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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2020; 9(9): 228-231

www.thepharmajournal.com
Received: 22-06-2020
Accepted: 20-08-2020

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Phytochemical Screening and diosgenin analysis of *Costus speciosus* (J. Koenig) Sm: An important medicinal plant of Andaman and Nicobar Islands

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Abstract
Costus speciosus (J.Koenig) Sm belonging to family Zingiberaceae is an important medicinal plant distributed in the Andaman and Nicobar Islands. The rhizome is the economic part and exploited medicinally for its stimulant, carminative, diuretic, digestive, anti-inflammatory and antiseptic properties. The steroidal sapogenin is also extracted from *Costus speciosus* and hence has wide industrial applications. An attempt was made to screen *Costus speciosus* for phytochemicals and diosgenin. Both tubers and roots showed the presence of carbohydrates, glycosides, saponins, proteins, aminoacids, phytosterols, saponins and phenolic compounds. Coumarins, fixed oils, gums and mucilages were absent in both roots and tubers. The diosgenin content was maximum in roots (1.45 mg/ml) when compared with tubers (0.98 mg/ml).

Keywords: *Costus speciosus*, phytochemicals, Andaman and Nicobar, Diosgenin

Introduction
Costus speciosus belonging to the family Zingiberaceae is an important medicinal plant widely distributed in Andaman and Nicobar Islands and widely used by the inhabitants of the Island for treating various ailments. The plant is succulent rhizomatous herb with erect or spreading stems reaching 2-3 m in height. The flowers look like crepe paper, thus commonly called crepe ginger. After the flowers fade away, the attractive red cone-shaped bracts remain (Abirami *et al*, 2017) [1]. The rhizome of *Costus speciosus* is traditionally medicinally mainly for its stimulant, carminative, diuretic, digestive, anti-inflammatory and antiseptic properties (Mandal *et al*, 2007) [3]. In Ayurveda, *Costus speciosus* is used to subdue *vata* and *kapha* and promotes complexion. It is one of the constituent of indigenous drug "*amber mezhugu*" useful in rheumatism. In addition to its medicinal properties, the species is also rich in one of the sapogenins, diosgenin. This sapogenin diosgenin is mainly used in the manufacture of steroidal hormones for use as antifertility drugs. The requirement of diosgenin was mainly met by the *Dioscorea sp*. In India, rhizomes of *Dioscorea deltoidea* and *Dioscorea Prazeri* are the only raw materials used by steroidal industry (Sarin *et al*, 1974) [4]. Because of considerable increase in demand of steroidal drugs, a large number of plant species were exploited as alternate sources like *Trillium govanianum, Balanites roxburghii* and *Parispolyphylla.* The commercial exploitation of diosgenin has less scope in these species due to very low diosgenin content or poor availability (Sarin *et al*, 1974) [4]. Das Gupta and Pandey, 1970 were the first to report that rhizomes of *Costus speciosus* (Koen.) Sm, a perennial herb distributed throughout India contains 2.12% diosgenin in 3.86% of total sapogenins. Nahak and Sahu, 2011 [6]. *Costus speciosus* is then valued very much for its diosgenin content which is a suitable material for synthesis of steroidal drugs and contraceptives (Nahak and Sahu, 2011) [6]. This gregarious plant is seen as weed growing in this Island in oil palm and coconut plantations, moist shady habitats, hill-slopes and cleared forests. It is desirable that commercial exploitation of this medicinal plant would be an ideal measure to control such weedy species (Abirami, *et al*, 2017) [2]. Large scale cultivation of this herb can also supplement the present requirement of diosgenin, the synthesis of costly steroidal drugs including cortisones, sex hormones and oral contraceptives, and in due course may become a substitute for yams. As an initiative study, *Costus speciosus* was screened for its phytochemicals and also diosgenin content in both roots and rhizomes, which may pave a way for commercial exploitation of this species in the Islands for industrial applications.

Materials and Methods

Plant Materials

The rhizomes of *Costus speciosus* were collected from Jirkatang, South Andaman and planted in the medicinal plant gene garden at Garacharma farm, ICAR-CIARI. The species identification was confirmed with Botanical Survey of India, Port Blair. The rhizomes with roots were collected from the field and washed thoroughly under running tap water. The rhizomes and roots were separated and were subsequently cut with a scalpel into slice of 0.5 mm thickness. The pieces were dried in an oven at 110 °C for 8 hours. The dried material was cooled and powdered in a blender. The powder was stored in a cool and dry place until further analysis.

Soxhlet extraction

50 g of dried samples of rhizomes and roots of *Costus speciosus* were extracted with ethanol as solvent in Soxhlet apparatus for 14 hours. The filterate was collected and concentrated by using rotary evaporator under controlled condition of temperature and pressure. The extracts were concentrated to dryness to yield crude residue. The residues were stored at -20 ^{o}C and were used for further phytochemical screening of secondary metabolites.

Phytochemical analysis

Phytochemical screening were performed to assess the qualitative chemical composition of different samples of crude extracts using commonly employed precipitation and coloration reactions to identify the secondary metabolites like alkaloids, flavonoids, glycosides, proteins, phenolic compounds, saponins, starch, steroid, tannins and terpenoids. The phytochemical analysis were done using standard procedures (Sofowara, 1993; Trease and Evans 1989) [7, 8].

Extraction of Diosgenin

Diosgenin was extracted from the dried tubers and roots of *Costus speciosus* following the method of Rishi *et al*, 1976 with some modifications. 20 g of powdered sample was dissolved in 100 ml of 20% aqueous ethanol. The suspension was dried in water bath and concentrated using rotary evaporator. Diosgenin was extracted thrice with 20 ml of hexane as solvent. The hexane extract was washed with 0.1 M aqueous sodium chloride to remove free fatty acids. After evaporation the samples were dried in an oven at 40^{o}C to a constant weight.

Spectrophotometric analysis of diosgenin

Diosgenin content in the tuber and root samples were analyzed spectrophotometrically. Diosgenin calibration curve was obtained using standard diosgenin and the absorbance was measured at 544 nm in spectrophotometer.

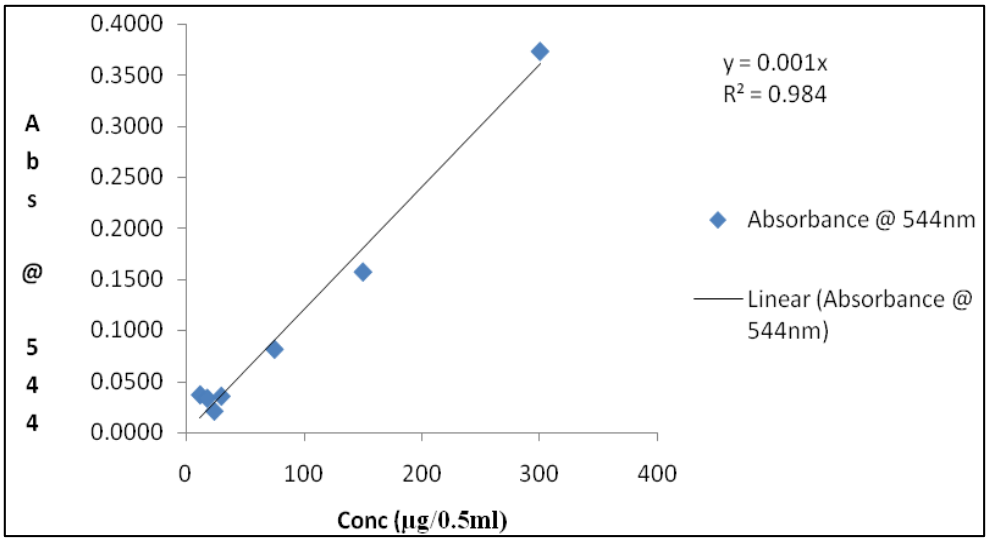

Fig 1: Calibration Curve-Diogenin

The extracted sample was then measured at 544 nm using a UV Spectrohotometer (Rsihi *et al*, 1974) against a blank with 50% aqueous methanol instead of sample extract. The diosgenin concentration in the sample were calculated from standard curve and expressed in mg/ml of sample.

Results and Discussion

Phytochemical Screening

Phytochemical screening of tubers and roots of *Costus speciosus* showed the presence of carbohydrates, glycosides, saponins, proteins, aminoacids, phytosterols and phenolic compounds (Table 1). The presence of phenolic compounds helps in biological activity of the species. The phenolic acids are directly absorbed by the body and their bioavailability is relatively high (Stevenson and Hurst, 2007) [9]. The presence of phenolic compounds also forms the base for the antioxidant potential and hence the presence of flavonoids and phenols make the plant species a good candidate for exploration of antioxidants (Abirami *et al*, 2014) [1]. Both the tubers and roots show the presence of spaonins. Saponins were reported to possess hypotensive and plasmolytic effect. Saponins are a special class of glycosides which have soapy characteristics and reported to be active antifungal agents (Jagtap and Satpute, 2014) [10]. Saponins are reported to have wide range of pharmacological and medicinal activities to treat diabetes, cancer, hepatitis, blood pressure and cholesterol (Soetan and Aiyelaagbe, 2009) [11]. Coumarins, fixed oils, gums and mucilages were absent in both roots and tubers of *Costus speciosus*.

Diosgenin analysis

The roots and tubers of *Costus speciosus* evaluated for their diosgenin content revealed the presence of diosgenin in both roots and tubers. The diosgenin content was maximum in

roots (1.452 mg/ml) and comparatively less in tubers (0.981 mg/ml). In addition to *Cosus speciosus*, diosgenin is also found in many plant species *Smilax menispermoidea*, *Trigonella foenum* and *Trillium*, and many species of *Dioscorea* (Patel *et al*., 2012; Chen *et al*., 2011) [12, 13]. This diosgenin is used in the preparation of steroidal drugs and contraceptives. In addition to the industrial production of steroidal drugs, diosgenin is also useful in treatment of various ailments like cancer, hypercholesterolemia and inflammation (Jesus *et al*, 2016) [14]. The diosgenin thus have various applications and can be exploited both in herbal and medical industry.

Thus the medicinal plant species, *Costus speciosus* is gaining more importance due to its rich phytochemicals and diosgenin content. This species is grown wild in different parts of the Andaman and Nicobar Islands and hence there exists a wide scope for commercial exploitation.

Table 1: Preliminary phytochemical screening of rhizome and root extracts of *Costus speciosus*

S. No	Compounds	Tests	Inference	Tubers	Roots
01	Alkaloids	Mayer's test	White precipitation	-	-
		Wagner's test	Reddish brown precipitation	-	-
		Hager's test	Prominent yellow precipitation	+	+
02	Carbohydrates	Molish's test	Violet ring	+	+
		Fehling's test	Red precipitation	+	+
		Benedict's test	Characteristic coloured precipitation	+	-
03	Glycosides	Borntrager's test	Pink colour	+	-
		Legal's test	Pink colour	+	+
04	Saponin	Froth test	2cm layer of foam	+	+
		Foam test	Foam persists for ten minutes	+	+
05	Proteins and aminoacids	Biuret test	Pink colour	+	-
		Xanthoproteic test	Yellow colour	+	+
06	phytosterols	Libermann – Burchard's test	Colour changes	+	+
07	Fixed oils	Spot test	Oil strain on the papers	-	-
08	Phenolic compounds	Ferric chloride test	Dark green colour	+	+
		Lead acetate test	Bulky white precipiteation	+	+
		Alkaline reagent test	Yellow fluorescence	+	+
		Magnesium and hydrochloric acid reduction test	Pink colour to crimson colour	+	-
09	Gums and mucilages		White or cloudy precipitation	-	-
10	Coumarin		Yellow colour	-	-

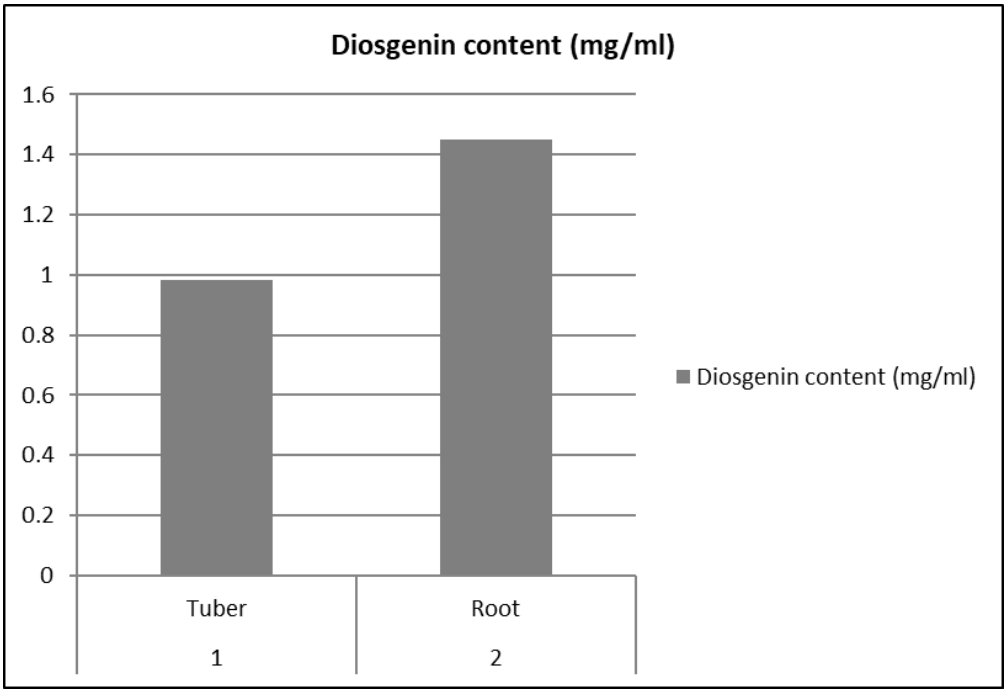

Fig 2: Diosgenin content in tubers and roots of *Costus speciosus*

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