

SOLANUM SPECIES AS A SOURCE OF STEROIDAL DRUGS

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Method for the quantitative estimation of steroidal alkaloids especially those from solanum species has been described. Solasodin contents of six species of solanum in leaves fruits and seeds of the plant are reported.

INTRODUCTION

Plant steroids are the main sources of corticosteroidal drugs of which the most important plants belong to genus *Dioscorea*. World requirement of these steroids are mostly met from Mexico, Central America and to a small extent from China and India.

Most of the production of the steroidal hormones depend on wild plants. *D. deltoidea*, *D. composita* and *D. floribunda* are the most common varieties available for the preparation of diosgenin. In Pakistan, *D. deltoidea* is the main variety commercially available. The percentage of diosgenin in *D. deltoidea* is 3 to 4%. *Dioscorea* species are slow growing and takes about 3 to 4 years to mature. Another plant which is still not commercially used is the seed of *Trigonella foenumgraecum* which also contains upto 1.14% [1] diosgenin. Its crop takes six months to mature. Soybean is the cultivated plant used for the manufacture of stigmaterol as a by-product of refined oil. However, the manufacturing process starting from stigmaterol is complicated and more expensive than that of diosgenin. The other source of sapogenin is the hecogenin obtained from various species of *Agave*. This sapogenin can be obtained as a by-product of fiber manufacturing industry from *Agave* species.

The rise in demand for steroidal drugs that has occurred in recent years has accelerated the development of alternative sources of raw material. India developed a new source of diosgenin obtained from *Costus speciosus* [2]. It contain 2.12% pure diosgenin. It has been calculated that the cultivation of *Costus speciosus* is much economical as compared to *Dioscorea*. *Kallstroemia pubescens* [3] which is a tropical American weed, contains 0.91–1.71% diosgenin in whole plant and 1.78–1.92% in the leaves. This weed belong to the family *zygophyllaceae* and has been introduced in India. *Balanites orbicularis* fruit contain 1.1% of a mixture of diosgenin and yamogenin [4].

The most promising plants which can compete with *Dioscorea* are the *Solanum* species, which contains glycoalkaloids, the solasonine and solamargine which differ only

in their sugar components. The common aglycon is solasodine a nitrogen analogue of diosgenin. Solasodine can be used generally like diosgenin for the preparation of corticosteroid. Various species of solanum have been analysed in order to select the best variety, giving maximum yield of glycoalkaloids. *Solanum aviculare* is a common variety introduced in some countries including India. The cultivation is also being tried in Pakistan. It gives three crops of the leaves in a year and the solasodine content is 0.74% on dry basis [5]. Another promising plant is *S. khasianum* of which solasodine content is 5.4% in mature berries and 2% in mucilage around the seed and 0.7% in leaves [6, 7]. Glycoalkaloids varies from place to place. Berries of *Solanum khasianum* from Madras, Assam and Nafa contains 5.4, 3.2 and 2.1% of glycoalkaloids respectively. *Solanum laciniatum* is growing on a large area in Russia, Australia and Newzealand. It contain 0.88% of solasodine in leaves [8]. In *S. laciniatum*, *S. aviculare* and *S. khasianum* the glycoalkaloids are concentrated in fruit, leaves and roots, whereas in other solanum species the active components are concentrated in fruit only. The solanum species which are being cultivated on commercial scale in different countries are given in Table 1.

Table 1.

Name of plant	Country	Yield kg/ha
1. <i>S. auriculatum</i>	Germany	20.0
2. <i>S. aviculare</i>	India	21.1
3. <i>S. laciniatum</i>	Hungary	20.0
4. <i>S. marginatum</i>	Eucador	32.5
5. <i>S. mammosum</i>	Puerto Rico	24.1

In view of the importance of solasodine, some wild species of solanum as well as *Solanum khasianum*, *S.*

mammosum and *S. aviculare* being cultivated on experimental scale at Pakistan Forest Institute, Peshawar were analysed for solasodine content.

QUANTITATIVE ESTIMATION OF STEROIDAL ALKALOIDS

Estimation was carried out according to the method of Birner [9].

Powdered plant sample (100 mg) is soxhlet extracted with ethyl alcohol (95 %), volume made to 50 ml. Five ml of this solution transferred to a test tube and solvent completely removed on a water bath. The residue is treated with 3 ml of 1N HCl and hydrolysed for two hrs on a boiling water bath. The resulting solution neutralized with 3 ml in 1N sodium hydroxide. Two ml conc. acetic acid added and volume is made 10 ml with H₂O. One ml of this solution is equivalent to 1 mg of dry material.

Method for the estimation of fresh berries is the same as that for leaves with minor differences. 100 g of fresh berries were homogenised in 100 ml 2 % acetic acid. Pulp thus obtained further diluted with 400 ml 2 % acetic acid, shaken and centrifuged. From the supernatant layer one tenth of the volume was heated to boiling and alkaloid precipitated by 1–2 drops of ammonia. The content centrifuged and precipitate dissolved in 1 N HCl and volume made to 100 ml. Five ml of this was hydrolysed for 2 hr. To the flask, 5 ml 1N NaOH and 20 ml conc. acetic acid was added and the solution was made 100 ml with water in a volumetric flask. Each milliliter of this solution was equivalent to 5 mg of the fresh berries.

Preparation of Standard Curve. 10 mg of pure solasodine is dissolved in 250 ml of 20 % acetic acid. The solution thus prepared contain 40 mcg/ml of solasodine. In four separating funnels are pipetted 0, 1, 2 & 3 ml of standard solution and volume of each made to 5 ml with 20 % acetic acid. Sodium acetate/acetic acid buffer (5 ml, pH 4.7), aqueous methyl orange (1.0 ml, 0.65 %) and 5 ml chloroform are added to each funnel and shaken. Chloroform layer separated and spectrophotometer reading taken at 420 U using 10 mm cell. From these readings standard curve was prepared.

RESULTS AND DISCUSSION

From Table 2 it is evident that the percentage yield of

Table 2. Analysis of solanum species.

Species	Leaf	Fruit	Seed
<i>Solanum mammosum</i>	0.5 %	1.8 %	—
<i>S. nigrum</i>	1.06 %	1.37 %	0.71
<i>S. aviculare</i>	2.3 %	2.6 %	—
<i>S. aviculare</i> (NARC* Islamabad)	2.2 %	5.2 %	—
<i>S. pseudocapsicum</i>	1.1 %	—	—
<i>S. khasianum</i>	0.3 %	2.3 %	—

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solasodine is higher in *S. aviculare* as compared to other species of solanum. This indicates that the leaves and fruits of this plant can form an important raw material for the commercial preparation of steroidal drugs. *S. mammosum*, *S. aviculare* and *S. khasianum* are not available in Pakistan and are to be introduced. *Solanum nigrum* contain 1.0 % solasodine in leaves and 1.37 % in fruit and is very commonly available in Pakistan. This can be a possible source for the preparation of solasodine. In the case of *Solanum mammosum* and *S. khasianum*, solasodine is present only in the fruits and very small amount present in the leaves.

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