

HPLC analysis of diosgenin in three species of Costus

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Abstract:

Medicinal plants form an essential part of indigenous pharmaceutical system. Costus species (Family: Costaceae), commonly called as spiral ginger or crepe ginger are important medicinal plants used in traditional system of medicine in India. These plants are used for their stimulant, carminative, diuretic, digestive and antiseptic properties. Some of the species of Costus are mainly used for treating diabetes. Species of Costus are known to contain a steroidal saponin- diosgenin as a major bioactive component, which is utilized as a precursor for the synthesis of various drugs. The present study is taken up to quantify the amount of diosgenin present in rhizomes and leaves of three species of Costus (*C.pictus*, *C.speciosus* and *C.igneus*) using HPLC analysis.

Introduction

Higher plants are the source of numerous chemicals of commercial significance. They have impressive biological properties and are employed in various systems of traditional and folk medicine. In recent years, there is a great demand for plant-based products because of broad biological activities, low impact on environment and safety to non-target organisms. During last few decades, many plant species were screened and plants with high bioactive compounds were identified. Species of Costus are important medicinal plants with a source of anti-diabetic and antimicrobial compounds.

The genus Costus is a tropical herbaceous plant belonging to the family Costaceae. The species of Costus are widely employed in folk, ayurvedic and homeopathic systems of medicine [1]. Some of the commonly used species in unani and ayurvedic systems of medicine are *C. speciosus* Koen., *C.pictus* D.Don. and *C.igneus* N.E.Br. The *C.pictus* and *C.igneus* are referred as insulin plants [2]. Costus species are perennial rhizomatous herbs with erect or spreading stems. Leaves are simple, smooth, persistent, spirally arranged on stems. Hence these plants are often referred as the spiral ginger. The leaves are sub sessile and appear dark green in colour, elliptic or obovate in shape. In case of *C.igneus*, the leaves have light purple undersides whereas *C.speciosus* had silky texture beneath. The inflorescence is a spike around 10 cms long with large bracts in sub terminal position. Bracts are ovate or mucronate forming a cone like structure. Bracts are bright red coloured in *C.speciosus* and flowers are white in colour, 5-6 cm long with a cup-shaped labellum and crest yellow stamens. Bracts are green in colour in *C.pictus* and *C.igneus* but flower colour varies. Flowers of *C.pictus* are yellow with red stripes whereas in *C.igneus* they are orange in colour. Fruit is an ellipsoidal capsule. Costus is traditionally used as a medicinal herb mainly for its stimulant, carminative, diuretic, digestive and antiseptic properties. The rhizome is used internally in the treatment of abdominal pain, chest pains, liver problems, jaundice, gall bladder pain etc [3]. The rhizomes are the major source of diosgenin, which is anti-diabetic in nature and is used in the treatment of diabetes mellitus [4] [5]. In Ayurveda, Costus speciosus is used to subdue vata and kapha and promotes complexion. It is reported to cure dyspepsia, fever, cough and other respiratory disorders. It is one of the constituent of indigenous drug "amber mezhu" useful in rheumatism [6]. The rhizome possesses antifertility, anticholinesterase, anti-inflammatory and antihelminthic activities [7]. Essential oil from rhizome showed antimicrobial activity [8]. Antifungal activity of steroid saponins and sapogenins from Costus speciosus was analysed by [9]. In Siddha medicine system *C. igneus* root has been used in the form of powder (chooranam), decoction (kudineer) and oil (thylam) [10]. Dasgupta and Pandey (1970) reported diosgenin as the major constituent isolated from rhizomes of Costus speciosus [5]. Other constituents isolated from Costus species are Tigogenin, dioscin, gracillin β -sitosterol glucoside [11].

In the earlier studies, diosgenin was isolated from tubers of *Dioscorea zingiberensis* cell cultures by microplate-spectrophotometry and HPLC analysis [12]. Preliminary screening and qualitative HPTLC separation of secondary metabolites was reported from the rhizome of Costus speciosus [13]. In the present work, diosgenin content was quantified from rhizomes and leaves of three species of Costus using HPLC method.

Collection of Plant Material:

The leaves and rhizomes of Costus speciosus, Costus pictus and Costus igneus were collected from Botanical garden, Osmania University College for Women, Hyderabad.

Diosgenin extraction:

Dry and powdered material (leaves/rhizomes) of *Costus* (500g) was mixed with a solution of sodium acetate (20 mg) in water (1 lit). The mixture was allowed to stand for 24hrs and hydrolyzed with 5% Hydrochloric acid (2 lit) for about 14 hrs. The hydrolysate mass was filtered and repeatedly washed with water till free from acid. The residue was dried and extracted with hexane in a soxhlet apparatus for 4hrs. Concentration of hexane extract gave crude diosgenin as slightly yellow solid. The solid was crystallized by adding 95% Ethanol to give colourless needles (diosgenin).

Preparation of stock solution:

10 mg of standard diosgenin was weighed and dissolved in 5 ml methanol and sonicated for 15 min. The solution was diluted up to 10 ml with methanol to obtain a concentration of 1 mg/ml. Standard diosgenin (20 μ l) was injected to carry out chromatographic separation on C₁₈ column. Similarly Diosgenin extracted from leaves and rhizomes of different *Costus* species were dissolved in methanol and subjected to HPLC analysis.

Chromatography:

HPLC consist of Make waters (USA) Analytical system with alliance 2690 pump, automatic injector, and UV-dual lambda observance detector and empower2 software. The stationary phase used is C18 column. Calibration of the system was done by accurately weighing 0.01 gms caffeine (Merck, Germany) dissolved in 100ml of HPLC grade water. 20 μ l of different concentrations made from the caffeine stock solution were injected through a C18 Column. The mobile phase consisting of water and methanol (70:30 v/v) was used for degassing before use. UV detection was performed at 203 nm. All the chemicals used were HPLC grade. Standard diosgenin was obtained from natural remedies Pvt. Ltd (Bangalore, India). 20 μ l of standard diosgenin was injected using acetonitrile- water in the ratio of 90:10 v/v as mobile phase, flow rate was maintained at 1 ml/min and standard peak was obtained. Similarly diosgenin extracted from different species were subjected to HPLC analysis. The percentage of diosgenin in different samples was calculated from the peak area and standard diosgenin concentration.

Results and discussion:

The diosgenin quantification in leaves and rhizome samples of *Costus speciosus*, *Costus pictus* and *Costus igneus* was performed using HPLC analysis. All the samples showed characteristic peaks of diosgenin at the same retention time as that of standard diosgenin. The concentrations of diosgenin in different samples were calculated by measuring the peak area comparing with peak area of standard diosgenin. In the present study, variation in diosgenin content among the three different species of *Costus* was found. The variation in diosgenin content was also observed among different parts (leaves and rhizomes) of the same species. High percentage of diosgenin was observed in *Costus pictus* rhizomes (2.54%) followed by *Costus speciosus* rhizomes (2.15%) and *Costus igneus* rhizomes (1.17%). The percentage of diosgenin was relatively low in leaf samples. *Costus pictus* leaves showed 0.83%, whereas *Costus speciosus* and *Costus igneus* showed 0.58% and 0.39% of diosgenin respectively. The heterogeneity found in the diosgenin contents from the different species of *Costus* and also among different parts analyzed in this study. This may be due to factors such as the genotype, the physiological state, the climatic conditions as well as the geographic localization of plants as pointed out by Dinan et al.[14]. The result of our study is correlating with that of Ganzera [15], who found significant differences in the steroidal sapogenin contents of different *Tribulus terrestris* samples depending on the origin and part of the plant used for extraction. The present study showed that *C.pictus* has higher content of diosgenin followed by *C.speciosus* and *C.igneus*.

Conclusion:

The bioactivity of plant products mainly depends on the amount of the major active constituents. In order to select plants with rich bioactive compounds, HPLC was performed in three species of *Costus* i.e *C.speciosus*, *C.pictus* and *C.igneus*. Variation in diosgenin content was observed not only among the three species but also within different parts of same species i.e rhizomes and leaves. The percentage of diosgenin is more in *C.pictus* followed by *C.speciosus* and *C.igneus*. Among the two parts studied, rhizomes showed higher amount of bioactive compound than the leaves. The present study on HPLC quantification of diosgenin is very much useful in selection of superior genotypes rich in bioactive constituent, diosgenin.

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