



**QUANTITATIVE ESTIMATION OF β SITOSTEROL AND STIGMASTEROL
IN *ASPARAGUS .RACEMOSUS* , AND, *TINOSPORA CORDIFOLIA***

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ABSTRACT

Ethanobotinacal & Phytopharmaceutical studies showed that the plants have good medicinal value for the tribes of Rajasthan. The primary screening of primary metabolites showed that the metabolite content were high in these plants. The amount of Quantitative data revealed that in *A.racemosus* the maximum amount of total sterols (β -sitosterol and Stigmasterol) in seeds (12.90 mg/gdw) and minimum in roots (7.82 mg/gdw) .In *T.cordifolia* the maximum amount of total sterols (β -sitosterol, stigmasterol) was observed in seeds (15.18 mg/gdw) and minimum was found in Stem (6.57 mg/gdw).



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INTRODUCTION

Plants are biochemical factories and have been component of many phytomedicines. 800 plants are known to be used in Ayurveda and 750 plants are being used in the system of folk medicines, some of these plants exhibit wider acceptability and used in Ayurveda, Siddha, Tebetan, Unani, Homoeopathy and folk medicines. Preliminary phytochemical screening for the presence of primary metabolites alkaloids, Flavonoids, steroids and terpenoids was carried out. The result for alkaloids (30.43%), Flavonoids (47.82%), steroids (65.21%) and terpenoids (43.47%) (Verma et.al.2011)

MATERIALS AND METHODS

1. Collection of plant materials and extraction procedure

Asparagus racemosus, and, *Tinospora cordifolia* were collected (July-August, 2011) from botanical garden of University of Rajasthan Jaipur (Rajasthan). The collected plants were shade dried and finely powdered. Different plant was extracted with constant agitation for 48 hrs. 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, Harborn and Harborn, 1998)

2. Extraction

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

3. 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% H₂SO₄ and anisaldehyde reagent, separately and heated at 110°C for 10 min.

4. Identification

Melting point and IR spectra of each of the isolated compound was taken and a comparison of the TLC colour reaction was made, which was found to be in accordance with those reported for authentic compounds.

RESULTS AND DISCUSSION

when both the *Asparagus racemosus*, and, *Tinospora cordifolia* plates were visualized under UV lamp two of the spots gave characteristic fluorescence and their R_f values were comparable to their respective standard compounds. (β -sitosterol - pinkish grey, R_f 0.90; Stigmasterol - greyish violet, R_f 0.83). The characteristic colours were also developed when TLC plates were sprayed with anisaldehyde reagent (β -sitosterol pink; Stigmasterol - greyish violet) and with 50% sulphuric acid (β -sitosterol - pink; Stigmasterol - greyish violet) corresponding to their authentic standard compounds. Melting points (β -sitosterol 135-136°C Stigmasterol 131-132°C) were also measured and compared with authentic standard compounds. IR spectra and authentic sample standard. Quantitative data revealed that in *A.racemosus* the maximum amount of total sterols (β -sitosterol and Stigmasterol) in seeds (12.90 mg/gdw) and minimum in roots (7.82 mg/gdw) (Table.1, Fig.1) In *T.cordifolia* the maximum

amount of total sterols (β -sitosterol, mg/gdw) and minimum was found in Stem (6.57 mg/gdw) was observed in seeds (15.18 mg/gdw) (Table.2 , Fig.2).

Table1
Total Sterols content (mg/gdw) in various plant parts of *A.racemosus*

S. No.	Plant parts	β -sitosterol (mg/gdw)	Stigmasterol (mg/gdw)	Total Sterol (β -sitosterol+ Stigmasterol) content (mg/gdw)
1	Leaves	07.43	4.22	11.65
2	Stem	06.71	03.52	10.19
3	Seed	8.23	4.67	12.90
4	Roots	04.92	2.90	7.82

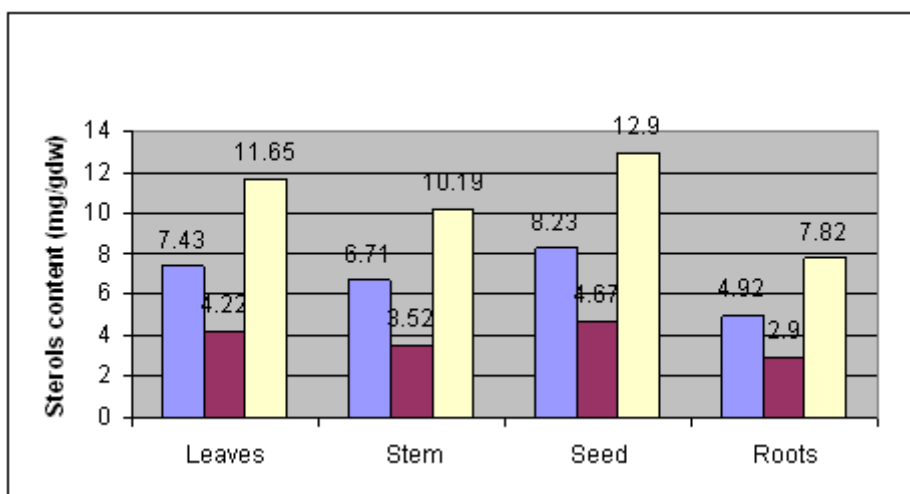


Figure 1
Total Sterols content (mg/gdw) in various plant parts of *A.racemosus*

Table 2
Total Sterols content (mg/gdw) in various plant parts of *T.cordifolia*

S. No.	Plant parts	β -sitosterol (mg/gdw)	Stigmasterol (mg/gdw)	Total Sterols (β -sitosterol + Stigmasterol) content (mg/gdw)
1	Leaf	05.67	03.79	9.46
2	Stem	04.23	02.34	6.57
3	Seeds	09.40	05.78	15.18

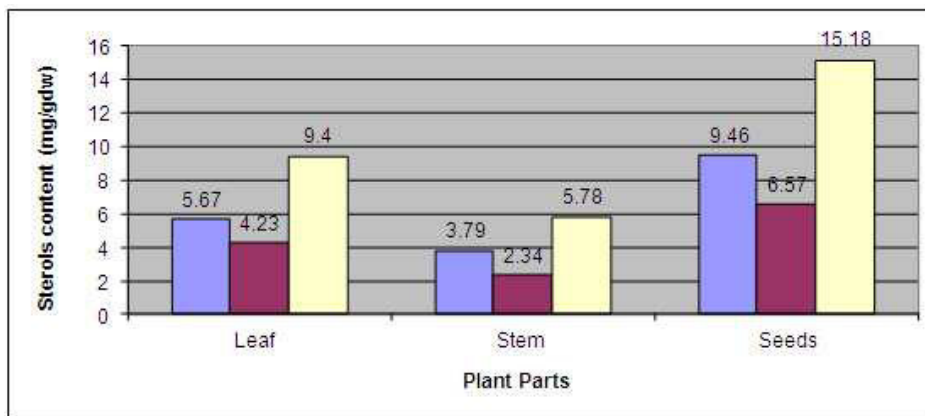


Table 2
Total Sterols content (mg/gdw) in various plant parts of *T.cordifolia*

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