PHYTOCHEMICAL INVESTIGATION OF THE MEDICINAL PLANT *COSTUS PICTUS* D. DON

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Abstract:

The plant *Costus pictus* is popularly known as insulin plant and it has been prescribed for diabetes. The diverged constituents present in the plant are responsible for the multiple potent to treat various diseases. The potential of the medicinal plants is evaluated on the basis of the presence of secondary metabolites by quantitative screening and identification of chemicals. The present study deals with the physical and chemical evaluation of the leaves and rhizomes of *C. pictus;* additionally, this research is to provide a toxic and trace element constituent of *C. pictus* which might plays vital role in the treatment of various ailments.

Keywords: phytochemicals, minerals, qualitative & quantitative analysis, GC-MS of C*ostus pictus.*

Introduction:

The plants have been continuing to be the source of all wealth. According to World Health Organization (WHO), nearly 4 billion people (66.6 percent of the world population) employ plants or plant products for primary health care in one form or the other (Penso, 1976). India is a emporium of medicinal plants and is one of the richest genetic resources in the world (Bagyalakshmi *et al*., 2009). Herbal medicines have been administered as early as 5000 BC in India to prevent and treat diseases. Though plants serve human to maintain their well being, the investigation of the chemprofile of the medicinal plants to combat various ailments becomes obligatory. Hence there is an urgent need for the constant screening of herbal drugs for better and affordable utilization. The potential of the medicinal plants is evaluated on the basis of the presence of secondary metabolites, by both qualitative and quantitative screening and identification of chemicals by GC-MS. The present study estimates the phytochemicals and physical, mineral and toxicological properties of the leaf and rhizome parts of the medicinal plant, *Costus pictus.*

The genus *Costus* of the family *Costaceae* was introduced from Mexico to India and consists of 150 species. *C. pictus* is an erect shrub with underground rhizome. In India, it has been cultivated for both ornamental and medicinal purposes. It is reported to have antidiabetic properties due to the presence of several bioactive compounds, elements and essential oils. Since it is a more exploited and precious medicinal plant, the prevention of deterioration and purity of crude substance by influence of physical factors are essential. Hence the phytochemical, trace elements along with toxicological evaluations are particularly focused in the present study.

MATERIALS AND METHODS

Collection of Materials

The rhizomes of *Costus pictus* were collected from Kerala Forest Research Institute, Peach, Kerala, India. The flowering twig of the same was authenticated by BSI, Southern Regional center, Coimbatore, Tamil Nadu, India.

Phytochemical screening: Qualitative Analysis

Different qualitative tests were carried out in the leaf and rhizome of *C. pictus* for the detection of various phytochemical constituents. The shade dried powder form of plant material was extracted with different solvents such as petroleum ether, chloroform, ethanol, methanol and water.

Quantitative analysis: The quantitative estimation for various phytochemicals was carried out by following the standardized methods with slight modifications (Siddhuraju and Becker 2003; Zhishen *et al.*, 1999; Makkar *et al.*, 2007; Harborne, 1973; Klein and Perry, 1982; Porter *et al.*, 1986).

GC-MS analysis: The identification and quantitation of organic substances were carried out by the analytical technique GC-MS. Unknown compounds were identified by matching full mass spectrum of unknown peaks with mass spectral library and data base.

Gas Chromatography/ Mass Spectrometry (GC/MS) was performed in a DB-5 MS column (30 m × 0.25 mm, 0.25 µm film thickness). The ion source temperature and interface temperature were at 230 °C and 240 °C respectively. Ionization mode electron impact was at 70 eV with a scan range of 40 – 700 m/z. The peaks in the chromatogram were identified based on their retention indices and mass spectra in comparison with the MS NIST spectral library database of known compounds (NIST, 2011).

Physical evaluation: In order to determine the physical standard of medicinal plant material, loss on drying and Ash value tests were carried out.

Determination of loss on drying: The percentage of loss on drying for the leaf and rhizome of *C. pictus* was estimated by Quality Control Methods for Medicinal Plant Materials, WHO, Geneva 1988. The percentage was calculated with reference to the amount of dried powder taken and tabulated.

$$\text{Percentage of loss on drying at } 105^{\circ}C = \frac{\text{loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

Determination of Ash content

The percentage of total Ash was calculated by the above WHO guidelines. The Ash value was calculated as per the following formula,

$$\text{The percentage of Ash} = \frac{\text{Weight of Ash} \times 100}{\text{Weight of sample taken}}$$

Toxicological evaluation: Presence of pathogens, aflatoxins and heavy metals were assessed to prevent chemical change and microbial contaminations.

Microbial contamination: This test was carried out as per the guidelines of the Ayurvedic Pharmacopoeia of India, Part II (API-II). Total viable aerobic count of pathogens such as *Eschericia coli*, *Pseudomonas aerogenosa*, *Staphylococcus aureus* and *Salmonella species* were studied. Total bacterial, yeast and mold counts were also studied.

Analysis of aflatoxins: The leaves and rhizomes of *C. pictus* were tested for the most potent carcinogenic aflatoxins B1, B2, G1 & G2 produced by soil fungi using AOAC, 2008.

Heavy metal analysis: Quantification of high potent heavy metals such as Arsenic, Cadmium, Lead and Mercury were made to eliminate their presence and harmful effects. The method of testing for Arsenic was BVILCH/NS/SOP – 053 by ICPOES and for Mercury was Mercury Analyser MA 5840D, EC make

Instrument Manual, Electronics Corporation of India Ltd. Heavy metal analysis was carried out as per AOAC – Official methods of Analysis of AOA C – International 18th edition, 2005.

RESULTS:

Qualitative phytochemical screening of *C. pictus*

The various extracts of *Costus pictus* leaf and rhizome were screened qualitatively for various phytochemicals including alkaloids, anthroquinones, carbohydrates, fatty acids, flavonoids, glycosides, proteins, phenols, saponins, sterols,steroids, tannins, terpenoids and volatile oils.

The qualitative screening of *C. pictus* leaf extracts showed the presence of almost all the studied phytochemicals except saponins, sterols and volatile oils. On the other hand it rhizome extracts were found to be positive for all phytochemicals except sterols (Table 1 and 2).

In the present study qualitative phytocemical screening showed the presence of phytochemicals including alkaloids, anthroquinone, carbohydrate, fatty acids, flavonoids, glycosides, proteins, phenols, saponins, sterols, steroids, tannins, terpenoids and volatile oils etc. Similarly, Srinivasan *et al*. (2016) performed the phytochemical screening of root, rhizome, stem, leaf and flower parts of *C. pictus* collected from Kozhikode District, Kerala and reported the presence of various chemicals supports the results of the study.

Quantitative phytochemical estimation of *C. pictus*

The quantity of various phytochemicals such as alkaloids, anthroquinones, carbohydrates, fatty acids, flavonoids, glycosides, proteins, phenols, saponins, sterols, steroids, tannins, terpenoids and volatile oils was estimated in leaf and rhizome extracts of the species.

The results revealed that proteins were present in major level in the leaf extracts of *Costus pictus* (156.7 mg/g) followed by carbohydrates (50.3 mg/g) and terpenoids (23.5 mg/g). *Costus pictus* rhizome extract had higher quantity of carbohydrates (56.7mg/g) followed by proteins (54.7mg/g) and glycosides (34.6mg/g) (Table 3)

GC- MS analysis

***Costus pictus* leaf**

GC-MS analysis of *C. pictus* leaf extract showed the presence of 32 various compounds accounting for 99.97%. The major compound was identified as undecane (11.36%) followed by 6,10,14-trimethyl-2-pentadecanone (9.35%), dodecane (8.01%) and decane (7.44%). There were 4 compounds identified to be present in very low percentage including butyl-cyclohexane (0.78%), pthalic acid (0.53%), cyclononanone (0.96%) and 3-methyl-2-(3.7.11-trimethyldodecyl) thiphene (0.72%) (Table 4 and Fig. 1).

***Costus pictus* rhizome**

It was revealed from the GC-MS analysis that tetraethyl silicate (26.39%), 3-(3,4-dihydro-6,7-dimethoxy-3,3-dimethyl-1-isoquinolinylamino)-propanoic acid (23.42%) and heptadecane (20.23%) were the major compounds of *Costus pictus* rhizome extract. Other compounds that were identified in the *Costus pictus* rhizome extract include 2H-Indeno(1,2-b)furan-2-one (14.30%), N.N'-bis(3-aminopropyl)-1,3-propanediamine (8.05%) and 2-bromo-ethanol (7.62%) (Table 5 and Fig. 2).

GC-MS analysis of leaf showed thirty two compounds with undecane (11.36%) as major compound. Several reports available on the presence of undecane in various plant species including *Symplocos crataegoides* (Govindarajan *et al*., 2016), *Aristolochia bracteolata* (Das, 2016) and *Aerva javanica* (Karthishwaran *et al*., 2018) support the present study. It was reported that fresh leaves of *Costus pictus* contains 18 chemical compounds and were identified by using GC-MS. From the chromatogram, it was evident that the major component in the ether fraction is bis (2'- ethyl hexyl)-1, 2-benzene dicarboxylate. The major components in the acid fraction were haxadecanoic acid and 4, 8, 12, 16- tetra methyl hepta decan 4-olide (George *et al*., 2007). Like leaf, rhizome also reported to have several unreported compounds.

Loss on drying: The percentage of loss on drying for the leaf of *C. pictus* was 11.03% and rhizome was 13.90%. The loss was more in rhizome than the leaf (Table 6). Loss on drying (LOD) of *C. pictus* leaf and rhizome was 11.03% and 13.90% respectively. Similar such results were obtained in the drying treatment of Mentha and Origano as 10% and 13.2% (Rajat and Dey, 2016).

Ash value: The percentage of total ash was estimated as 10.85% and 12.90% for the leaf and rhizome respectively. The results showed that ash value of rhizome was greater than the leaf (Table 6). The ash value was assessed as 10.85% and 12.90% for the leaf and rhizome of *Costus pictus* and it matched with the determined ash value in *Coriandrum staivum* (10%), *Mentha piperita* (11%), *Calendula offianale* (12%), (Muller and Heindl) and *Butea mono sperma* which ranges from 13.35% to 14.45% (Tambe *et al*., 2012)

Toxicological Evaluation:

The microbial contamination tests for pathogens viz., *Eschericia coli; Pseudomonas aerogenosa, Staphylococcus aureus and Salmonella species* were found to be negative. Total yeast and mold count were also reported to be absent. The bacterial count for the leaf and rhizome was 15 Cfu/gm and was found to be within the permissible limit (Table 7). The microbial contamination for pathogens, yeast and mold were negative and the leaf and rhizome of *Costus pictus* was free from the above microbes. The microbial load of some medicinal plants were already worked out by earlier workers with similar results (MacDonald Idu *et al*., 2010) and (Oprea *et al*., 2015)

The analysis of main types of aflatoxins namely B1, B2, G1 and G2 were found to be below the limit of quantification for both leaf and rhizome. The detection limit was reported to be 0.3 µg/kg (Table 8). Heavy metal analysis of leaf and rhizome of *C. pictus* was within the maximum permissible limit. Thus the detected volume for cadmium in the leaf and rhizome was 0.27 ppm and 0.099 ppm, for lead 2.50 ppm and 3.80 ppm and for mercury 0.22 ppm and 0.78 ppm. Arsenic was not detected from the leaf and rhizome of *Costus pictus* (Table 9). The levels of aflatoxins AFB1, AFB2, AFG1 and AFG2 were found to be below the level of detection limit. Several reports are also available in the detection of Aflatoxins in plant species viz., *Mucuna prieriens, Delphinium denudatum* and *Portulaca obraceae.* (Siddique *et al.,* 2013).

Trace element analysis showed that the leaves of *C. pictus* contained appreciable amounts of the elements K, Ca, Cr, Mn, Cu, and Zn (Jayasri *et al*., 2008). In the present study the volume of Arsenic, Cadmium, Lead and Mercury present in the leaf & rhizome were estimated. Arsenic was not detected from both the samples. The detected values of cadmium, lead and mercury were also found to be below the permissible value, thus ruling out the toxicity of the above metal contamination. The above four heavy metals

were detected in the leaves of *Cassia alata, Moringa, olbifera, Oamum gratissimum* and *Cymbopogaon ctratus*. (Kofi Annan *et al.*, 2013).

Table 1: Qualitative phytochemical screening of *Costus pictus* leaf extracts

S. No	Phytochemicals	Aqueous	Ethanol	Petroleum ether	Chloroform
1.	Alkaloids	+	+	+	+
2.	Anthroquinone	+	+	+	+
3.	Carbohydrate	+	+	+	+
4.	Fatty acids	+	+	+	+
5.	Flavonoids	+	+	+	+
6.	Glycosides	+	+	+	+
7.	Proteins	+	+	+	+
8.	Phenols	+	+	+	+
9.	Saponins	-	+	-	-
10.	Sterols	-	-	-	-
11.	Steroids	+	+	+	+
12.	Tannins	+	+	+	+
13.	Terpenoids	+	+	+	+
14.	Volatile oils	-	+	+	-

Table 2: Qualitative phytochemical screening of *Costus pictus* rhizome extracts

S. No	Phytochemicals	Aqueous	Ethanol	Petroleum ether	Chloroform
1.	Alkaloids	+	+	+	+
2.	Anthroquinone	+	+	+	+
3.	Carbohydrate	+	+	+	+
4.	Fatty acids	+	+	+	+
5.	Flavonoids	+	+	+	+
6.	Glycosides	+	+	+	+
7.	Proteins	+	+	+	+
8.	Phenols	+	+	+	+
9.	Saponins	+	+	+	+
10.	Sterols	-	-	-	-
11.	Steroids	+	+	+	+
12.	Tannins	+	+	+	+
13.	Terpenoids	+	+	+	+
14.	Volatile oils	+	+	+	+

Table 3: Quantitative phytochemical estimation of *Costus pictus* leaves and rhizome

S. No	Phytochemicals	Ethanol Extract	
		Leaf(mg)	Rhizome(mg)
1.	Alkaloids	0.003	0.25
2.	Anthroquinone	Bdl	0.033
3.	Carbohydrate	50.3	56.7
4.	Fatty acids	12.7	13.6
5.	Flavonoids	2.1	1.56
6.	Glycosides	12.5	34.6
7.	Proteins	156.7	54.7
8.	Phenols	2.1	3.37
9.	Saponins	2.1	1.77
10.	Sterols	Not detected	Not detected
11.	Steroids	0.45	0.145
12.	Tannins	1.67	4.67
13.	Terpenoids	23.5	0.566
14.	Volatile oils	2.1	2.44

Table 4: GC-MS analysis of *Costus pictus* leaf

S.No.	Retention time	Compound	Peak %
1.	3.35	Nonane	3.43
2.	3.95	Propyl cyclohexane	1.09
3.	4.48	1-ethyl3-methyl benzene	2.26
4.	4.64	Mesitylene	1.67
5.	5.14	Decane	7.44
6.	5.53	4-methyl decane	2.39
7.	5.60	Mesitylene	2.07
8.	5.78	Butyl-cyclohexane	0.78
9.	6.11	1-methyl-3-propyl-benzene	1.58
10.	6.23	1-ethyl-3,5-dimethyl- benzene	2.22
11.	6.30	2-methyl-decane	1.52
12.	6.62	O-cymene	3.03
13.	6.94	Undecane	11.36
14.	7.18	1-ethyl-4-(1-methylethyl) benzene	2.05
15.	7.27	1,2,3,5-tetramethyl-benzene	2.94

16.	7.58	Pentyl-cyclohexane	1.69
17.	7.86	1,2,3,4-tetramethyl-benzene	3.16
18.	8.01	8-methyl-heptadecane	1.70
19.	8.49	Naphthalene	3.60
20.	8.59	Dodecane	8.01
21.	8.78	2,6-dimethyl-undecane	2.25
22.	9.68	2-methyl-decane	1.16
23.	10.10	Tridecane	1.54
24.	16.71	6,10,14-trimethyl-2-pentadecanone	9.35
25.	16.93	Pthalic acid	0.53
26.	17.89	1,2-benzenedicarboxylic acid, butyl 2-methyl propyl ester	7.17
27.	18.25	Cyclononanone	0.96
28.	19.34	5-dodecyldihydro-2(3H)-furanone	1.24
29.	21.37	3-methyl-2-(3.7.11-trimethyldodecyl)thiphene	0.72
30.	21.46	4,8,12,16-tetramethylheptadecane-4-olide	5.89
31.	22.91	Bis(2-ethylhexyl)pthalate	4.17
32.	24.66	N-methyl-N-acetyl-3,4-methylenedioxybenzylamine	1.00

Table 5: GC-MS analysis of *Costus pictus* rhizome

S.No.	Retention time	Compound	Peak %
1.	4.442	Tetraethyl silicate	26.39
2.	12.521	2H-Indeno(1,2-b)furan-2-one	14.30
3.	16.164	2-bromo-ethanol	7.62
4.	17.732	3-(3,4-dihydro-6,7-dimethoxy-3,3-dimethyl-1-isoquinolinylamino)-propanoic acid	23.42
5.	18.903	N.N'-bis(3-aminopropyl)-1,3-propanediamine	8.05
6.	19.282	Heptadecane	20.23

Table 6: Determination of loss Drying

S.No.	Species	Parts	Loss on Drying (%)	Ash (%)
1.	*Costus pictus*	Leaf	11.03	10.85
		Rhizome	13.90	12.90

Table 7 : Total microbial load for the Leaf & Rhizome of *Costus pictus*

S. No.	Parameters	*Costus Pictus*	
		Leaf	**Rhizome**
1.	Escherchia coli	Negative	Negative
2.	Pseudomonas aeruginosa	Negative	Negative
3.	Staphylococus aureus	Negative	Negative
4.	Salmonella sp	Negative	Negative
5.	Total Bacterial count	15 cfu/gm	15 cfu/gm
6.	Total yeast and mould	Nil	Nil

Table 8: Analysis of aflatoxins for the leaf and rhizome of *Costus pictus*

Afloxtoxins	Detection (µg/kg)	Costus Pictus	
		Leaf	**Rhizome**
B1	0.3	BLQ	BLQ
B2	0.3	BLQ	BLQ
G1	0.3	BLQ	BLQ
G2	0.3	BLQ	BLQ

BLQ – Below Limit of Quantification

Table 9: Determination of Heavy Metals in the Leaf and Rhizome of *Costus pictus*

S. No	Heavy metal	Maximum permissible limit (ppm)	Observed value (ppm)	
			Leaf	**Rhizome**
1.	Arsenic	3	ND	ND
2.	Cadmium	0.3	0.27	0.099
3.	Lead	10	2.50	3.80
4.	Mercury	1	0.22	0.78

***** ND – Not Detected

Conclusion:

On the whole, the quantitative phytochemical studies proved that the leaf and rhizome of *Costus pictus* is having alkaloids, terpenoids, phenols and other important constituents. Meanwhile GC-MS report of *C. pictus* reported to have some unknown and familiar compounds with varying percentage. Further, detailed phytochemical analysis would reveal the presence of some interesting compounds in *C. pictus*. In the present study the leaves and rhizomes of *C. pictus* were assessed for physical evaluation by loss on drying and Ash value method. The toxicological evaluation was also supported through microbial contamination, aflatoxins and heavy metal analysis to prove the purity and safety of the plant, *Costus pictus*.

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