

QUANTITATIVE ESTIMATION OF β -SITOSTEROL AND STIGMASTEROL IN VIVO AND IN VITRO TERMINALIA CHEBULA RITZ

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ABSTRACT

In all the plant parts contain high amount of sterols the quantitative data revealed that in *Terminalia chebula* the total content sterols (β -sitosterol and Stigmasterol) in seed (22.12 mg/gdw) and minimum in stem (15.82 mg/gdw). The *in vitro* studies shows that the highest amount of sterols was found at the age of 6 week old cultures (27.22mg/gdw) and the lowest amount was found in the 8 week old tissue (21.69mg/gdw)

KEYWORDS: *T. chebula*, *in vitro*, β -sitosterol, stigmasterol,

INTRODUCTION

Terminalia chebula (Family: Combretaceae) was one of the traditional medicine used in many folkclaims and it is called as "King of medicine". It is an middle-sized tree leaves are ovate, or elliptic, flowers are yellowish white, fruits are yellowish brown in colour distributed through out in India^{1,2}. *Terminalia chebula* contains tannin, chebulic acid, glycosides, sugar, triterpenoids, steroids and small quantity of phosphoric acid. The pharmacological activities previously reported are Antibacterial, Antifungal, Antiviral, Anticarcinogenic³.

MATERIALS AND METHODS

Collection of plant material

Terminalia chebula plants were collected (July-August, 2008) from state forest nursery Jabalpur Madhyapradesh Plant was identified by comparing with those available in the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India. The collected plants were shade dried and finely powdered. Different plant was 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.^{4,5}

Extraction

Dried and powdered plant test materials were defatted in petroleum ether (60-80°C) for 24 hr 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 examination⁶.

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; benzene : ethyl acetate (3 : 1) were also used but hexane acetone (8 : 2) gave better separation.⁷

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.

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

The T.L.C plate of *T. chebula* extracts, 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^oC Stigmasterol 131-132^oC) were also measured and compared with authentic standard compounds. IR spectra and authentic sample standard Quantitative data revealed that in *T. chebula* the maximum amount of total sterols (β -sitosterol and Stigmasterol) in seed (22.12 mg/gdw) and minimum in stem (15.82 mg/gdw) (Table.1). The *in vitro* studies shows that the highest amount of sterols was found at the age of 6 week old cultures (27.22mg/gdw) and the lowest amount was found in the 8 week old tissue (21.69mg/gdw) (Table.2)

However, till date there was no report on the presence of sterols from *T. chebula in vivo* and *in vitro*. In the present

study β -sitosterol and Stigmasterol have been confirmed in *T. chebula*. of family Combretaceae

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Table 1: Total Sterols content (mg/gdw) in various plant parts of *T. Chebula*

S. No.	Plant parts	β -sitosterol (mg/gdw)	Stigmasterol (mg/gdw)	Total Sterol (β -sitosterol + Stigmasterol) content (mg/gdw)
1	Leaf	12.43	09.22	21.65
2	Seed	11.82	10.30	22.12
3	fruit	09.71	07.52	17.23
4	Stem	08.92	6.90	15.82

Table 2: Growth indices and total sterol content *in vitro* (β -sitosterol + Stigmasterol) (mg/gdw) in various plant parts of *T. Chebula*

S. No	Age of tissue in weeks	GI	β -sitosterol (mg/gdw)	Stigmasterol (mg/gdw)	Total Sterol (β -sitosterol + Stigmasterol) content (mg/gdw)
1	2	0.22	13.56	8.42	21.98
2	4	0.54	14.58	9.53	24.11
3	6	1.10	16.57	10.65	27.22
4	8	0.94	15.38	8.31	21.69

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