxyl groups (3427 cm^{-1}), absorption band of C=C stretching (1655 cm^{-1}) and C-H stretching (2990 cm^{-1}). The mass spectrum of 2 exhibited a molecular ion peak at *m/z* 426 consistent with the molecular formula $C_{30}H_{50}O$.

The ^{1}H NMR spectrum of 2 showed multiple peaks at δ values from 0.75 to 1.14 ppm assign to methyl protons. One multiplet proton peak at δ value 3.80 ppm attributes to alkene protons. The ^{13}C NMR spectrum exhibited signals between δ value 14.08 - 55.2 ppm indicating the presence of several methyl and methylene proton in the structure. ^{13}C NMR spectrum also exhibited signal at δ 79.1 (C-3) indicates the presence of electronegative group like OH. ^{13}C NMR spectrum showed signal at δ 134.8 ppm (C-13) indicates the presence of quaternary carbon.

On the basis of physical characteristics ^{1}H NMR, ^{13}C NMR, Mass, and IR spectral evidence, the structure of compound 2 was elucidated as β- amyrin (Fig 2).

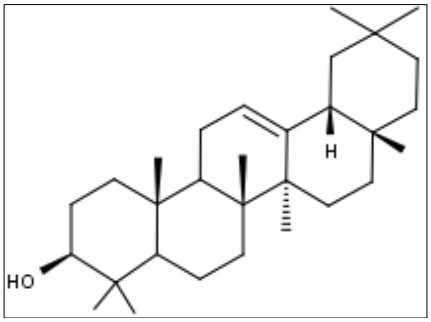

Fig 2: Chemical structure of compound 2 (β- amyrin)

Compound 3

Elution of the column with PE: EA (95: 05) furnished colorless crystals compound 3. It gave blue colour with AS reagent indicating the triterpenoid nature of the molecule. It was soluble in PE indicating non polar nature of compound. Melting point of compound was found to be in the range of 132-139º (Table 2). IR spectrum showed characteristic absorption bands for hydroxyl groups (3427 cm^{-1}), absorption band of C=C stretching (1655 cm^{-1}) and C-H stretching (2930 cm^{-1}). The mass spectrum of compound 3 exhibited a molecular ion peak at *m/z* 414 consistent with the molecular formula $C_{29}H_{50}O$.

Table 2: Physical characteristics of isolated compounds from *Momordica cochinchinensis*

Compound	Physical State	Melting point range	R_f (Cm)
Compound 1	White crystals	210-219^{0}C	0.51 (Toluene: Ethyl Acetate) (93: 7)
Compound 2	White crystals	192-200^{0}C	0.38 (Petroleum ether: Ethyl acetate) (9:1)
Compound 3	Colourless crystals	132-139^{0}C	0.21 (Petroleum ether: Ethyl acetate) (9:1)

The ^{1}H NMR spectrum of 3 showed several singlet peaks at δ values from 0.91 to 1.21ppm assign to methyl protons. One multiplet proton peak at δ value 3.56 ppm attributes to alkene protons. The ^{13}C NMR spectrum exhibited signals between δ value 12.08 - 51.3 ppm indicating the presence of several methyl and methylene proton in the structure. ^{13}C NMR spectrum also exhibited signal at δ 71.1 (C-3) indicates the presence of electronegative group like OH. ^{13}C NMR spectrum showed signal at δ 138.2 ppm (C-5) indicates the presence of quaternary carbon.

On the basis of physical characteristics ^{1}H NMR, ^{13}C NMR, Mass, and IR spectral evidence, the structure of compound 3 was elucidated as β - sitosterol (Fig 3).

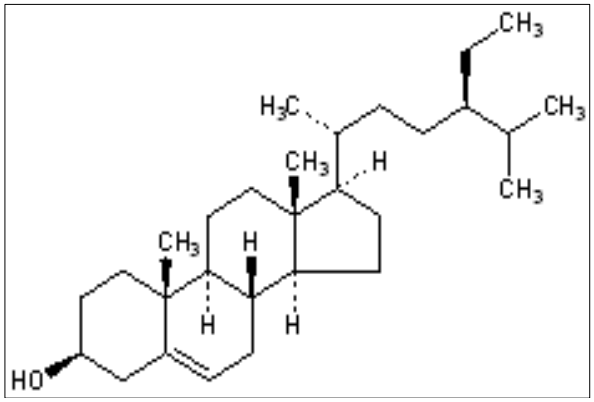

Fig 3: Chemical structure of compound 3 (β- Sitosterol)

Conclusion

From the physical, chemical and spectral evidences compound 1, 2 and 3 were confirmed as Lupeol (Fig 1), β-amyrin (Fig 2) and β - sitosterol (Fig 3).

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