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Identification of chemical compounds in the essential oil from *Costus pictus* D. Don plant parts antimicrobial studies isolation and quantification of Diosgenin from its root

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ABSTRACT

The chemical compounds present in the essential oil of the different plant parts viz., rhizome, root, stem, leaves and flowers of Costus pictus D. Don were identified by GC MS method. The antimicrobial studies of these essential oils were carried out by disc diffusion method. Diosgenin, a steroidal saponin has been isolated for the first time from the roots of Costus pictus. The diosgenin content in the root of Costus pictus was quantified by HPLC method.

Keywords: Costus pictus, GC MS, antimicrobial activity, diosgenin, HPLC.

INTRODUCTION

The genus *Costus* Linn. belongs to family *Costaceae*, which has been separated from the family *Zingiberaceae* on the basis of the presence of spirally arranged leaves and rhizomes being free from aromatic essential oils. More than 100 species of the genus are distributed in the tropics all over the world. *Costus pictus* D. Don syn. *Costus mexicanus* (DC.) Greene commonly known as Spiral ginger, Stepladder or Insulin plant is a plant originated in Mexico. It was introduced from Mexico to India (Kerala) very recently. In India it is grown in gardens as ornamental plant especially in Kerala almost in every home. The major attraction of this plant is its stem with spiral leaves and light airy and tissue paper like flowers. Red painted stem enhances the beauty of the glossy linear leaves and strongly spiralling canes [1].

In the present work, we describes the extraction of essential oil from the different plant parts of *C. pictus* viz., rhizome, root, stem, leaves and flowers, identification of the chemical compounds present in them by GC-MS analysis and the screening of antimicrobial activity of the essential oils by disc diffusion method. We also describe the isolation, identification and quantification of diosgenin from the root of *C. pictus*.

The literature survey revealed that only a very few phytochemical works has been reported in this plant. Even though there are few reports on the chemical compounds from the essential oil of *C. pictus*, it has not covered all the plant parts. Moreover their antibacterial studies have been limited to the leaf essential oil. Hence in the present work, all the plant parts – root, rhizome, leaf, stem and flower of *C. pictus* has been taken and their antifungal activities has been studied for the first time in addition to their antibacterial activities. A number of compounds has bee identified by GC-MS analysis in addition to the already reported ones from the different plant parts namely stem, leaf and flower essential oil of *C. pictus*. The antibacterial and antifungal activities of essential oil from all plant parts were also investigated by disc diffusion method and a made it a comprehensive work. Diosgenin, a steroid saponin has been isolated for the first time from the root of *C. pictus*. It was identified by m.p., mixed m.p & co-TLC with the reference standard. The quantification of diosgenin in root of *C. pictus* was also carried out by HPLC method.

Costus pictus is well known for its antidiabetic properties [1].The antidiabetic effect of the methanol extract of *C. pictus* leaves has been reported [2]. The earlier studies of the plant extracts on alloxan induced diabetic rats have shown the prevention of diabetes and related complications. The methanolic leaf extract of *C. pictus* is used to lower blood glucose level in alloxan induced diabetic rats [3]. Powdered leaves of *C. pictus* is known to possess therapeutic effect, when supplemented to streptozotocin induced diabetic rats, is found to reduce blood glucose level by 21% after 15 days of supplementation [4]. The antioxidant and anticancer properties of the bark of *C. pictus* has been reported. The chloroform fraction of the bark of *C. pictus* possesses highest antioxidant activity. Chloroform and methanol extracts of *C. pictus* has been reported to possess potent anticancer properties against HT29 and A549 cells [5]. The chloroform extract *of C. pictus* leaf has been reported to possess superoxide radical scavenging activity, inhibition of lipid peroxidation and potent antioxidant activity *in vitro* [6]. The antibacterial activities of the aqueous and methanol extracts of the leaf, root, stem and flower of *C. pictus* has been reported. The antioxidant activities of these different extracts were evaluated by DPPH free radical scavenging activity and in all case methanol extract was reported to have highest antioxidant activity. The total phenolic content in the leaf, root, stem and flower of *C. pictus* has been reported and ranged from 148 to 233 μg of gallic acid/g of plant extracts. Also the flavonoid content has been reported varies from 370 to 930 μg of quercetin/g of plant extracts [7].

Jayasri et al. estimated the presence of trace elements in *C. pictus* and showed that the leaves and rhizomes contain appreciable amounts of various elements such as K, Ca, Cr, Mn, Cu and Zn, which may be responsible for potentiating insulin action [8]. *C. pictus* leaf has been reported to contain various phytochemicals like alkaloids, glycosides, carbohydrates, saponins, proteins and phenols [9, 10]. Shilpa et al. isolated methyl tetracosanate from bioactivity guided purification of methanolic extracts of *C. pictus* leaves which showed an optimum glucose uptake at 1 ng/mL in 3T3-L1 adipocytes [11].

Beena and Reddy analyzed the volatile constituents of *C. pictus* stems, leaves and rhizomes and palmitic acid was found to be the major component in the stem, leaf and rhizome [12]. The essential oils of the stems, leaves and rhizomes of *C. pictus* from southern India were reported. The essential oil of *C. pictus* stem is rich in hexadecanoic acid (28.3%), 9, 12-octadecadienoic acid (18.33%), linalyl propanoate (6.03%), dodecanoic acid (5.62%), tetradecanoic acid (4.82%), α-eudesmol (3.55%), γ-eudesmol (3.21%) and 4-ethoxy phenol (3.06%). The leaf essential oil contains hexadecanoic acid (24.51%), 2-pentanol (22.48%), β-ionone (8.69%), α-ionone (8.01%), farnesyl acetone (7.04%) and dodecanoic acid (3.96%). Hexadecanoic acid (25.26%), dodecanoic acid (16.56%), tetradecanoic acid (10.20%), linalool (8.48%), 9, 12-octadecadienoic acid (7.74%), and α-terpineol (4.44%) are the main constituents of the rhizome essential oil [12]. Shiny et al. isolated a glycosidic compound from the methanol extract of *C. pictus* leaf which was similar to β-L-Arabinopyranose methyl glycoside and suggested that it might be the inducer molecule of its antidiabetic property [13].

The antibacterial property of the *C. pictus* leaf oil was checked against ten pathogenic bacteria namely *Bacillus cereus, Enterobacter faecalis, Salmonella paratyphi, Staphylococcus aureus, Escherichia coli, Streptococcus faecalis, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Serratia marcescens* and showed varying degrees of antibacterial activity on the microorganisms tested [14].

EXPERIMENTAL SECTION

Plant Material

The root, rhizome, stem, leaf and flower of *Costus pictus* were collected from Kozhikode District, Kerala, India during May 2014 and duly identified by Dr. P. S. Udayan, Professor, Dept of Botany, Sri Krishna College, Guruvayoor, Kerala. A voucher specimen was deposited at the herbarium of the University of Calicut for future references. The root, rhizome, stem and leaf were washed with water, cut into small pieces and grinded in a mixer grinder. The flowers were also washed with water and grinded in a mixer grinder.

The root of *C. pictus* collected as in above was used for the isolation of diosgenin. The roots were washed with tap water, dried in the shade and coarsely powdered in a mixer grinder.

GC-MS analysis

GC-MS analysis was performed by the split injection (1:10) of 1.0 μL of the essential oil in hexane on a Perkin Elmer GC (Clarus 680) fitted with Elite 5 cross bonded 5% diphenyl-95% dimethyl polysiloxane capillary column (30 m x 0.25 mm x 0.25 μm coating thickness), coupled with a mass detector (Clarus 600 T) and Turbo Mass version 5.4.2 software. GC-MS operating conditions are as follows: injector temperature: 250 ºC for all plant parts; transfer line temperature: 280 ºC for all plant parts; detector temperature: 280 ºC (for flower & stem), 300 ºC (for leaf, root & rhizome).

Oven temperature programme: (i) for flower: 100 ºC hold for 5 min, 250 ºC with ramping 5 ºC min^{-1}, hold 250 ºC for 10 min. (ii) for leaf: 150 ºC hold for 10 min, 250 ºC with ramping 3 ºC min^{-1}, 275 ºC with ramping 1 ºC min^{-1}, hold 275 ºC for 5 min. (iii) for stem: 60 ºC hold for 5 min, 110 ºC with ramping 5 ºC min^{-1}, 220 ºC with ramping 3 ºC min^{-1}, 250 ºC with ramping 5 ºC min^{-1}, hold 250 ºC for 5 min. (iv) for root & rhizome: 100 ºC hold for 5 min, 280 ºC with ramping 10 ºC min^{-1}, hold 280 ºC for 10 min. Carrier gas: helium at a flow rate of 1.0 mL min^{-1}, mass spectra: electron impact (EI+) at 70 eV, ion source temperature: 250 ºC (for flower & stem), 280 ºC (for leaf, root & rhizome). Individual components were identified by NIST database matching. The percentage composition was determined by area normalization from TIC.

Chemicals and Reagents used
Solvents such as methanol, petroleum ether, ethyl acetate etc. used were of analytical/ HPLC grade (E. Merck). Precoated silica gel 60 F_{254} plates (E. Merck) of uniform thickness 0.2 mm were used for TLC. HPLC water was obtained from Millipore water purification system. The standard diosgenin was purchased from Sigma Aldrich. Antimony trichloride was purchased from S. D. Fine Chemicals, Mumbai.

HPLC instrument
The Shimadzu (Japan) HPLC system consisted of LC-10AT VP pump, SPD M10A VP photodiode array detector, SCL-10A system controller, CLASS-VP 6.12 SP5 integration software and a Rheodyne model 7725 i syringe-loading sample injector fitted with a 20 µL injection loop, was used for the analysis. Baseline resolution of diosgenin was obtained at 26 ± 2 °C using a Phenomenex Luna C_{18} column (250 x 4.6 mm i.d; 5 µm) and Phenomenex guard column (4 x 2 mm i.d; 5µm). HPLC method was validated by performing the sensitivity, linearity and recovery studies.

Isolation of the essential oil
The essential oil was obtained by the hydrodistillation of the rhizome (2 Kg), root (300 g), leaf (1.5 Kg), stem (1.8 Kg) and flower (100 g) of *C. pictus* separately in a Clevenger apparatus for 3 h. The essential oil obtained was dissolved in diethyl ether (10 mL). The organic phase was dried over anhydrous sodium sulphate and filtered. After removal of the solvent, yellow transparent oil with pleasant odour in the case of leaf (0.02% v/w), stem (0.10% v/w) and root (0.05% v/w) and colourless oil with pleasant odour in the case of rhizome (0.03% v/w) and flower (0.06% w/w) was obtained which were stored in refrigerator at 4 ºC until analyzed.

Antimicrobial assay
Test microorganisms employed for *in vitro* antimicrobial assay were obtained from Department of Microbiology, Kuvempu University, Shankarghatta, Shimoga, Karnataka and antifungal assay were obtained from Department of Microbiology, University of Mysore, Manasagangothri, Mysore, Karnataka. The antimicrobial assay of the essential oil was performed by standard filter paper disc diffusion technique [15, 16]. A total of two Gram positive, three Gram negative bacteria and five pathogenic fungi were used for this antimicrobial screening. The test solution at a concentration of 2% was prepared by dissolving the essential oil in hexane and Minimum Inhibitory Concentration (MIC) was reported. 0.1% streptomycin (Hi Media) and bavistin (0.2%) were used as standards for antibacterial and antifungal activities respectively. A blank disc impregnated with hexane followed by drying off was used as a negative control.

Briefly, the test discs, standard discs and blank discs were placed in the Petri dishes that contain the specific bacterial or fungal culture and then left in a refrigerator at 4 ºC for 12-18 h in order to diffuse the material from the discs to the surrounding media. The Petri dishes were then incubated at 37 ºC for overnight to allow the bacterial growth and 48-72 h for fungal growth. The antibacterial and antifungal activities of the essential oil were then determined by measuring the respective zones of inhibition in mm.

Isolation and identification of diosgenin
200 g of the coarsely powdered roots of *C. pictus* was completely defatted with petroleum ether (b.p. 60-80 ˚C) in a Soxhlet apparatus. The dried defatted marc was extracted with MeOH (1 L) in a Soxhlet apparatus for 12 h. MeOH extract was then evaporated to dryness in a rotary evaporator under vacuum at 50 °C. About 200 mL of 4N HCl was added to the residue, heated to hydrolyse on a water bath for 30 min. It was cooled, filtered and the aq. layer was partitioned with 50 mL of $CHCl_3$. The lower chloroform layer was collected. This was repeated 6 times. The combined chloroform extract was evaporated to about 25 mL in a rotary evaporator and allowed to cool. On cooling, colourless solid was separated out. It was recrystallized from hot methanol which yielded crystalline diosgenin (16 mg) with m. p. (205-207 °C). The diosgenin was confirmed by m. p., mixed m. p. and co-TLC with the authentic standard.

Thin Layer Chromatogram of the plant extract was developed using the solvent system toluene: ethyl acetate: formic acid (5:2:1). The analyte detection was done under Visible and UV light. The developed plates were derivatised using 10% $SbCl_3$ reagent in $CHCl_3$.

Quantification of diosgenin by HPLC method

Preparation of standard solution of the extract and diosgenin for HPLC analysis

10 g of the powdered root of *C. pictus* was extracted with 100 mL 80% aqueous MeOH (acidified with 4N HCl) in a soxhlet extractor for 8 h. The solvent was removed under suction. The residue was redissolved in MeOH and made up to the mark in a 25 mL volumetric flask with MeOH so that 1 mL of standard solution contains 1 mg of the extract. It was then filtered through 0.20 µm nylon-6, 6 membrane. This solution was used for the HPLC analysis.

A stock solution containing 1 mg/mL of standard diosgenin was prepared in methanol. The working standards were prepared by suitably diluting the stock solution with methanol.

HPLC analysis

Baseline resolution of diosgenin was obtained at 25 ± 2 °C using a Phenomenex Luna C-18 column using the solvent system 70:30 v/v acetonitrile: water (containing 0.1% HCOOH). The mobile phase was passed through 0.45 µm PVDF filter and degassed before use. The flow rate was kept constant at 1.0 mL/min and the detection was done at 203 nm. For calibration, standard solutions of diosgenin were prepared at concentrations of 50, 100, 200, 300, 400 and 500 µg/mL using methanol as solvent. The standard solution (20 µL) was injected in triplicate and the average detector response was measured. The root extracts were assayed in triplicate, peak areas corresponding to diosgenin (RT: 3.91 min) were compared with the calibration curve and thus the amount of diosgenin was determined. The amount of diosgenin present in the root was found to be 1.32% w/w (dry weight basis).

The developed HPLC method was validated for specificity, linearity, precision and accuracy. The limit of quantification for diosgenin was 0.1 µg/mL. A correlation coefficient of 0.998 indicates good linearity between the concentration and the area. The recovery studies were also carried out and the recovery value of diosgenin by this method was 83.1 ± 0.5%. The proposed method is simple, accurate and reliable for quantitative monitoring of diosgenin in the root *C. pictus*.

RESULTS AND DISCUSSION

Identification of chemical compounds in the essential oil from the C. pictus D. Don plant parts & its antimicrobial studies

The essential oil obtained by the hydrodistillation of flower, leaves, stem, rhizome and root of *C. pictus* gave a yield of 0.06% v/w, 0.02% v/w, 0.10% v/w, 0.03% v/w and 0.05% v/w respectively on fresh plant material basis. Table 1 shows the chemical composition of the essential oil from the different plant parts of *C. pictus*. The chemical compounds were identified by comparison of the mass spectra of each GC component with those of the standards available by library search. Palmitic acid (n-hexadecanoic acid) was found to be the major constituent in all the five essential oils together with other fatty acids, hydrocarbons and mono, di and sesquiterpenes. TIC of the essential oil from the rhizome of *C. pictus* is shown in Figure 1.

The essential oil from the flowers of *C. pictus* contains mono and sesquiterpenes such as thymol (5.23%), nerolidol (0.91%), α–farnesene (0.67%), caryophyllene (0.28%) and trans-Z-α-bisabolene epoxide (0.22%) together with other compounds. The leaf essential oil contains β–sitosterol acetate (0.18%), diterpenes such as phytol (2.16%) isophytol (1.64%) and geranyl geraniol (1.21%) together with other compounds. The stem essential oil contains 5.66% of phytol together with other compounds. The essential oil from the rhizome of *C. pictus* contains mono terpenes such as thymol (0.66%) and β-myrcene (0.08%) together with other compounds. The essential oil from the root of *C. pictus* contains mainly fatty acids and long chain hydrocarbons.

Phytol, a diterpene alcohol is used in the fragrance industry. It is also used in the cosmetics, shampoos, toilet soaps, household cleaners and detergents. Phytol can be used as a precursor for the manufacture of synthetic forms of Vitamins E and K1. β-myrcene and δ-nerolidol are also used in the perfumery. Gernayl geraniol is an intermediate in the biosynthesis of vitamins E and K. It is pheromone for a variety of insects and a potent inhibitor of *Mycobacterium tuberculosis*. Trans-Z-α-bisabolene epoxide and α-farnesene are also act as pheromone.

The antibacterial and antifungal activities of the essential oil of *C. pictus* plant parts have been assayed at concentration of 2% against strains of both Gram positive and Gram negative bacteria and pathogenic fungi. The susceptibility testing was carried out by measuring the inhibitory zone diameters on nutrient agar, with conventional disc diffusion method and the inhibitory zone diameters were read and rounded off to the nearest whole number

(mm) for analysis (Figure 2). The inhibitory effects of the essential oil against these microorganisms are given in Tables 2 and 3.

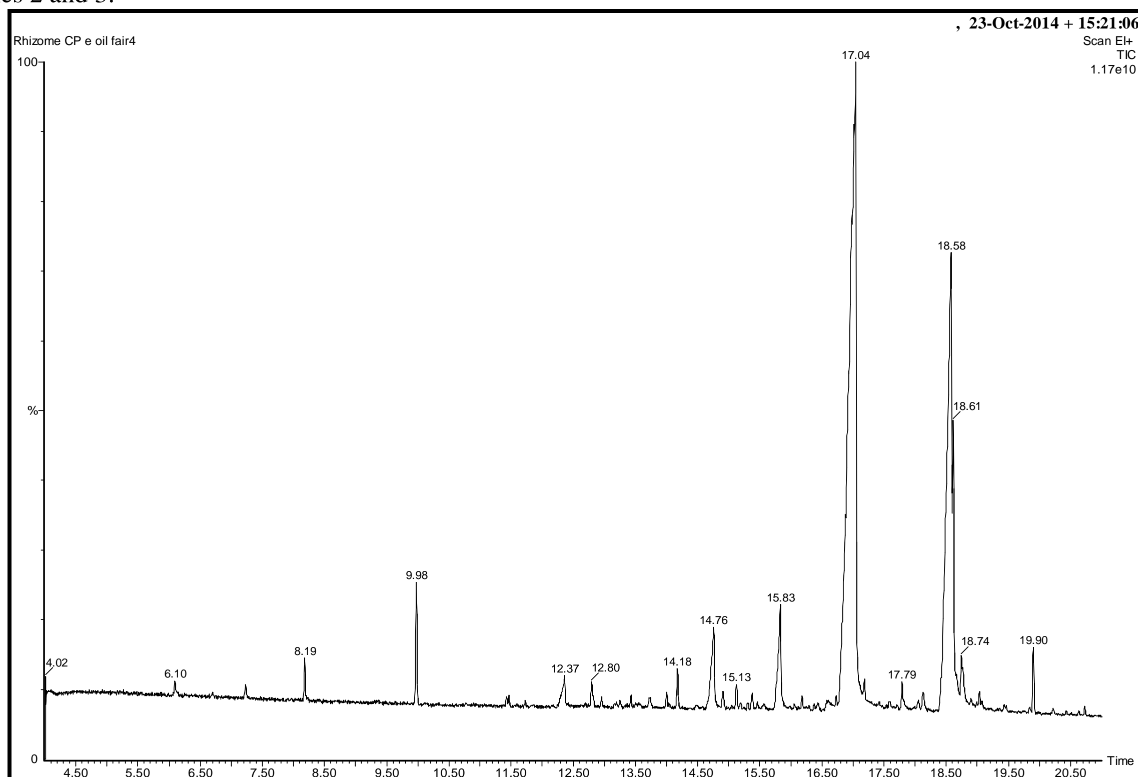

Figure 1. TIC of the essential oil of *C. pictus* rhizome

Table 1. Chemical compounds identified in the essential oil of Costus pictus plant parts

Sr.no	Name of the compound	Flower (RT min, Conc %)	Leaf (RT min, Conc %)	Stem (RT min, Conc %)	Rhizome (RT min, Conc %)	Root (RT min, Conc %)
1	Thymol	10.06, 5.23	-	-	8.19, 0.66	-
2	β-caryophyllene	13.22, 0.28	-	-	-	-
3	Trans-Z-α-bis- abolene epoxide	15.42, 0.22	-	-	-	-
4	α –farnesene	16.70, 0.67	-	-	-	-
5	δ-nerolidol	24.28, 0.91	-	-	-	-
6	Homopiperazine	20.22, 0.52	-	-	-	-
7	Geranylgeraniol	-	27.27, 1.21	-	-	-
8	Isophytol	-	28.11, 1.64	-	-	-
9	Phytol	-	31.53, 2.16	27.14, 5.66	-	-
10	β-myrcene	-	-	-	7.23, 0.08	-
11	Kaur-16-ene	-	-	-	-	16.96, 11.04
12	Thunbergol	-	-	-	-	19.41, 4.24
13	β –sitosterol acetate	-	21.74, 0.18	-	-	-
14	n-decanoic acid	12.12, 0.26	-	-	-	-
15	Tetradecane	12.67, 0.51	22.66, 0.13	-	9.98, 1.87	-
16	Dodecanoic acid	17.25, 0.80	17.73, 0.11*	-	12.37, 0.14*	-
17	Tetradecanoic acid	21.54, 1.71	24.46, 1.55	-	14.76, 1.72*	14.67, 0.52
18	E-7-octadecene	22.89, 0.06	-	-	-	-
19	Pentadecanoic acid	23.58, 1.85	26.70, 0.68	-	16.83, 2.33	-
20	5-methyl heneicosane	24.10, 0.05	-	-	-	-
21	Heptacosanoic acid methyl ester	24.55, 0.03	-	-	-	-
22	n-hexadecanoic acid	26.55, 36.82	30.12, 43.50*	23.14, 46.86*	17.04, 46.23*	16.85, 38.17
23	Eicosanoic acid	27.50, 0.88	25.35, 1.24	15.17, 2.84	15.38, 0.02	15.75, 0.65
24	1-octadecyne	27.78, 0.97	-	-	-	12.95, 0.12
25	Octacosane	27.87, 0.72	40.67, 0.15	-	19.90, 2.58	17.16, 0.20
26	Tritetracontane	27.93, 0.04	34.78, 0.03	-	-	-
27	9,12-octadeca-dienoic acid	29.51, 28.68	-	28.30, 18.94*	18.58, 14.03	-
28	Octadecanoic acid	29.69, 0.35	-	-	-	-
29	17-pentatri-acontene	30.93, 0.24	25.79, 0.04	26.92, 0.06	-	-
30	Hexacosane	31.61, 5.64	-	-	-	-
31	Hexatriacontane	33.07, 2.15	38.79, 0.12	29.97, 0.48	12.80, 0.09	15.11, 0.21
32	1-octacosanol	34.19, 1.28	-	-	-	-
33	Tetratetra- contane	34.74, 3.22	-	32.86, 0.14	-	16.17, 0.04
34	1-heneicosanol	37.62, 0.50	-	-	-	-
35	1-decosene	37.78, 0.21	-	-	-	-
36	1-tetracosanol	38.02, 0.43	-	-	-	-
37	Tetracosane	38.18, 0.55	-	-	14.0, 0.02	-
38	2-aminonona-decane	-	14.68, 0.05	-	-	-

39	Dotriacontane	-	15.43, 0.94	23.67, 1.08	16.18, 0.01	19.02, 0.11
40	Hexadecane	-	18.35, 0.09	-	-	9.97, 5.83
41	Hexadecyloxira-ne	-	26.25, 0.02	21.71, 0.52	-	-
42	2-hexyl-1-decanol	-	26.36, 0.76	-	-	-
43	Z-5,17-octadecadien-1-ol acetate	-	32.86, 19.89	-	-	-
44	1-pentacontanol	-	33.01, 0.48	-	-	-
45	Pentatriacontane	-	35.97, 0.06	-	-	-
46	n-butylmyristate	-	-	18.88, 0.26	-	-
47	2-methyl-octadecane	-	-	20.14, 0.08	-	-
48	9,12-tetradecadien-1-ol	-	-	28.43, 6.32	-	-
49	1-hexacosene	-	-	28.95, 0.75	-	-
50	14-hepta- decenal	-	-	-	12.96, 0.03	-
51	Octadecanal	-	-	-	14.18, 0.89	-
52	Heneicosane	-	-	-	15.13, 0.07	13.99, 0.08
53	Oleic acid	-	-	-	15.46, 0.02	-
54	Heptadecanoic acid	-	-	-	17.79, 0.07	-
55	9,17-octadecadienal	-	-	-	18.61, 4.65	-
56	Heptacosane	-	-	-	20.73, 0.03	18.11, 0.02
57	11-tridecen-1-ol	-	-	-	-	10.11, 0.02
58	2-heptadecenal	-	-	-	-	13.72, 0.10
59	Hexadecanal	-	-	-	-	14.17, 3.96
60	Octadecanol	-	-	-	-	15.30, 0.02
61	Tetradecanal	-	-	-	-	16.36, 0.01
62	9-octadecenal	-	-	-	-	18.48, 0.17
	Total isolate	95.78	75.62	84.01	75.54	65.51
	Unidentified	4.22	24.38	15.99	24.46	34.49

** Previously reported compounds*

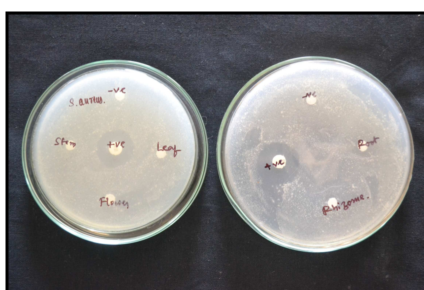

Figure 2. Antibacterial activity of essential oil of *C. pictus* plant parts against *Staphylococcus aureus*

Table 2. *In vitro* antibacterial activity of essential oil of *C. pictus* plant parts

Sample[a]	*Escherichia coli*	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Salmonella typhimurium*
	Diameter of Inhibition zone (mm)[b]				
Positive Control (Streptomycin)	20 ± 0.05	25±0.06	20 ± 0.03	15 ± 0.06	25 ± 0.08
Negative Control (Hexane)	-	-	-	-	-
Leaf essential oil	5 ± 0.02	-	5 ± 0.06	6 ± 0.02	-
Stem essential oil	5 ± 0.02	-	4 ± 0.02	8 ± 0.05	-
Flower essential oil	5 ± 0.04	6 ± 0.03	6 ± 0.05	6 ± 0.05	-
Root essential oil	-	5 ± 0.01	8 ± 0.04	10 ± 0.08	6 ± 0.03
Rhizome essential oil	-	6 ± 0.03	15 ± 0.09	7 ± 0.04	-

[a]Standard and test solutions prepared in hexane
[b]Values are the mean of three replicates

Table 3. *In vitro* antifungal activity of essential oil of *C. pictus* plant parts

Sample[b]	*Fusarium oxysporum*	*Aspergillus tamarii*	*Rhizopus stolonifer*	*Cladosporium cladosporioides*	*Nigrospora oryzae*
	Diameter of Inhibition zone (mm)[a]				
Positive Control (Bavistin)	30 ± 0.09	36 ± 1.0	40 ± 0.07	38 ± 0.06	52 ± 1.2
Negative Control (Hexane)	-	-	-	-	-
Leaf essential oil	-	-	5 ± 0.03	-	-
Stem essential oil	-	-	-	-	-
Flower essential oil	-	-	-	-	-
Root essential oil	-	7 ± 0.05	-	-	-
Rhizome essential oil	-	-	-	-	-

[a]Values are the mean of three replicates
[b]Standard and test solutions prepared in hexane

The screening results indicate that the leaf and stem essential oils showed moderate antibacterial activity against both Gram-negative bacterial strains *Escherichia coli* (MTCC-2125), *Pseudomonas aeruginosa* (MTCC-1688) and Gram-positive bacterial strain *Staphylococcus aureus* (MTCC-3160) and no activity against Gram-negative bacterial strain *Salmonella typhimurium* (MTCC-1357) and Gram-positive bacterial strain *Bacillus subtilis* (MTCC-1789). The flower essential oil showed moderate antibacterial activity against all the four bacterial strains except *S. typhimurium*. The root essential oil showed good antibacterial activity against *S. aureus*; moderate antibacterial

activity against *B. subtilis*, *P. aeruginosa* and *S. typhimurium* and no activity against *E. coli*. The rhizome essential oil showed good antibacterial activity against *P. aeruginosa*; moderate antibacterial activity against *B. subtilis* and *S. aureus* and no activity against *E. coli* and *S. typhimurium*.

E. coli strain was highly susceptible with MIC to 13.5 ± 0.3 mg/mL with leaf, stem and flower essential oils. *B. subtilis* strain was highly susceptible with MIC to 11 ± 0.6 mg/mL with flower, root and rhizome essential oils. *P. aeruginosa* was susceptible with MIC to 14.2 mg/mL with stem essential oil; 13 ± 0.5 mg/mL with leaf and flower essential oils; 8.39 mg/mL with root essential oil and 2.6 mg/mL with rhizome essential oil. *S. aureus* was susceptible with MIC to 12.8 ± 0.2 mg/mL with flower and leaf essential oils; 8.65 ± 0.7 mg/mL with stem and rhizome essential oils and 4.7 mg/mL with root essential oil. *S. typhimurium* was susceptible with MIC to 14 mg/mL with root essential oil.

Similarly, the screening results against the pathogenic fungi indicated that only root and leaf essential oils showed moderate antifungal activity against *Aspergillus tamarii* (MTCC-8841) and *Rhizopus stolonifer* (MTCC-2591) respectively and showed no activity against other pathogenic fungi tested namely *Fusarium oxysporum* (MTCC-4118), *Cladosporium cladosporioides* (MTCC-3507) and *Nigrospora oryzae* (MTCC-8465). The essential oil from other plant parts showed no activity against these five tested fungi.

Aspergillus tamarii strain was highly susceptible with MIC to 11.5 ± 0.3 mg/mL with root essential oil. *Rhizopus stolonifer* strain was highly susceptible with MIC to 13 ± 0.6 mg/mL with leaf essential oil.

In the present study, we may attribute the antibacterial and antifungal activities of the essential oil of *C. pictus* plant parts are due to the higher percentage of various mono, di and sesquiterpenes present in it. The antifungal activity of sesquiterpenes such as caryophyllene oxide has been well established [17]. The antimicrobial activities of essential oils are well documented, which are found application as antibacterial, antifungal or antiviral agents for food preservatives, in clinical microbiology or in pharmaceutical preparations [18, 19]. According to literatures, these components exert antimicrobial effects through the disruption of bacterial or fungal membrane integrity and the inhibition of respiration and ion transport processes. With the alarming incidence of antibiotic resistance in microorganisms of medical importance, there is increasing interest in plants as sources of agents for the treatment of infectious diseases [20, 21, 22, 23, 24]. Additionally, the main advantage of naturally occurring antimicrobial agents is that they do not enhance resistance among microorganisms, a phenomenon commonly encountered with the use of synthetic antibiotics [25].

The antimicrobial properties of the mono, di and sesquiterpenes isolated from different plants were reported earlier. Trans-Z-α-bisabolene epoxide, thymol, β- Caryophyllene etc. are reported to have good antibacterial and antifungal properties. Essential oil with phenols such as thymol, eugenol, carvacrol etc. as main compounds expresses the highest and broadest activity against both bacteria and fungi. The highest activity of the phenols is explained by the acidic character of the –OH group forming hydrogen bond with an enzyme active centre. The antimicrobial activity of hydrocarbons is lower than that of oxygenated essential oil components [26, 27, 28, 29].

Thymol has been reported to show antibacterial activity against bacterial strains including *Aeromoans hydrophila* and *Staphylococcus aureus*. The antibacterial activity is caused by inhibiting growth and lactate production and by decreasing cellular glucose uptake [26]. The antifungal nature of thymol is caused by thymol's ability to alter the hyphal morphology and cause hyphal aggregates, resulting in reduced hyphal diameters and lyses of hyphal wall [30].

Isolation and identification of diosgenin from the root of Costus pictus

The preliminary phytochemical investigation of the root of *C. pictus* revealed the presence of steroid and saponin compounds among other secondary metabolites. In line with the presence of diosgenin in the root of *Costus speciosus* (a closely related species), an attempt has been made to check the presence of diosgenin in the root of *C. pictus*. The TLC profile of the 80% aq. methanol extract (acidified with HCl) gave a greenish blue spot corresponding to standard diosgenin at R_f 0.38 on spraying with 10% antimony trichloride reagent in $CHCl_3$ (Figure 3). Diosgenin was successfully isolated from the root of *C. pictus* by adopting the reported method [31]. The diosgenin (Figure 4) was confirmed by its m.p. (205-207 °C), mixed m.p. and co-TLC with the authentic standard.

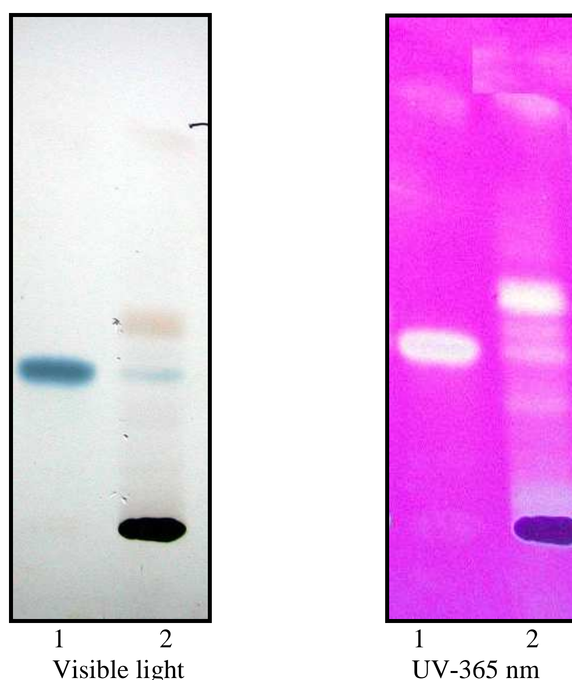

Figure 3. TLC Plate dipped in 10% antimony trichloride reagent
Track 1. Standard diosgenin
Track 2. Methanolic extract of *C. pictus* roots

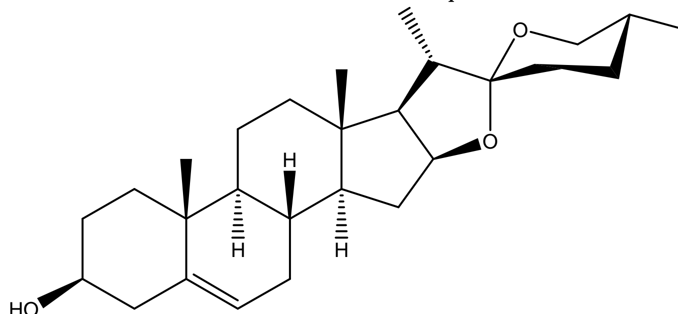

Figure 4. Structure of Diosgenin ($C_{19}H_{18}O_{11}$)

Quantification of diosgenin by HPLC method

HPLC method was developed for the quantification of diosgenin in the *C. pictus* roots. 1 mg/mL solution of standard diosgenin was prepared. From the stock solution different concentrations viz. 50, 100, 200, 300, 400 and 500 µg/mL were prepared and calibration curve was plotted to check the linearity. 1 mg/mL solution of methanolic extract of *C. pictus* root was used as the test sample. The mobile phase used was 70:30 v/v acetonitrile: water (containing 0.1% HCOOH) as at a flow rate of 1.0 mL/min under isocratic conditions. The chromatogram was viewed at a wave length of 203 nm. Diosgenin was eluted at RT of 3.91 min.

Calibration curve was plotted to check the linearity range and diosgenin content was found to be 1.32% w/w in the dried roots of *C. pictus*. The HPLC chromatogram of the std diosgenin and the methanol extract of the root of *C. pictus* are given below (Figures 5 & 6).

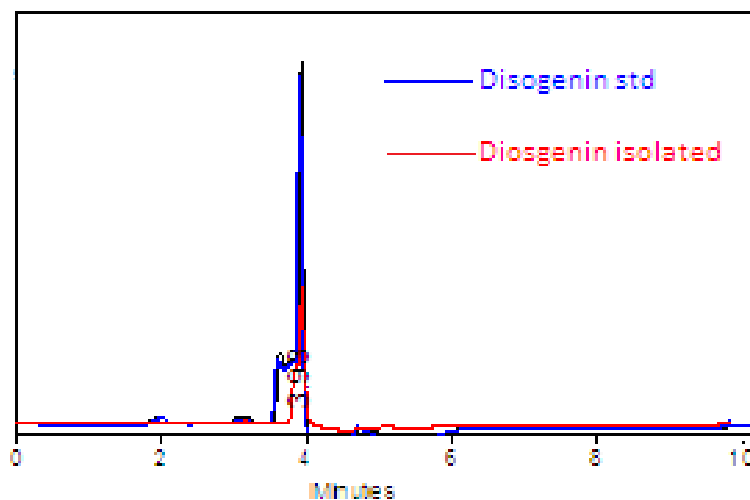

Figure 5. HPLC chromatogram of standard diosgenin

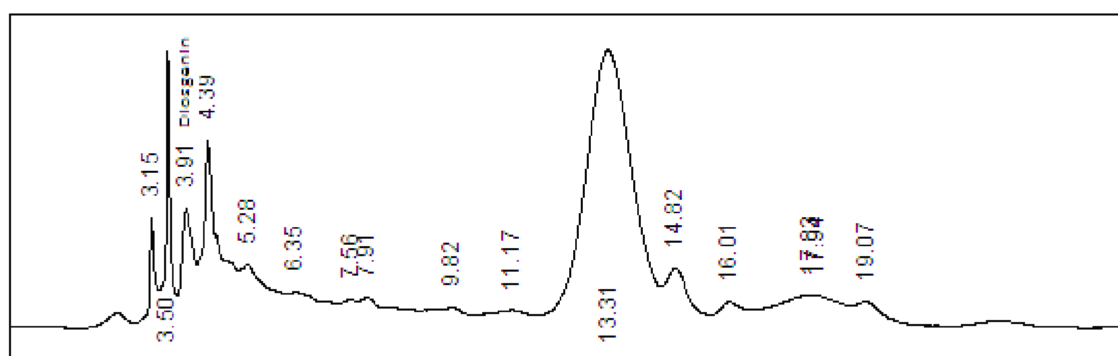

Figure 6. HPLC chromatogram of *Costus pictus* root extract

CONCLUSION

The present investigation has justified the folklore use of *Costus pictus*. The potential for developing antimicrobial drug through screening of higher plants is rewarding as it will lead to the development of a phytomedicine to act against microbes. The plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic compounds.

C. pictus is regarded as a valuable plant source in folklore and modern drug development. The various compounds identified from the essential oil of the plant parts of *C. pictus* are reported to possess a wide range of pharmacological activities.

The essential oil from *C. pictus* was found effective against some bacterial and fungal pathogens which are associated with fever, skin diseases, dysentery and respiratory problems. The antimicrobial activity results presented here demonstrate that this plant essential oil has a commercial potential. The antimicrobial activity shown by the oil may be due to a synergistic effect of various components present in it. It is usually found that such combined effect of various components is responsible for therapeutic activities of the plant extracts.

Even though the essential oil from the different parts of *C. pictus* shows promising results for antimicrobial activity against various bacterial and fungal strains, the higher palmitic acid (n-hexadecanoic acid) content in them is worrisome. The essential oils from the flowers, leaves, stem, rhizome and root of *C. pictus* contained palmitic acid as the major constituent with concs. 36.82%, 43.5%, 46.86%, 46.23% and 38.17% respectively. Palmitic acid increases Low Density Lipoprotein (LDL) cholesterol levels and raise the LDL to HDL ratio in healthy adults. High levels of LDL cholesterol increase the risk for coronary heart diseases. Consumption of foods rich in saturated fatty acids and cholesterol has long been recognized as an important precursor for the development of coronary heart diseases and saturated fatty acids elevate LDL concentration in the blood. Moreover palmitic acid induces

degeneration of myofibrils in rat adult cardiomyocytes. Exposure of adult rat cardiomyocytes to palmitic acid for 18 hours has shown destroyed both the contractile elements and the cytoskeleton. The deleterious effect of palmitic acid on myofibrils preceded DNA fragmentation and was detectable 3 h after administration of palmitic acid. Palmitic acid, being the major component of the essential oil from the different parts of *C. pictus*, the constant use of especially *C. pictus* leaves for diabetic treatment may cause serious health hazards and it must be avoided.

Diosgenin, a steroid saponin has been reported for the first time from the roots of *C. pictus.* Diosgenin is a major bioactive constituent of various edible pulses and roots. It is well characterized in the seeds of fenugreek (*Trigonella foenum graecum* Linn) as well as in the root tubers of wild yams (*Dioscorea villosa* Linn). Diosgenin is structurally similar to cholesterol and other steroids. Since its discovery, diosgenin is the single main precursor in the manufacture of synthetic steroids in the pharmaceutical industry. Diosgenin is used for the commercial synthesis of cortisone, pregnenolone, progesterone and other steroid products [32]. Diosgenin is reported to have antitumour, hypocholesterolemic, antidiabetic, antioxidant, antiobesity antiinflammatory etc activities [33].

In the present scenario, China and Mexico are the top two countries with the richest yam resource in the world, the yield of diosgenin accounts for 67% of world production. *C. speciosus*, a closely related species of *C. pictus* is otherwise known as the ‘Indian source of diosgenin’. The outcome of the present study is that, the root of *C. pictus* contain diosgenin. Hence root of *C. pictus* can also be used an important raw material for the production of diosgenin along with *C. speciosus*. In order to find out for getting maximum yield of diosgenin from the root of *C. pictus*, different factors which may contribute the production of diosgenin such as geo climatical conditions, harvesting time, age of the plant etc has to be investigated in detail.

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