

Phytochemical and Nutritional Evaluation of *Amorphophallus campanulatus* (Roxb.) Blume Corm

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Abstract

Amorphophallus campanulatus (Roxb.) Blume (Araceae) is commonly known as Elephant foot yam. Corms are used in India in curries and pickles and are ascribed in vitiated conditions of vata and kapha, arthralgia, elephantiasis, tumours, inflammations, haemorrhages, vomiting, cough, bronchitis, asthma, anorexia, dyspepsia, flatulence, colic constipation, helminthiasis hepatopathy, splenopathy, amenorrhoea, dysmenorrhoea, seminal weakness, fatigue, anaemia and general debility. The present communication deals with the detailed pharmacognostic evaluation of the corm sample using light microscopy, WHO recommended physico-chemical determinations and phytochemical procedures. The physico-chemical, morphological and histological parameters presented in this paper may be proposed as parameters to establish the authenticity of *A. campanulatus* corms. A detailed nutritional analysis has also been carried out for quantitative evaluation of active nutrient components to determine calorific value for edible usage. HPTLC analysis showed the presence of β -sitosterol as marker compound in different extracts and fractions.

Keywords: *Amorphophallus campanulatus*; Elephant foot yam; Pharmacognosy; HPTLC; Nutritional value

Introduction

Elephant foot yam, botanically equated to *Amorphophallus campanulatus* (Araceae). The corms are dry, acrid, pungent; increases both appetite and taste; digestive, anthelmintic and aphrodisiac; useful in vitiated conditions of vata and kapha, elephantiasis, inflammations, haemorrhoids, haemorrhages, abdominal pain, asthma, piles, dysentery, splenopathy, amenorrhoea, seminal weakness, fatigue, anaemia and general debility [1,2]. The fresh corms are applied externally as an irritant to treat acute rheumatism. The corm is a hot carminative in the form of a pickle. It contains betulinic acid, β - sitosterol, stigmasterol, triacotane, lupeol, and β -sitosterol palmitate [2]. Besides these, glucose, galactose, sharp crystal of calcium oxalate, rhamnose and xylose are also present [3]. The corms are reported to possess antibacterial, antifungal and cytotoxic activities due to presence of a diterpenoid namely salviasperanol and amblyone, a triterpenoids [4,5]. The ethanol and aqueous extract of corms showed antioxidant and hepatoprotective activity (CCl₄ induced hepatic damage)

In view of its diverse medicinal applications and edible usage, the present study was undertaken to quantification of medicinally important biomarker compound and evaluation of nutritional values; this type of studies in this species is not reported so far. This study will be useful to herbal industries for successful commercial exploitation and to maintain batch to batch consistency of the raw material.

Methodology

Collection of plant material

Plant material was collected from Jaunpur (Uttar Pradesh), India in October, 2013. It was authenticated and Field Voucher No. 262534 has been deposited in herbarium, National Botanical Research Institute, Lucknow (India).

Microscopic analysis

Fresh corm pieces were preserved in 70% ethyl alcohol for histological studies. Transverse sections were cut using YSI-118 Yorco Cryostat Microtome Automatic Deluxe Model (Yorco Scientific

Industries Pvt. Ltd. Delhi, India). Sections were stained with safranin and counterstained with fast green [6]. For study of powdered elements fine powder of seeds was treated with chloral hydrate for about 10 min, followed by a gentle heating and then finally mounted in glycerine for observation. All preparations were observed under Olympus CX-31 microscope. Photomicrographs were taken using Olympus digital camera Model No. E- 420.

Physicochemical analysis

Reagents used for physico-chemical and phytochemical analysis were procured from Loba Chemie laboratory reagents and fine chemicals Mumbai, India. Physico-chemical and phytochemical studies like total ash, water soluble ash, acid insoluble ash, extractive values, sugar, starch, total phenolics, total protein, and tannins were calculated from the shade dried and powdered (60 mesh) plant material [7].

High Performance Thin Layer Chromatographic Analysis (HPTLC)

Reagents and chemicals

Standard β -sitosterol was procured from Sigma-Aldrich (Steinheim, Germany). Solvents used to prepare mobile phase solutions were of HPLC grade and purchased from Merck (Germany).

Sample preparation

Air dried (35-40°C) powdered corm (100 mesh) of *A. campanulatus*

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(5.0 g) was extracted in a Soxhlet apparatus with hexane, chloroform, acetone, alcohol and then water successively and evaporated to dryness through rotary evaporator and then lyophilized. Accurately weighted extracts of each solvents is reconstitute in its native solvents and dissolved to prepare 1 mg mL⁻¹ of solution for HPTLC studies.

Standard preparation

A stock solution of standard compounds (1 mg/ml) was prepared by dissolving 1 mg of accurately weighted standard (β -sitosterol) in 1 ml of methanol and further working solution of 100 ng /ml was prepared by adding 900 μ l HPLC grade methanol in to 100 μ l of stock solution.

Chromatography conditions

Chromatography was performed on Merck HPTLC precoated silica gel 60 GF254 (20 \times 10 cm) plates. Soxhlet fractions of samples and standard compound (β -sitosterol) of known concentrations were applied to layers as 6 mm wide bands positioned 15 mm from bottom and 10 mm from side of plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl /s from application syringe. These conditions were kept constant throughout analysis of samples.

Detection and quantification of marker compound

Following sample application, layers were developed to a distance of 80mm, with Toluene: Ethyl acetate: Formic acid, (80: 20: 2, v/v), as mobile phase, in a Camag glass twin-through chamber (20 cm \times 10cm) previously saturated with mobile phase vapor; temperature was 28°C. After removal of plates from chamber, completely dried in air at room temperature and peak areas for samples and standard were recorded by densitometry in absorbance/reflectance mode at λ max=600 nm, by means of a Camag TLC Scanner 3 with winCATS version [3.2.1] software [8,9].

Nutrient, Proximate and chemical analysis

Air dried (35-40°C) powdered corm (100 mesh) of *A. Campanulatus* were taken. The moisture contents were determined by drying the

sample at 105°C in the oven up to constant weight. Nutrient analysis was done by AAS method and crude protein value of the sample was assessed by determining the total organic nitrogen using Micro-Kjeldahl's apparatus. The crude lipids were extracted in petroleum ether at 40 to 60°C, using Soxh let apparatus, and then evaporating the solvent up to dryness by using rotary evaporator [10,11]. For the estimation of the fiber contents, the dry outcome of lipid estimation was ignited and the ash contents were determined and taken as equivalent to fibre contents [10]. Carbohydrate contents of each sample were calculated using the difference method as follows:

$$\text{Carbohydrate (\%)} = 100 - [\text{moisture (\%)} + \text{protein percentage (\%)} + \text{lipid (\%)} + \text{ash contents (\%)}]$$

Whereas, the energy values of each sample were determined using the following formula.

$$\text{K calories/100 g} = 9 (\text{crude fats (\%)} + 4 (\text{carbohydrates (\%)} + \text{proteins (\%)}).$$

Results

A brief taxonomic description of the plants

A stout herbaceous plant with underground hemispherical depressed dark brown corm; leaves compound, large, solitary, petiole stout, mottled, 60-90 cm long, leaflets 5-12.5 cm long of variable width, oblong, acute, strongly and many-nerved; male and female inflorescences contiguous, neuters absent, appendage of spadix subglobose or amorphous, equalling or longer than the fertile region, spathe campanulate, pointed, strongly, closely veined, greenish pink externally, base with purple, margins long; fruits ovoid 2-3 seeded red berries.

Microscopic characters of the corm

Corm shows a wide zone of cork consisting of 5-25 tangentially elongated, rectangular, thin-walled cells, a few inner layers containing rosette crystals of calcium oxalate, and plenty of simple and compound starch grains; ground tissue very wide consisting of thin-walled,

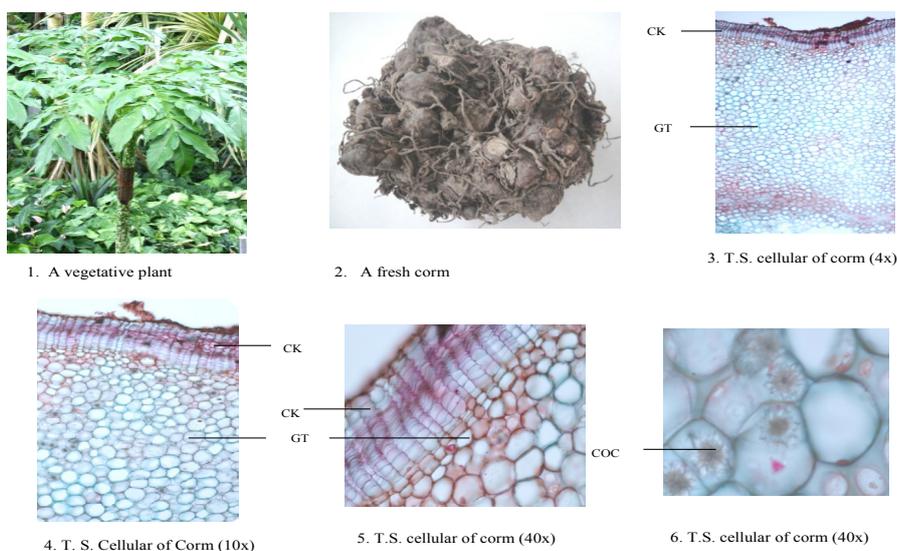


Figure 1: Macro and Microscopic characters of the corm of *Amorphophallus campanulatus* L. CK, cork; COC, calcium oxalate crystals; GT, ground tissue & SG, starch grain.

parenchymatous cells; a few cells containing both rosette and acicular crystals of calcium oxalate; starch grains both simple and compound, spherical in shape consisting of 2-4 components, measuring 3-31 μ in diameter; vascular bundles poorly developed, scattered in ground tissue; vessels arranged in groups of 2-3, having spiral thickenings; a few parenchyma cells of ground tissue containing yellowish cell contents (Figure 1).

Powder creamish-grey; shows abundant simple and compound starch grains, measuring 3-31 μ in diameter, fragments of cork cells, a few rosette and acicular crystals of calcium oxalate.

Physico-chemical studies

Soxh let extraction: Air dried material was used for quantitative determination of different physico-chemical values & primary metabolites. A known quantity of dried plant material was extracted in a Soxh let apparatus with hexane, chloroform, acetone, alcohol and then water successively. For this purpose, transfer a weighed quantity (depending on chemical content) of the air dried, crushed drug to an extraction thimble, extract with Hexane, Chloroform, Acetone, methanol (at 40°C to 60°C) and water (at 100°C) in a continuous extraction apparatus (Soxh let extractor) for 6 hours for each solvent. Filter the extract quantitatively into a evaporating dish and evaporate off the solvent on a water bath. Dry the residue at 105°C to constant weight and calculated the extract amount for each solvent (Figure 2).

Determination of total ash: Incinerate about 2 to 3 g accurately

weighed, of the ground drug in a tared platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cool and weigh. If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, incinerate the residue and filter paper, add the filtrate, evaporate to dryness, and ignite at a temperature not exceeding 450°C. Calculate the percentage of ash with reference to the air-dried drug (Figure 3).

Determination of acid insoluble ash: Boil the Total Ash obtained above for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug (Figure 3).

Determination of extractive values: Macerate 5 g of the air dried drug, coarsely powdered in three conical flask, and added 100 ml of Hexane, Alcohol and water in each closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug and calculated the extractive values of each extract (Figure 3).

Total phenolics content, tannin, sugar, starch, total protein [12] is found in plant sample in various concentrations (Figure 4).

Nutrient and chemical analysis: The concentration of N, P, K, Mg,

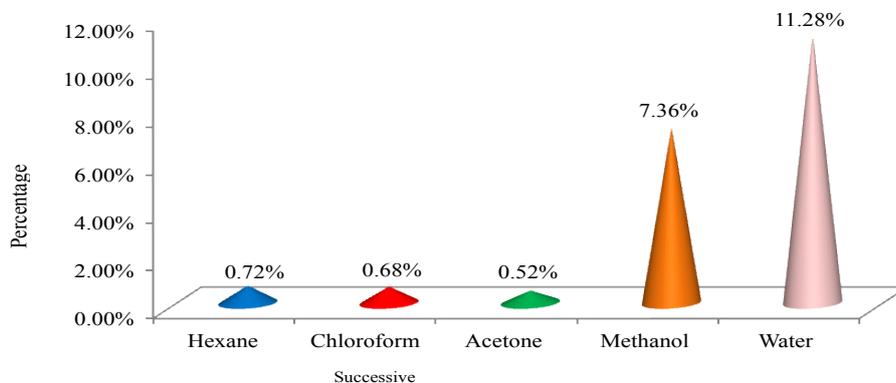


Figure 2: Successive Soxhlet extractive values of *A. campanulatus* corm.

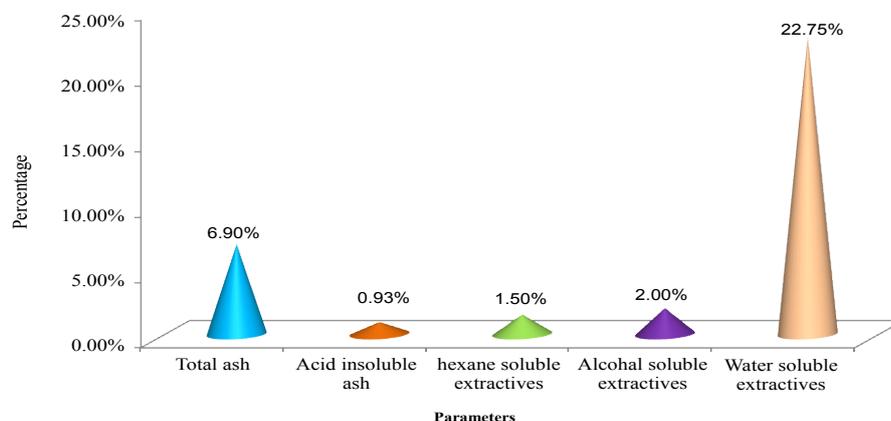


Figure 3: Quantitative physico-chemical analysis of *A. campanulatus* corm.

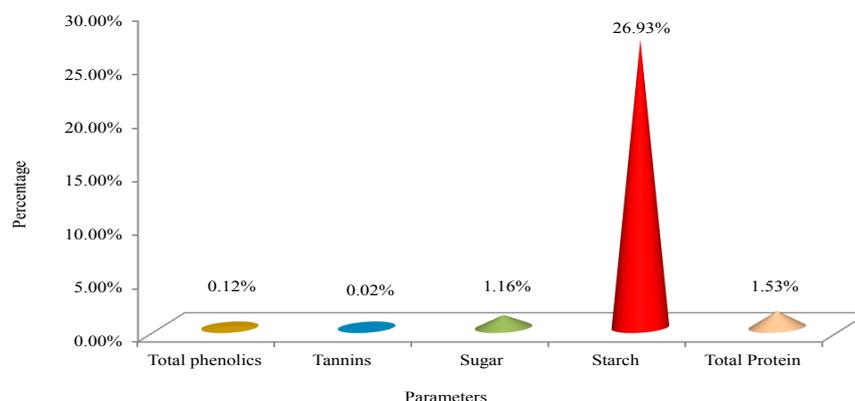


Figure 4: Quantitative estimation of metabolites of *A. campanulatus*.

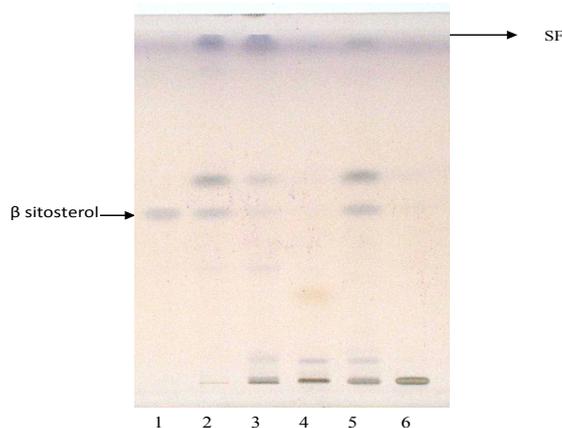


Figure 5: HPTLC Profile of *A. campanulatus* samples along with β -sitosterol as standard under 600nm 1, β -sitosterol; 2, Hexane fraction; 3, Chloroform fraction; 4, Methanol fraction; 5, Acetone fraction; 6, Water fraction.

Parameter	Result
Nitrogen ($\mu\text{g/g}$)	2.24 ± 0.10
Phosphorus ($\mu\text{g/g}$)	4.53 ± 0.07
Potassium ($\mu\text{g/g}$)	38.1 ± 0.14
Magnesium ($\mu\text{g/g}$)	119.2 ± 9.3
Zinc ($\mu\text{g/g}$)	23.1 ± 1.1
Chromium ($\mu\text{g/g}$)	8.9 ± 1.3
Copper ($\mu\text{g/g}$)	32.6 ± 8.1
Iron ($\mu\text{g/g}$)	340.2 ± 21.3
Manganese ($\mu\text{g/g}$)	6.6 ± 1.0

Table 1: Nutrient analysis of *A. campanulatus*.

Constituents	Value (%)	RSD (%)
Moisture	7.3	1.118
Ash	6.9	1.183
Protein	11.53	0.987
Fat	3.52	0.864
Carbohydrate	70.75	1.085
Crude fibre	14.32	1.098
Energy (kcal/100 gm)	359.08	1.207

Table 2: Proximate and chemical analysis of *Amorphophallus campanulatus* (Roxb.) Blume corm.

Zn, Cd, Cr, Cu, Fe, Mn in plants are appended (Table 1) and Proximate and chemical analysis result of *Amorphophallus campanulatus* is tabulated in Table 2.

Quantification of standard compound in plant sample: HPTLC method was subsequently applied for the analysis and quantification of β -sitosterol in different extract of plant sample were observed at R_f 0.54 in standard track (Figure 5) as well as at same R_f in sample tracks (Figures 6 and 7). It was analyzed that free β -sitosterol content in the plant samples were found in range 0.013% to 0.096% in various solvents in plant sample (Figure 8).

Discussion

From the above studies corm can easily be identified on the basis of macroscopic characters like scars on external surface and creamish white internal portion. Taste of corm is quite characteristic and is acrid. On microscopical examination rosette and acicular crystals of calcium oxalate, and plenty of simple and compound starch grains containing cells are observed in the corm.

Physicochemical values viz. total ash, acid insoluble ash, alcohol and water-soluble extractives are observed. The total ash (6.9%) and acid insoluble ash (0.93%) are considered to be an important and useful parameter for detecting the presence of inorganic substances

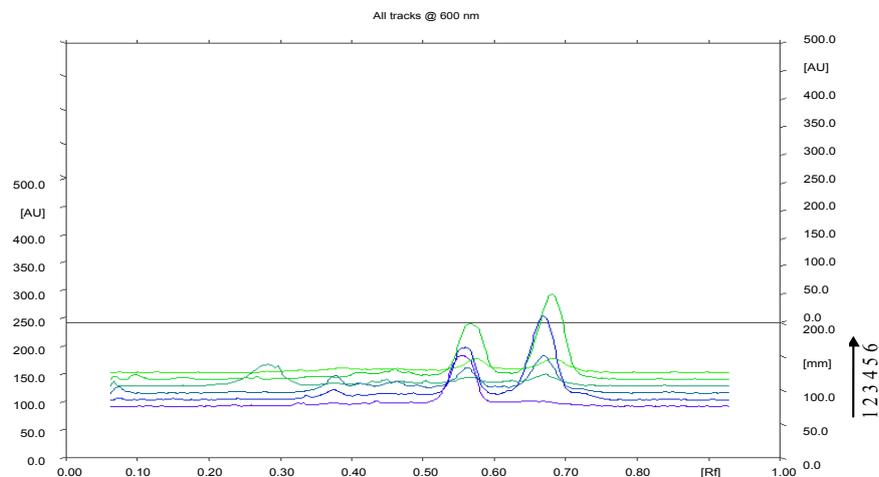


Figure 6: Three dimensional chromatogram of *A. campanulatus* samples (2-6) along with standard.

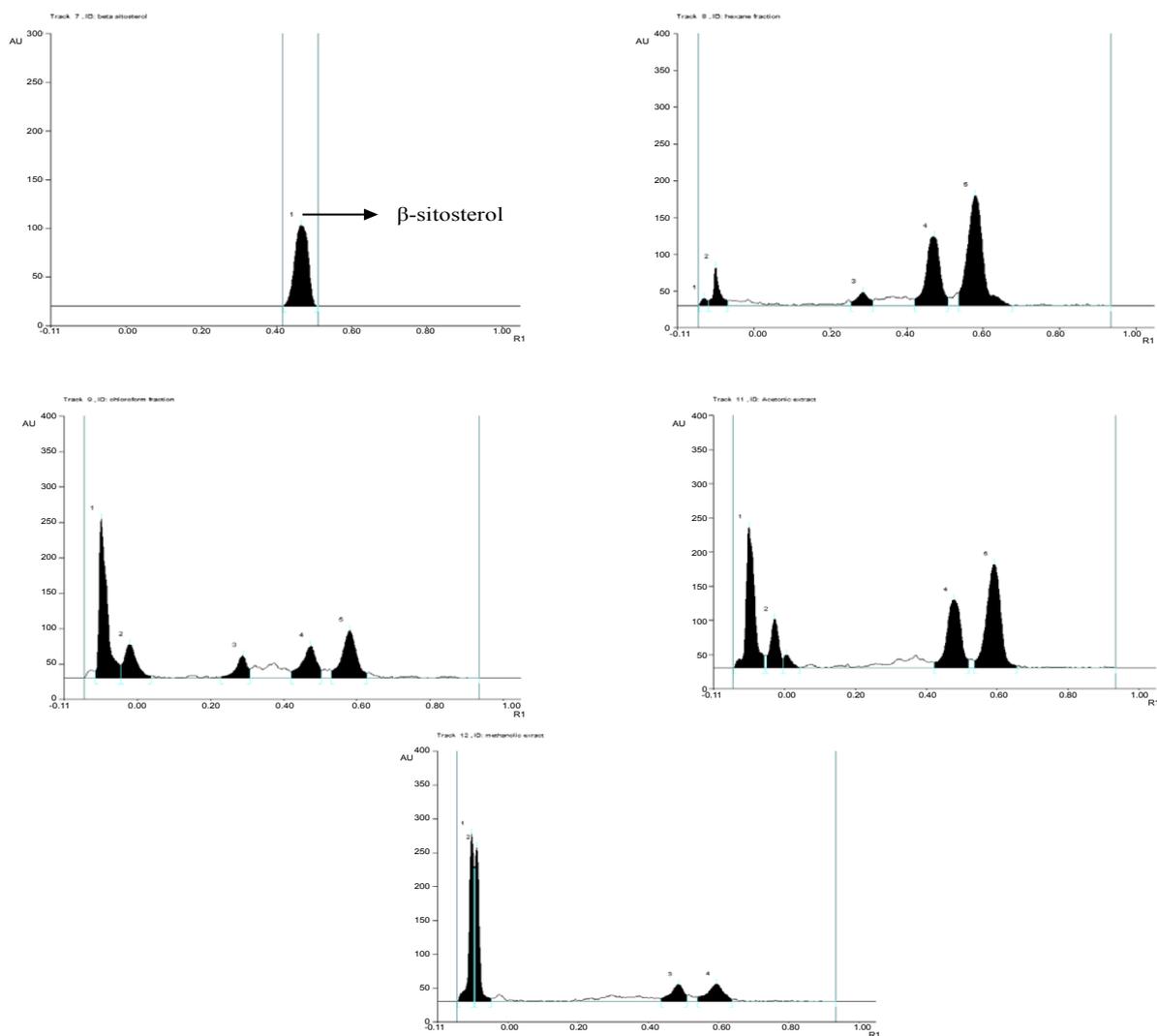


Figure 7: HPTLC densitometric scan (at 600 nm) of *A. campanulatus* samples with reference compound β -sitosterol.

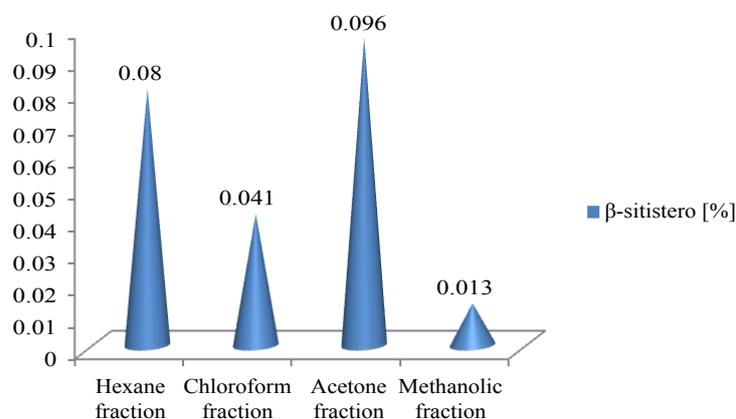


Figure 8: Quantitative analysis of compound (β -Sitosterol) in *A. campanulatus* plant sample.

like silicate ion. Similarly the hexane (1.5%), alcohol (2.0%) and water-soluble extractives (22.7%) are indicators of the total solvent soluble components. On quantitative estimation of primary metabolite showed the presence of total phenolics (0.012%), tannins (0.02%), sugar (1.16%), starch (26.93%) and total protein (1.53%). The proximate analysis showed the percentage moisture content, ash content, crude protein, crude fibre, crude fat and carbohydrate of the *A. campanulatus* corm as 7.30, 6.90, 11.53, 14.32, 3.52 and 70.75% respectively while its calorific value is 359.08 Kcal/100 g (Table 2). This shows its high nutritional significance as food supplement.

Above study on this species may be useful to industries for the commercial exploitation with pharmaceutical and nutraceutical attributes to herbal formulations. Nutrient analysis also suggests that this medicinal plant can be used for human consumption or for preparation of herbal products and standardized extracts.

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