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Quantitative estimation of β-sitosterol and stigmasterol in *Gloriosa superba* L. and *Urginea indica* (Roxb.) Kunth

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The present study was done to reveal the quantitative content of total sterols in various plant parts of *Gloriosa superba* and *Urginea indica.* In *G. superba* the maximum amount of total sterols (β-sitosterol and stigmasterol) was observed in flowers (22.12 mg/gdw) and minimum in rhizome (15.82 mg/gdw). In *U. indica* the maximum amount of total sterols (β-sitosterol, stigmasterol) was observed in leaf (23.46 mg/gdw) and minimum was found in bulb (18.18 mg/gdw).

Key words: *Gloriosa superba, Urginea indica,* β-sitosterol, stigmasterol.

INTRODUCTION

Gloriosa superba L. (commonly called Glory lily) is a member of the family Liliaceae. The plant is used to treat acute attacks of gout and familial Mediterranean fever. Colchicine is the main alkaloid found in *G. superb* (Shivakumar, 2003). *Urginea indica* (Roxb.) Kunth (commonly called Indian Squill) also belongs to the family Liliaceae. Traditionally it is used to treat asthma, bronchitis, rheumatism and leukemia (Duke, 1992). Proscillaredin, scillarenin and scillarens are the major constituents of *U. indica* (Duke, 1992).

Sterols have been isolated from large number of plant species and probably occur in all angiosperms and gymnosperms. 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 (Davidson and Banerjee, 1980). The common phytosterols reported from plants are β-sitosterol, stigmasterol and campesterol.

MATERIALS AND METHODS

Collection of plant materials and extraction procedure

G. superba L. and *U. indica* was collected during July and August, 2009, from botanical garden of Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh). The collected plant was 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 (Harborn, 1984; Harborn and Harborn, 1998).

Extraction

Dried and powdered plant test materials were first defatted in petroleum ether (60 to 80°C) for 24 h in 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

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Table 1. Total sterols content (mg/gdw) in various plant parts of *G. superb*.

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	Flower	11.82	10.30	22.12
3	Stem	09.71	07.52	17.23
4	Rhizome	08.92	6.90	15.82

Table 2. Total sterols content *(*mg/gdw) in various plant parts of *U. indica.*

S/No.	Plant parts	β-sitosterol (mg/gdw)	Stigmasterol (mg/gdw)	Total sterols (β-sitosterol + stigmasterol) content (mg/gdw)
1	Leaf	12.67	10.79	23.46
2	Bulb	09.40	8.78	18.18

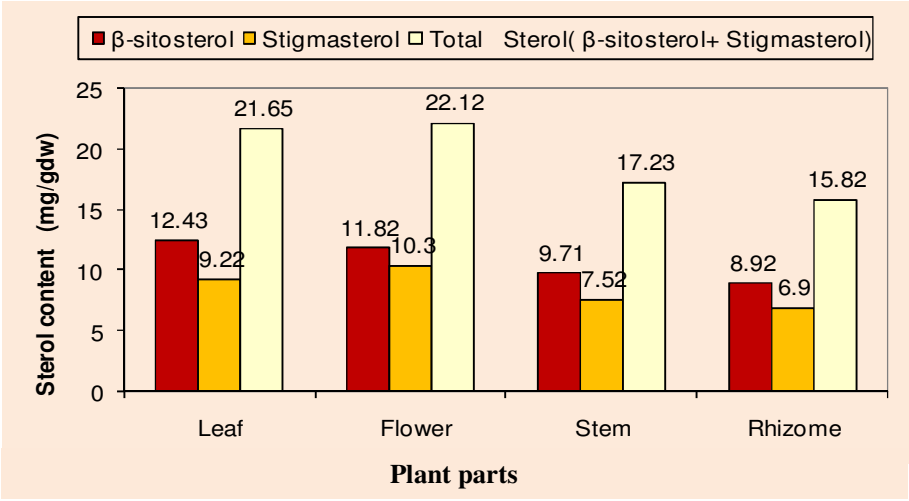

Figure 1. Total sterol content (β-sitosterol + stigmasterol) (mg/gdw) in various plant parts of *G. superba.*

examination (Kaul and Staba, 1968).

Thin layer chromatography (TLC)

Glass plates coated with silica gel-G as described 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) was also used but hexane: acetone (8:2) gave better separation (Heble et al., 1968; Kaul and Staba, 1968). These plates were air-dried and visualized under ultra violet (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 Infrared spectroscopy (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.

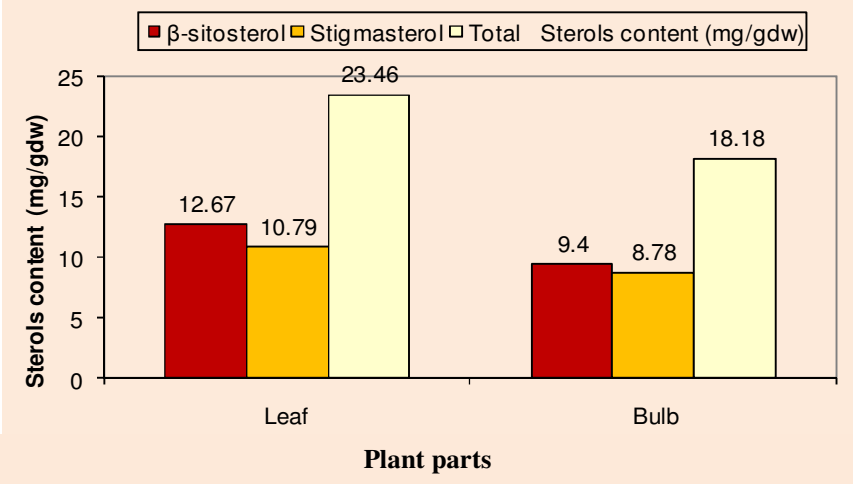

Figure 2. Total sterol content (β-sitosterol+ Stigmasterol) (mg/gdw) in various plant parts of *U. indica*.

RESULTS

Production of sterols from their structure, composition, effects, biochemistry, identification and their function has also been reported in several plants since last few years. In the present study, quantitative estimation of β-sitosterol and stigmasterol was done on two members of Liliaceae family G*. superba* and *U. indica*. The results indicated that maximum β-sitosterol and stigmasterol content was present in *U. indica* as compared to *G. superba.* Out of leaf and bulb of *U. indica*, maximum content was found in leaf followed by bulb. While in *G. superb*, maximum content of β-sitosterol and stigmasterol was found in flower (22.12 mg/gdw) followed by leaf (21.65 mg/gdw), stem (17.23 mg/gdw) and rhizome (15.82 mg/gdw).

DISCUSSION

In both *G. superba* and *U. indica* extracts, when the plates were visualized under UV lamp, two of the spots gave characteristic fluorescence and their Rf values were comparable to their respective standard compounds (β-sitosterol - pinkish grey, Rf: 0.90; Stigmasterol - greyish violet, Rf: 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 to 136°C, Stigmasterol 131 to 132°C) were also measured and compared with authentic standard compounds. IR spectra and authentic sample standard quantitative data revealed that in *G. superba* maximum amount of total sterols (β-sitosterol and stigmasterol) was present in flowers (22.12 mg/gdw) and minimum in rhizome (15.82 mg/gdw) (Table 1 and Figure 1). In *U. indica*, the maximum amount of total sterols (β-sitosterol, stigmasterol) was observed in leaf (23.46 mg/gdw) and minimum was found in bulb (18.18 mg/gdw) (Table 2 and Figure 2). However, till date, there have been no reports on the presence of sterols from *G. superba* and *U. indica*. In the present study, β-sitosterol and stigmasterol have been confirmed in two plant species, namely *G. superba* and *U. indica* of family Liliaceae.

Conclusion

In the present study, presence of stigmasterol and β-sitosterol in *G. superba* and *U. indica* and its quantitative estimation was reported. The maximum content was found in leaf of *U. indica* (23.46 mg/gdw) followed by flower *of G. superba* (22.12 mg/gdw) as compared to other plant parts.

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