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PHYTOCHEMICAL SCREENING OF *COSTUS MEXICANUS* LIEBM. - AN INSULIN PLANT

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ABSTRACT

Phytochemicals are extensively found at different levels in many medicinal plants. *Costus mxicanus* an important medicinal plant belongs to the family Costaceae. It is used for diabetics by traditional healers. The present study was undertaken to investigate the preliminary phytochemicals in the petroleum ether, acetone and ethanol extracts of leaves, stem and rhizome of *Costus mexicanus* Liebm. and the study revealed the presence of alkaloid, flavanoid, terepenoid in the *Costus mexicanus.*

Keywords: *Costus mexicanus*, preliminary phytochemical**.**

1. INTRODUCTION

Medicinal plants are of great importance to the health of individual and communities. The medicinal value of these plants lies in some chemical active substances that produce a definite physiological action on the human body. The most important of these chemically active (bioactive) constituents of plants are: alkaloids, tannin, flavonoid and phenolic compounds. Many of these indigenous medicinal plants are also used for medicinal purposes (Edeoga *et. al.*, 2005).

A knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic materials as tannins, oils, gums, precursors for the synthesis of complex chemical substances, etc. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Farnsworth, 1966).

Costus is a genus of perennial tropical herbaceous plants from the costus family (Costaceae). *Costus mexicanus* Liebm. is also known as Insulin plant. It is used as a munching supplementary food for the treatment of diabetes. In Mexico, it is used to treat renal disease (Martinez, 1996; Rzedowski, 1979; Caceres *et. al.*, 1987; Argueta *et. al.*, 1994). It is reported to have effects on renal functions and its anti-inflammatory, hypoglycemic actions (Martinez, 1996 and Merina, 2004) and ant diabetic property (Merina, 2005 and Nandhakumar *et. al.*, 2007).The plant is also rich in antioxidants (Shubha, 2010). The plant was reported to contain flavonoids, saponins, reduced sugars and tannins (Comargo *et. al.*, 2006). Hence, this study was designed to undertake the physicochemical and phytochemical screening of various extracts of *Costus mexicanus*.

2. MATERIALS AND METHODS

2.1. Collection and identification of plant materials

The leaves, stem and rhizome of *C.maxicana* were collected from Kannur, Kerala and authenticated by Botanical survey of India, Coimbatore. The voucher specimen of the plant was deposited at the college for further reference.

2.2. Extraction of plant materials

The plant materials (leaves, stem and rhizome of *C.maxicana*) were air-dried at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. The petroleum ether, acetone and ethanol extracts were prepared by soaking 50 g each of the dry powdered plant materials in 300 L of ethanol at room temperature for 48 h. The extracts were filtered after 48 h, first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the water bath set at 40°C.

2.3. Physicochemical Analysis:

The coarse powder of leaves, stem and rhizome of *Costus mexicanus* was subjected to various physicochemical studies for determination of ash values and extractive values.

2.3.1. Qualitative screening

The dried, pulverized leaves and roots were subjected to phytochemical analysis to screen for the presence of secondary metabolites such as Alkaloids, Tannins, Saponin, Resins, Flavonoids, Glycosides,

Steroids, Phenols, Terpenoid, Cardiac glycosides and Triterpenoids. The phytochemical screening was carried out using standard procedure (Sofowora, 1993 and Trease and Evans, 2002).

2.3.2. Quantitative screening

2.3.2.1. Alkaloid determination

2.5 g of the powder was extracted using 100 ml of 20% acetic acid in ethanol. The solution was covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to attain precipitation. The whole solution was kept as such so that precipitate will settle. Collected precipitate was washed with dilute ammonium hydroxide and finally filtered. Filtrate was discarded and pellet obtained was dried and weighed (Edeoga *et al*., 2005 and Okwu and Josiah, 2006).

2.3.2.2. Saponin determination

10 g of sample was mixed with 100 ml of 20% aqueous ethanol. The mixture was kept for 4 hours on water bath shaker at 55° C. Filtrate was again extracted in same manner. The combined extract was concentrated to 40 ml over water bath at 90°C. Concentrate obtained was transferred into a separating funnel and 10 ml of diethyl ether was added to it. After shaking vigorously aqueous layer was recovered and ether layer was discarded. The process was repeated. To the aqueous layer n-butanol was added. The whole mixture was washed in separating funnel twice with 10 ml 5% of aqueous NaCl. Upper part was retained and heated in water bath until evaporation. Latter it was dried in oven to a constant weight (Obadoni and Ochuko, 2001; Edeoga *et al*., 2005).

2.3.2.4. Tannins determination

2g of plant powder was extracted thrice in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume to 3 ml was adjusted by distilled water. The solution after vortexing were mixed with 1 ml of 0.016M $K_3Fe(CN)_6$, followed by 1 ml of 0.02M $FeCl_3$ in 0.10 M HCl. Vortexing was repeated and the tubes were kept as such for 15 min. 5 ml of stabilizer (3:1:1 ratio of water, H_3PO_4 and 1% gum arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. Standard curve was plotted using various concentrations of 0.001M gallic acid (Graham, 1992).

2.3.2.5. Carbohydrate determination

0.5 g of plant material was extracted with 80% ethanol. Extract was dissolved in 10 ml water. Different aliquots were prepared and final volume was made to 1 ml by water. 5 ml of 96% of concentrated $H_2SO_4$was added followed by shaking and incubation for 40 min at room temperature. 1 ml of 5% phenol was added to each tube and absorbance was taken at 490nm. Standard curve using different concentrations of 25 mg% glucose (Krishnaveni *et al*., 1984).

2.3.2.6. Proteins determination

1g plant material was extracted using 10 ml water added with few drops of triton X- 100. Supernatant was extracted in acetone and the pellet obtained was dissolved in 0.1 M NaOH. Aliquots were prepared and final volume was made to 1 ml by distilled water. 5 ml of copper reagent was added to tubes, mixed well and incubated for 10 minutes. 1 ml of folin's reagent was mixed. Tubes were incubated for 30 min at room temperature and absorbance was taken at 700 nm. Standard curve was prepared using 50 mg % BSA (Lowry *et al*., 1951).

2.3.2.7. Lipids determination

1g plant sample was dissolved in ether and stirred for a hour. Mixture was centrifuged, supernatant dried and dissolved in ethanol. 0.1 ml of alcohol was taken as blank, olive oil as standard and test sample as unknown respectively. 2 ml of concentrated H_2SO_4 and 5 ml of phosphovanillin reagent was added and mixed well, incubated for 30 min. Absorbance was read at 540nm (Ganai *et al*., 2005).

3. RESULTS AND DISCUSSION

The results of preliminary phytochemical study were tabulated in Table-1. The phytochemical study revealed the presence of steroids, flavonoids, alkaloids, coumarins, triterpenoids, tannins and carbohydrate. The table-2 shows the Quntitative Phytochemical Screening which is measured in g %. The Physicochemical Analysis is described with physical nature, extractive value and ash value in table-3.

Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). Just *et al.* (1998) revealed the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *C.maxicana* extracts and has supported the usefulness of this plant in managing inflammation. Flavonoids, another constituent of *C.maxicana* leaves, stem and rhizome extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-

angionic, analgesic, anti-allergic, cytostatic and antioxidant properties .The result justifies the use of these plants in traditional medicine for the treatment of various kinds of diseases including infectious disease (Idu *et al.*, 2006).

4. CONCLUSION

This study has shown the scientific basis for some of the therapeutic uses of *C.maxicana* plant in traditional medicine. The preliminary phytochemical tests are helpful in finding chemical constituents in the plant material that may lead to their quantitative estimation and also in locating the source of pharmacologically active chemical compound.

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Table 1: Qulalitative Phytochemical Screening of *Costus Maxicana*

Bioactive Agents	Petroleum ether			Acetone			Ethanol		
	L	S	R	L	S	R	L	S	R
Alkaloid	+	+	+	+	+	+	+	+	+
Flavanoid	+	+	+	+	+	+	+	+	+
Saponin	-	-	+	-	-	-	-	-	+
Glycoside	+	+	-	+	+	-	+	-	-
Tanin	-	-	+	-	-	+	-	-	-
Terpenoid	+	+	+	+	+	+	+	+	+
Resin	+	+	+	+	+	-	-		-
Steroid	+	-	+	+	+	+	+	+	+
Phenol	+	+	-	-	+	-	-	+	-
Cardiac glycoside	+	+	+	-	+	+	-	+	+
Tri-terpenoids	+	-	-	-	-	-	+	-	+

Table 2: Quntitative Phytochemical Screening of *Costus Maxicana*

Bioactive Agents	Quantity/100g of plant material* (i.e. g %)		
Alkaloid	L	S	R
Tannins	1.53 ± 0.02	1.18 ± 0.05	1.73 ± 0.01
Saponins	0.05±0.001	0.03 ± 0.03	0.06 ± 0.003
Flavonoids	0.35±0.0025	0.47 ± 0.04	0.21 ± 0.03
Cardiac Glycosides	1.73±0.036	1.33 ± 0.064	1.47 ± 0.043
Carbohydrates	0.056±0.005	0.067 ± 0.007	0.032 ± 0.003
Lipids	0.375±0.0012	0.474 ± 0.006	0.659 ± 0.004
Proteins	2.44±0.002	2.96 ± 0.003	4.69 ± 0.006

*Results are mean ± SD of triplicate determination on the basis of dry weight.

Table 3: Physicochemical Analysis of *Costus Maxicana*

S.No.	Parameters	Observation		
		Leaves	Stem	Rhizome
I	Physical test			
	Nature	Smooth	Scaly	Scaborous
	Colour	Dark green	Greenish yellow	Brown
	Odour	Odourless	Pungent smell	Pungent smell
II	Extractive value			
	Petroleum ether	8.45	6.82	7.5
	Acetone	9.29	7.34	9.26
	Ethanol	11.86	10.62	9.36
III	Loss of Drying	9.53 %		
IV	Ash Value			
	Total ash	9.2	7.8	8.8
	Acid insoluble ash	2.7	3.9	2.3
	Water soluble ash	3.1	3.6	2.4