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Phytochemical Investigation of Insulin Plant (*Costus pictus*)

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Insulin plant (*Costus pictus*) a folk medicine used for the treatment of diabetes mellitus is subjected to physico-chemical analysis. The saponified extract is analyzed using GC-MS for the identification of the chemical constituents. 18 Chemical compounds are identified from the plant leaves.

Key Words: Phytochemical, *Costus pictus*.

INTRODUCTION

Insulin plant (*Costus pictus*) is one of the folk medicines used for the treatment for Diabetes mellitus. This plant belongs to Costaceae family, which has been separated from Zingiberaceae on the basis of the presence of spirally arranged leaves and rhizomes being free from aromatic essential oils[1]. The plant commonly known as spiral ginger is originated in Mexico and is found to have antidiabetic properties[2]. Dichloromethane and methanol extracts of the plants belonging to Zingiberaceae, from Alpinia, Costus and Zingiber genera were found to be antibacterial, while methanolic extract of *Costus discolour* exhibited potent antifungal activity against *Aspergillus ochraceous* (MIC-15.6 μ/disc)[3,4]. All these extracts showed antioxidant activity comparable or higher than that of α-tocopherol. The methanolic extract of the leaves of the insulin plant showed no toxicity in both acute and sub acute toxicity studies conducted in mice and rats, respectively[2]. Since this is a newly identified medicinal plant not much work are reported on its phytochemical studies. Hence this plant is selected for the phytochemical investigation.

EXPERIMENTAL

Leaves of the insulin plant were collected locally and authenticated. Air-dried leaves were used for the investigation. All chemicals including solvents used for extraction were of AR grade (BDH/Merck). Solvents used

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for chromatography were of HPLC grade. Physico-chemical parameters such as moisture content, volatile oil content, ash content, fibre content, sugar content and water soluble extractives are determined by standard analytical methods[5]. Plant material was also subjected to successive solvent extraction using a Soxhlet extractor with solvents in the ascending order of polarity of such as petroleum ether, cyclohexane, acetone and ethanol. Extracts were subjected to qualitative tests for the identification of various plant constituents such as alkaloids and steroids[6]. TLC analysis of the extracts was done in suitable solvent systems using silica gel G coated plates. The positions of the spots were detected in iodine chamber. GC-MS analysis was done using Shimadzu-QP 2010 GC-MS with a DB-5 column of 0.25 mm thickness and 30 m length. Oven temperature held at 50ºC for 5 min and then programmed up to 300ºC at the rate of 10ºC/min and held at that temperature for 10 min. Helium was used as the carrier gas.

Air-dried, powdered plant material was saponified with alcoholic KOH (0.5 N). The saponified fraction was extracted with ether. The ether layer was evaporated to remove ether and the residue dissolved in ethanol (ether fraction). The aqueous layer was acidified with dil. HCl and extracted with ether, evaporated and dissolved in ethanol (acid fraction). Both the fractions were subjected to GC-MS analysis.

RESULTS AND DISCUSSION

Preliminary physico-chemical analysis of the air-dried leaves is as given in Table-1. The fibre contents of the leaves is 21.1 %, which indicates that the leaves contain more fibers which are essential for diabetic patients. Qualitative ash analysis showed that it contains carbonate, oxalate, chloride, phosphate and sulphate as anions and sodium, potassium, magnesium and iron as cations.

TABLE-1
ANALYTICAL DATA OF AIR-DRIED LEAVES

Constituent		%
Moisture content		10.00
Fibre content		21.10
Sugar content:	Reducing	10.40
	Total	16.77
Ash content		15.02
Water-soluble extractives		15.93

Successive extractions of the leaves gave 5.2 % extractives in petroleum ether, 1.06 % in cyclohexane, 1.33 % in acetone and 2.95 % in ethanol. Analysis of successive extracts showed the presence of steroids in all

extracts. The ethanol extract contained alkaloid also. These extracts were subjected to TLC analysis using different solvent systems. In *n*-hexane: acetone (9:1) system the petroleum ether extract gave 6 spots (R_f : 0.12, 0.27, 0.35, 0.54, 0.65 and 0.98) while acetone gave 3 spots (R_f : 0.13, 0.54 and 0.92) and in ethanol only 1 spot, (R_f : 0.52).

The MS fragmentation pattern of the compounds are assigned and are given in Table-2[7,8]. From the chromatogram, it was evident that the major component in the ether fraction is *bis*(2′-ethylhexyl)-1,2-benzenedicarboxylate (59.04 %). Presence of α-tocopherol in this fraction may be the cause of the antioxidant property of the leaf extract[3,4]. A steroid, ergastanol present in the ether fraction supports the TLC analyses of the successive extracts.

TABLE-2
GC-MS ANALYTICAL DATA OF THE ETHER FRACTION

Retention time (min)	Percentage	m.w.	Base peak	Compounds assigned
40.073	1.99	296	71	Phytol
50.579	1.58	332	271	Xanthen-3-one
51.632	59.04	390	149	*Bis*(2′-ethylhexyl)-1,2-benzene dicarboxylate
54.335	1.55	394	71	Octacosane
55.775	2.25	400	135	α-Ergastanol
59.494	5.09	–	71	Derivative of octacosane
61.206	8.16	402	402	α-Tocopherol
68.292	20.34	436	71	Derivative of octacosane

TABLE-3
GC-MS ANALYTICAL DATA OF THE ACID FRACTION

Retention time (min)	Percentage	m.w.	Base peak	Compounds assigned
20.237	1.20	228	73	Tetradecanoic acid
21.277	1.19	242	73	Pentadecanoic acid
22.380	44.53	256	73	Hexadecanoic acid
23.233	1.30	270	73	Heptadecanoic acid
23.985	13.39	282	69	Heptadecene-[8]-carbonic acid [1]
24.171	5.47	284	73	Octadecanoic acid (Stearic acid)
25.864	27.86	324	99	4,8,12,16-Tetramethyl-heptadecan-4-olide
27.036	1.50	198	113	7-Tridecanone
27.484	1.40	340	73	Docosanoic acid (Behenic acid)
29.009	1.35	368	73	9-Octadecenoic acid (Oleic acid)

The analysis of acid fraction gave 10 peaks in the chromatogram and the compounds present in this fraction are assigned and given in Table-3. From the data obtained, the major components in the acid fraction are hexadecanoic acid (44.53 %) and 4,8,12,16-tetramethylheptadecan-4-olide (27.86 %). This fraction also contains decosanoic acid. Presence of octacosanoic acid and tetracosanoic acid were reported from Costus speciousus and Costus tonkinensis, respectively[9]. Further phytochemical investigations are in progress.

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