An Approach to Identify Sterol Entities from *Abrus Precatorius*’s Seeds by GC-MS

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Sterols are most important compounds in living organisms and it is found in unsaponifiable fraction of plant. The objective of this investigation was to identify the chemical constituents of the seeds of *Abrus Precatorius*. A plant used for medicinal purpose in folklore. Petroleum ether extract of the seeds was used for this study. Saponifiable fraction and unsaponifiable fractions were separated. Compounds extracted in di-ethyl ether and separated using column chromatography. Stigmasterol, gamma sitosterol, campesterol were identified from the seeds by using gradient elution. The eluted chemical compounds were analysed by GC-MS. The present analysis revealed that seeds of *Abrus Precatorius* contain three sterols compound. Principal themes of the study were to highlight the development and application of chromatographic techniques for the detection of the sterols compounds by single injection which will be used for natural product and pharmaceutical industries.

Keywords: *Abrus Precatorius*, GC-MS, Stigmasterol, Campesterol, Gamma Sitosterol

Introduction

The plant sterols are most important compound for human diet. Plant sterols have likewise been reported to have anti-inflammatory effects in frequently used animal models for colitis.¹ Most of plant sterol compounds are unsaturated and fully saturated plant sterols (stanols) are available in much lesser concentrations. Campesterol, Stigmasterol, β-sitosterol, and brassicasterol are the major sterols found in plants but their quantity depends on species of plant.² *Abrus precatorius* is a well-known plant in Ayurveda³ and Unani medicine. *Abrus Precatorius* belongs to *fabaceae* family and inborn to India. It’s growing in tropical and sub-tropical areas, dry deciduous and scrub forests⁴,⁵. General names of plants are crab’s eye, precatory pea, rosary pea, jequirity etc.⁶. In West Tropical Africa, *Abrus precatorius* leaves have been active to sweeten foods and medicines used for stomach complaints, to treat cough, fever and cold. The leaves are casually chewed and the vine sometimes sold as a masticatory in Curacao⁷. The plant is primarily known for seeds and available in various type red, black, white seeds. Seeds are toxic because of the presence of abrin⁸. The present paper describes a simple, fast method based on a GC-MS technique for the easy determination and identification sterols in petroleum ether extracts of *Abrus precatorius* seeds.

Material and methods

Chemicals and reagents

The used chemicals and reagents like Libermann-buchard, Petroleum ether AR grade, Methanol, KOH, Anhydrous Na₂SO₄, n- hexane, Ethyl acetate, Diethyl ether and Glacial Acetic acid were purchased from Merck (India) Ltd. without further purification.

Plant Materials

An *A. Precatorius* seed were purchased from local, Rajkot (Gujarat), India and was authenticated by Dr. Vivek Vegda, Botanist, School of Science, RK University, Rajkot (Gujarat- India)

Preparation of extracts⁹

The *A. Precatorius* seeds were dried and grinded to make grainy powder. Grinded grainy powder (299.6 gm) was extracted with petroleum ether (60° – 80°C) by soxhlet extraction method for 24 hrs. Solvent was distilled off and the extract was concentrated and dried under reduced pressure, which
yielded 4.9706 gm yellow brownish mass. This crude extract was used for further investigation.

**Experimental**

Accurately weighed 4.9706 gm of petroleum extract was taken in flask and 50 ml of 20% methanolic KOH was added and kept overnight at room temperature. On next day, the mixture was refluxed for 8 hrs and cooled at room temperature. Twice volume of distilled water was added and extracted with ether. Collected ethereal extract was washed with distilled water till extract becomes neutral and dried over anhydrous sodium sulphate. Afterward, the ether was evaporated and unsaponifiable fraction obtained. (1.0025 gm).

**Investigation of unsaponifiable fraction**

The unsaponifiable fraction was tested for sterol with libermann-buchard reagent which gave strong positive test. The unsaponifiable fraction (1.0025 gm) was concentrated and dried. For a given separation, column chromatography was carried out on Kieselgel 60, long narrow column, but the resultant flow rate was lower. A fritted-glass disk may be seated in the end of the tube to act as a support for the packing material. The column is fitted at the end with a stopcock. A small fraction of dried sample was chromatographed on 30-50 times of its volume of silica gel 60 - 120 mesh size and build up column with hexane. A gradient solvent systems were used for elution as in sequence of 95:05, 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 50:50 with n-hexane: ethyl acetate which give fractions F1 to F40. Obtained fractions were monitored on thin layer chromatography and selected fractions were analysed by GC-MS.

**Fraction: F23 to F25**

Fraction 23 to 25 were obtained same spot compound in TLC after libermann-buchard spraying reagent named as AKC-1. In GC-MS analysis compound identified as Stigmasterol, Campesterol and Gamma-Sitosterol.

**GC-MS protocol**

“Analysis was carried out using a HP5 Agilent Technology 5977B (Santa Clara, US) model no. 7820A MS coupled to an 5977B equipped with an HP-5 fused silica capillary column (30 m × 0.320 mm i.d. × 0.25 μm film thickness). The column temperature was initially held at 44°C for 5 min. and then increases it 10°C/min. to 300°C for 15 min. The helium flow-rate was 1 ml/min. the ion source of the MS was operated at 260°C and the transfer line at 260°C. Electron impact (EI) ionization was carried out at 965 Volt; quantitative determination was based on the total ion current corrected for the detector response of each individual sterol. The mass range from 50-550 amu was scanned at a rate of 1562 [N=2] unit/second. For analysis, the steroids fraction of 10 mg were dissolved in diethyl ether 10.0 ml and 1.0 µL aliquots by auto injector. Steroids were identified by direct comparison of their MS and retention time with those of authentic sample and with data from NIST 14 Library.”

**Result and Discussion**

Interpretation of mass spectrum in GC-MS was concluded by using the database of National Institute Standard and Technology (NIST) having more than 2, 50,000 patterns repository. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library.

**Unsaponifiable fraction**

**Compound-1: Stigmasterol**

Molecular formula C_{29}H_{48}O, Molecular weight 412.4, RT (Retention time) - 45.418. The peak at 45.418 minutes had a mass [M'] 412.4. The daughter
ion spectra of these compounds (inserts) revealed the characteristic fragments $m/z$ 55.1, 133.1, 159.1, 207, 255.2, 300.2, 351.3 and 412.4. (Figure 2)

**Compound- 2: Gamma sitosterol**
Molecular formula $C_{29}H_{50}O$, Molecular weight 414.39. GC-MS Fragment: The peak at 46.091-46.365 minutes had a mass $[M^+]$ 412. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments $m/z$ 55.1, 57.1, 81.1, 91, 95.1, 105, 107.1, 145.1, 329, 414.4. (Figure 3)

**Compound- 3: Campesterol**
Molecular formula $C_{28}H_{48}O$, Molecular weight 400.37. GC-MS Fragment: The peak at 44.871-45.186 minutes had a mass $[M^+]$ 400.37. The daughter ion spectra of these compounds (inserts) revealed the characteristic fragments $m/z$ 55.1, 73.0, 96.0, 135.0, 207.0, 253.0, 281.0, 341.0, 400.4. (Figure 4). The results showed unsaponifiable matter was separated through extraction with mid-polar solvents like diethyl ether. From the unsaponifiable fraction, three sterols compounds Stigmasterol, campesterol and gamma-sitosterol were identified. It was difficult to separate sterol compounds from seeds through simple chemical methods; hence, separation through various chromatographic techniques like adsorption TLC, reversed phase TLC and GC-MS. In the present study GC-MS technique has been employed for studying the unsaponifiable fraction. For Mass Spectrum of Stigmasterol (Figure 2), Gamma sitosterol (Figure 3) and Campesterol (Figure 4).

**Conclusion**
In the present study by using soxhlet extraction and simple column chromatography three sterol entities identified. There are many chemical constituents are available in *A. Precatorius* seeds. However our aim was to develop rapid method to identify only sterol entities. GC-MS technique very powerful tool for developing such kind of method. After many trials method was developed and stigmasterol, campesterol and gamma sitosterol were identified. Using GC-MS Method sterols can be identified quite easily which will be used for industrial and research purpose.

**Conflict of interest statement**
Authors confirms that there no any conflict of interest.

**Reference**


