

**PRELIMINARY PHYTOCHEMICAL SCREENING AND GAS  
CHROMATOGRAPHY MASS SPECTROMETRY ANALYSIS OF  
*COSTUS PICTUS* D. DON**

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**ABSTRACT**

Plants have always been source of drugs and many of the currently available synthetic drugs were derived directly or indirectly from them. The bio active constituents present in the plant are responsible for the medicinal properties of the plant. Bioactive compounds or phytochemicals are non-nutritive chemicals naturally occurring in plants. The study was aimed to investigate the qualitative phytochemical constituents present in ethanol, aqueous and petroleum ether extracts and GC-MS analysis of *Costus pictus* leaves. The preliminary screening showed presence of alkaloids, steroids, terpenoids, glycosides, carbohydrates, tannins, saponins, phenols, flavonoids and proteins. GC-MS analysis confirmed the presence of biologically active compounds like terpenes, diterpenes, fatty acid,

essential oils, acyclic alkanes, phenols and triterpene. These results provide scientific support for further research in the development of novel drug.

**KEYWORDS:** *Costus pictus*, Bioactive compounds, GC-MS, Alkaloids, Terpenoids, Flavonoids.

**INTRODUCTION**

Medicinal plants have a long history of use as therapeutics and their use is widespread in both developing and developed countries. The rich knowledge base of countries like India and China, medicinal plants and health care has led to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development programs in the pursuit of discovering novel drugs.<sup>[1]</sup> Plants synthesize a wide range of bioactive compounds

that are responsible for the medicinal properties of the plant. Bioactive compounds or phytochemicals are non-nutritive chemicals naturally occurring in plants that have therapeutic actions, which can be refined to the synthesis of effective drug.<sup>[2]</sup> Conventional drugs tend to use the bioactive ingredients of plants rather than the whole plant. These can be derived from any part of the plant like leaves, flower, bark, roots, fruits and seeds. Analysis of these compounds provides insight into the biological activity of plants.<sup>[3]</sup> Gas Chromatography Mass Spectrometry (GC-MS) is one of the most commonly used technique for the identification of bioactive compounds present in medicinal plants.

Great numbers of plant species that have the opportunities to discover the potent drug are present in our nature. One of these is *Costus pictus* D.Don belonging to Costaceae family, commonly known as "spiral ginger" or "insulin plant". This plant is originated from Mexico and introduced to India. Recently, this plant gains more medicinal interest due to its medicinal properties, leaves of the plant has anti-diabetic activity in humans.<sup>[4,5]</sup> Scientific studies on *Costus pictus* have shown that they possess a range of pharmacological properties such as hypolipidemic<sup>[4]</sup>, antispasmodic, anti fungal<sup>[6]</sup>, antibacterial and antioxidant<sup>[7,8]</sup> effects apart from its anti-diabetic activity. Therefore, based on the ethno medical claims, the present study was planned to screen *Costus pictus* leaves for the phytocompounds and was analyzed by GC-MS analysis.

## MATERIALS AND METHODS

### Collection of plant material

Fresh and healthy leaves of *Costus pictus* were collected and authenticated from Botanical Survey of India (BSI). Leaves were washed, shade dried and powdered.

### Preparation of plant extract

The powdered samples were extracted with petroleum ether, ethanol and distilled water separately in a stoppered container for a defined period with continuous agitation. The extract is then filtered, condensed and stored for further studies.

### Phytochemicals analysis of *Costus pictus* leaves

#### Alkaloids

##### Wagner's test

About 0.5ml of extract was treated with 2- drops of Wagner's reagent (solution of Iodine in potassium iodide) and the formation of reddish brown precipitate indicated the presence of alkaloids.

**Steroids**

2 ml of acetic anhydride was added to 0.5g of extract with 2ml sulphuric acid. The colour change from violet to blue or green in samples indicated the presence of steroids.

**Terpenoids****Salkowski test**

5ml of each extract was mixed in 2ml of chloroform and 3ml of concentrated sulphuric acid was carefully added to form a layer. A reddish brown colouration of the interface was found to show positive results for the presence of terpenoids.

**Glycoside**

To the solution of the extract in Glacial acetic acid, few drops of Ferric chloride and Concentrated Sulphuric acid are added, and observed for reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer.

**Test for carbohydrates****Fehling's test**

Equal volume of Fehling A and Fehling B solutions was mixed. 2ml of this solution was added to each extract and boiled. Formation of red brick precipitate at the bottom of the test tube indicates the presence of carbohydrates.

**Benedict's test**

2ml of Benedict's solution was added to each extract and boiled. Formation of reddish brown precipitate indicates the presence of carbohydrates.

**Iodine test**

2ml of iodine solution was treated with each plant extract. Dark blue or purple coloration indicates the presence of carbohydrates.

**Tannins**

About 0.5g of the powdered samples was boiled in 20ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

### Saponins

About 2g of the powdered sample was boiled in 2ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously then observed for the formation of emulsion.

### Phenols

The 0.5g extract was dissolved in 5ml of distilled water. To this, few drops of neutral 5% ferric chloride solution were added. A dark green colour indicated the presence of phenolic compounds.

### Flavonoids

5ml of dilute ammonia solution were added to a portion of the aqueous plant extract followed by addition of concentrated Sulphuric acid. A yellow colouration was observed in extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

### Proteins

#### Ninhydrin test

Each extract was treated with 2ml of 0.2% ninhydrin solution. Presence of violet colouration indicated amino acids and proteins.

### GC-MS analysis

GC-MS was performed on a Thermo GC – Trace ultra Ver: 5.0, with a split injector and a Thermo MS DSQ, mass selective detector fused with silica capillary column having a dimension of 30 mts, 0.25 ID and 0.25  $\mu\text{m}$  thickness. The oven was programmed from an initial temperature 70°C raised to 260 C at 6°C/ min. Helium gas was used as the carrier gas at a constant flow rate of 1mL/min and volume of 1 $\mu\text{l}$  was injected at a split ratio 10:1. The identification of phyto-components was confirmed based on the peak area (%), molecular formula and retention time (RT).

## RESULTS AND DISCUSSION

The preliminary phytochemical screening of ethanol, petroleum ether and aqueous extracts of *Costus pictus* leaves are presented in Table 1. Ethanol extract was found to be rich in biological active compounds such as alkaloids, steroids, terpenoids, glycosides, carbohydrates, tannins, phenol, flavonoids and proteins. Similarly, petroleum ether extract

indicated the presence of alkaloids, steroids, terpenoids, glycosides, phenols and flavonoids. Phytochemical compounds like alkaloids, terpenoids, glycosides, carbohydrates, phenols, flavonoids, saponin and proteins were found in aqueous extract. Alkaloids derived from medicinal plants show biological activities like antimicrobial, antioxidant, cytotoxicity.<sup>[9]</sup> Alkaloids represent a class which affects the central nervous system, reduces appetite and behaves as diuretic.<sup>[10]</sup> Terpenoids also possess antimicrobial, antiparasitic, antiviral, antispasmodic, antihyperglycemic, anti-inflammatory and immunomodulatory properties.<sup>[11]</sup> Studies have shown flavonoids possess a wide range of biological and pharmacological activities such as antibacterial<sup>[12,13]</sup> antifungal<sup>[14]</sup> and anticancer.<sup>[15,16]</sup>

**Table 1: Qualitative phytochemical analysis of *Costus pictus* leaf extracts.**

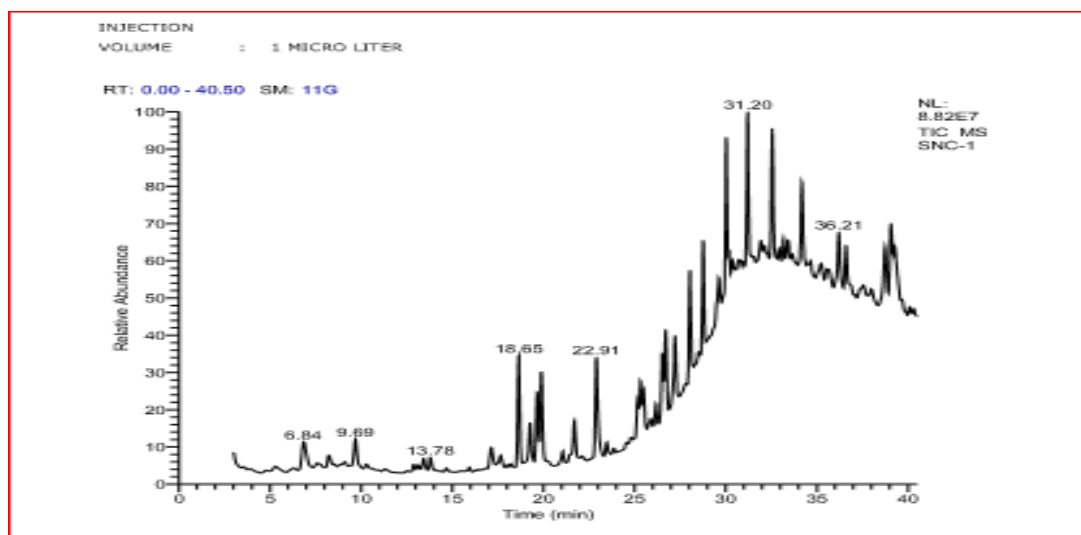
Phytochemicals	Solvents		
	Aqueous	Ethanol	Petroleum ether
Alkaloids	+	+	+
Steroids	-	+	+
Terpenoids	+	+	+
Glycosides	+	+	+
Carbohydrates	+	+	-
Tannins	-	+	-
Saponins	+	-	-
Phenols	+	+	+
Flavonoids	+	+	+
Proteins	+	+	-

+ = Presence of constituents; - = Absence of constituents.

GC-MS analysis of ethanol and petroleum ether led to the identification of phytoconstituents which was confirmed based on the molecular weight (MW), molecular formula (MW), peak area and retention time (RT) (Table 2). The spectrum profile confirmed the presence of number of phyto compounds in ethanol extract of *C. pictus* leaves (Fig 1). The most prevailing compounds are Neophytadiene, Phytol acetate, 3,7,11,15- Tetramethyl-2-hexadecen-1-ol, 2-Pentadecanone, Hexadecanoic acid ethyl ester, Octadecanoic acid ethyl ester, Linoleic acid ethyl ester, Pentacosane, Octacosane, phytadiene, (S)-4-Hydroxymethyl-2-phenyloxazoline, Squalene, Pyranthrene and Methyl 2,4-dimethyltetradecanoate.

**Table 2: Detection of phytochemicals and their pharmacological properties of ethanol extract of *Costus pictus* leaves by GC-MS.**

S no	RT	Peak area (%)	Compound	Molecular formula	Molecular weight	Nature	Activities
1.	8.22	0.82	1-Tridecanol	C <sub>13</sub> H <sub>28</sub>	184	Fatty acid alcohol	Herbicidal
2.	18.65	4.79	Neophytadiene	C <sub>20</sub> H <sub>38</sub>	278	Terpene	Anti-bacterial, antipyretic, analgesic, anti-inflammatory, antimicrobial, antioxidant
3.	18.65	4.79	Phytol, acetate	C <sub>22</sub> H <sub>42</sub> O <sub>2</sub>	338	Diterpene	Anxiolytic, antidepressant, anti-nociceptive, anti-oxidant, hypolipidemic, anti-microbial, anti-inflammatory, anti-cancer, anti-diuretic
4.	19.27	2.05	3,7,11,15- Tetramethyl-2-hexadecen-1-ol	C <sub>20</sub> H <sub>40</sub> O	296	Diterpene	Anxiolytic, antidepressant, anti-nociceptive, anti-oxidant, hypolipidemic, anti-microbial, anti-inflammatory, anti-cancer, anti-diuretic
5.	22.91	5.43	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	Fatty acid	Anti-oxidant, hypocholesterolemi, nematocide, anti-inflammatory
6.	26.69	6.15	Octadecanoic acid/ Stearic acid	C <sub>20</sub> H <sub>40</sub> O <sub>2</sub>	312	Fatty acid	Anti-microbial, anti-cancer, anti-inflammatory, antiarthritic
7.	26.69	6.15	Linoleic acid ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	Fatty acid	Hypocholesterolemic, hepatoprotective, nematocide, anti-arthritis, antihistaminic
8.	30.02	8.36	Pentacosane	C <sub>25</sub> H <sub>52</sub>	352	Essential oil	Anti-oxidant, anti-microbial
9.	30.67	0.84	Nonacosane	C <sub>29</sub> H <sub>60</sub>	408	Acyclic alkanes	Anti-microbial, role in regulatory tissue rebuilding mechanism, biosynthesis of androgens, estrogens and corticoids.
10.	31.91	1.75	Pyranthrene	C <sub>30</sub> H <sub>16</sub>	376	Phenol	Antioxidant
11.	35.66	1.21	Methyl 2,4dimethyltetradecanoate	C <sub>17</sub> H <sub>34</sub> O <sub>2</sub>	270	Fatty acid	Antioxidant, Cancer preventive, hypercholesterolemic, nematocide
12.	36.59	2.38	Squalene	C <sub>30</sub> H <sub>50</sub>	410	Triterpene	Antibacterial, antioxidant, antitumor, drug carrier, detoxifier



**Fig 1: GC-MS chromatogram of ethanol extract of *Costus pictus* leaves.**

Terpenes, diterpenes, fatty acid, essential oils, acyclic alkanes, phenols and triterpene are the main compounds identified in *C. pictus* ethanol extract. The bioactive compounds exert a wide range of biological activities on physiological systems.<sup>[17]</sup> Hexadecanoic acid has antioxidant, hypocholesterolemic, nematicide and anti-inflammatory properties.<sup>[18]</sup> Phytol, a diterpene acts as anti-oxidant, hypolipidemic, anti-microbial, anti-inflammatory, anti-cancer, antidiuretic. Squalene, a triterpene acts as antibacterial, antitumor and cancer preventive agents.<sup>[19]</sup> The compounds identified by GC-MS analysis are medicinally valuable and can be used as potential source for the discovery of herbal drugs.

## CONCLUSION

In the present study qualitative phytochemical screening showed that leaves of *Costus pictus* are extensively rich in secondary metabolites. The GC-MS analysis confirmed the presence of bioactive compounds, which are reported to have biological activities in previous findings. These phytoconstituents are considered as active compounds that have commercial interest in pharmaceutical industries for the development of natural drugs.

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