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Research Paper

QUANTITATIVE ESTIMATION OF PHYTOSTEROL FROM TWO MEDICINALLY IMPORTANT PLANTS OF CUCURBITACEAE

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Two medicinal plants of the family Cucurbitaceae viz. *Luffa Cylindrica* Linn. and *Citrullus colocynthis* Linn. were investigated for their phytosterol contents. Three sterols, β-sitosterol, stigmasterol, campesterol have been isolated from parts of plants and callus culture of *L. Cylindrica* and *C. colocynthis.* In both the plants quantitative data revealed that sterols content was found to be higher in leaf of *C. colocynthis* and lower in stem of *L. cylindrica. In vitro* studies showed maximum growth indices in both the plants. Amount of sterols in 6 weeks old calli of *C. colocynthis* and minimum in 2 weeks old calli of *L. cylindrica.*

Keywords: *Luffa cylindrica*, *Citrullus colocynthis,* β-sitosterol, Stigmasterol, Campesterol

INTRODUCTION

Cucurbitacece is a large family of plants possessing medicinal properties. *Luffa cylindrica* Linn. and *Citrullus colocynthis* Linn**.** are two important plants of this family, which have not been much investigated for their phytosterols though considerable progress has been achieved regarding the biological activity and medicinal application on their plants.

Sterols are major plasma membrane components of most eukaryotic membranes, although their priecise structure differs between the kingdoms; animals contain cholesterol, and plants have β-sitosterol, campesterol, and stigmasterol, whereas fungi use mainly ergosterol. As sterols appear only in eukaryotes, it is likely that they confer properties to membranes or provide function that may not be required in prokaryotes and possibly not even in all eukaryotes. Cholesterol has been shown to modulate membrane thickness in artificial membranes, and this property has been proposed to play a role in membrane protein localization *in vivo*. Sterols are the starting material for the biosynthesis of plant steroids (Heftman, 1971). A rapid method for quantification of sterols after thin layer chromatography has also been established (Sharma and Sarin, 2012).

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Sterols have been isolated from large number of plant species and probably occur in all angiosperms and gymnosperms. The common phytosterols reported from plants are b-sitosterol, stigmasterol and campesterol.

MATERIALS AND METHODS

Collection of Plant Materials and Extraction Procedure

Luffa cylindrica Linn. and *Citrullus colocynthis* Linn. were collected from campus of University of Rajasthan, Jaipur.

Tissue Culture

Unorganized static cultures of *Luffa Cylindrica* Linn. and *Citrullus colocynthis* Linn were established from their seeds, leaves and nodal segments on Murashige and Skoog's (1962) medium supplemented with 3% *(w/v)* sucrose , 0.8% (*w/v*) agar and various growth hormones. Callus initiation took place after 12-18 days of inoculation in both the plants. The callus cultures thus obtained were maintained on MS medium for 12 months by subculturing the tissue in to fresh MS-medium with same growth hormone concentration at 3-4 weeks intervals. At different time intervals of subculturing growth indices of both plants calli were calculated. Three replicates of each of the tissue samples were examined and their mean values were calculated.

The callus was harvested at different time intervals of 2, 4, 6, 8 and 10 weeks of subculturings, dried at 60°C and Growth Indices (GI) were calculated, separately. The dried samples were subjected to extraction of sterols.

The collected plant parts (leaf and stem) of both the plants were also shade dried and finely powdered. Different plant parts were extracted with constant agitation for 48 h. The extracts were filtered using Whatman filter paper No. 1 and then concentrated *in vacuo* at 40°C using a Rotary evaporator and stored at 4°C (Harborn, 1984 and Harborn and Harborn, 1998).

Extraction

Dried and powdered plant test materials were defatted in petroleum ether (60-80°C) for 24 h on a water bath. Defatted material was air-dried and hydrolyzed in 30% HCl (v/v) for 4 h. Each hydrolyzed sample was washed with water till pH 7 was obtained and dried. The dried preparation was again extracted with benzene for 24 h. The extract was filtered and dried *in vacuo*. The crude extract was dissolved in chloroform before chromatographic examination (Kaul and Staba, 1968).

Thin Layer Chromatography (TLC)

Glass plates coated with silica gel-G as described above were used. Each of the extract was co-chromatographed separately with authentic sterols (β-sitosterol and stigmasterol) standard. These plates were developed in an air-tight chromatographic chamber, saturated with solvent mixture (hexane: acetone: 8:2; and Other solvent systems such as benzene and ethyl acetate (85:15; Heble *et al*, 1968) benzene : ethyl acetate (3:1, Kaul and Staba, 1968) were also used but hexane acetone (8:2) gave better separation.

These plates were air-dried and visualized under UV light and fluorescent spots corresponding to that of standard markers were marked. These developed plates were sprayed with 50% H_2SO_4 and anisaldehyde reagent, separately and heated at 110°C for 10 min.

Identification

Melting point and IR spectra of each of the isolated compound was taken and a comparison of the TLC color reaction was made, which was found

to be in accordance with those reported for authentic compounds.

RESULTS AND DISCUSSION

In the present investigation ,three sterols viz., β-sitostirol, Stigmasterol, and Campesterol were isolated and identified from various plant parts and calli of *Luffa cylindrica and Citrullus colocynthis* which were confirmed on the basis of their Rf value, TLC behavior, color, melting point, UV, IR and spectral studies (Table 1).

In *L. Cylindrica and C. colocynthis* extracts, when the TLC plates were visualized under UV lamp three of the spots gave characteristic fluorescence and their Rf values were comparable of their respective standard compounds. β-sitosterol (Rf value: 0.92, color: pinkish grey; Stigmasterol (Rf value : 0.88, color: grayish violet) and Campesterol (Rf value 0.76, color dark purple) were isolated and identified from various plant parts and calli of both plants. Melting points (β-sitosterol 135-136°C, Stigmasterol 140-142°C and Cempesterol 137-138°C) were also measured and compared with authentic standards compounds. IR spectral peaks of three isolated sterols from *L. Cylindrica and C. colocynthis* were found to be super imposable with those of their respective standard.

Maximum growth indices were also observed in six weeks old tissue (Tables 3 and 5).

The result obtained in both the plants of Curcurbitaceae are as follows:

Luffa cylindrica: In *L. cylindrica* plant part maximum amount of total sterol (b-sitosterol, stigmasterol and cempesterol) was found in the leaves (1.55 mg/g.dw) in comparison to stem (1.38 mg/g.dw) (Table 2).

In calli total amount of sterols was found to be higher in 6 weeks old callus (2.69 mg/g.dw) while minimum in 2 weeks old callus culture (0.25 mg/g.dw) (Table 3)

Citrullus colocynthis: In *C. colocynthis* plant part maximum amount of total sterol (β-sitosterol, stigmasterol and campesterol) was found in the leaves (2.25 mg/g.dw) and in comparison to stem (1.71 mg/g.dw; Table 4).

In calli total amount of sterols was found to be higher in 6 weeks old callus (2.83 mg/g.dw) while minimum in 2 weeks old callus culture (0.34/g.dw) (Table 5).

Table 1: Chromatographic Behavior and Chemical Characteristics of Isolated Sterols from *L. Cylindrica and C. colocynthis*

Isolated Compounds	Chemical Formula	Rf Value	Colour After Spray	Melting Point °C
β-sitosterol	$C_{29}H_{50}O$	0.92	Pinkish grey	135-136°C
Stigmasterol	$C_{29}H_{48}O$	0.88	Grayish violet	140-142°C
Campesterol	$C_{28}H_{48}O$	0.76	Dark purple	137-138°C

Table 2: Yield of Sterols Isolated (mg/g.dw) from Various Plant Parts of *L. cylindrica*

Plant Parts	β-sitosterol	Stigmasterol	Campesterol	Total(mg/g.dw)
Leaf	0.51	0.55	0.49	**1.55**
Stem	0.47	0.50	0.41	1.38

Table 3: Yield of Sterols Isolated (mg/g.dw) from *In Vitro* Cultures of *L. cylindrica*				
Age of tissue (Weeks)	**β-sitosterol**	**Stigmasterol**	**Campesterol**	**Total(mg/g.dw)**
2	0.07	0.13	0.05	0.25
4	0.22	0.27	0.16	0.65
6	0.45	0.52	0.32	1.24
8	0.32	0.38	0.25	0.98

Table 4: Yield of Sterols Isolated (mg/g.dw) from Various Plant Parts of *C. colocynthis*				
Plant Parts	**β-sitosterol**	**Stigmasterol**	**Campesterol**	**Total(mg/g.dw)**
Leaf	0.80	0.75	0.70	2.25
Stem	0.70	0.53	0.48	1.71

Table 5: Yield of Sterols Isolated (mg/g.dw) from *In Vitro* Cultures of *C. colocynthis*				
Age of tissue (Weeks)	**β-sitosterol**	**Stigmasterol**	**Campesterol**	**Total(mg/g.dw)**
2	0.14	0.12	0.08	0.34
4	0.38	0.23	0.20	0.81
6	0.98	1.10	0.75	2.83
8	0.78	0.85	0.65	2.28

CONCLUSION

Quantitative data revealed that maximum amount of sterols was observed in leaves of *C. colocynthis* and minimum in stem of *L. Cylindrica*. Among the two plant parts, leaves contained higher percentage of sterols as compared to stems. Total and individual amount of sterol was found to be higher in 6 weeks old tissue.

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