HRGS' Ayurveda Journal
Journal of Ayurveda Medical Sciences
Peer Reviewed Journal of Ayurveda and other Traditional Medicines
ISSN: 2456-4990|www.jayumedsci.com|jayumedsci@gmail.com

Original Article
In vitro screening

Comparative Phytochemical and Antioxidant Properties of *Costus pictus* and *C. speciosus*

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ABSTRACT

***Introduction*:** *C. pictus,* known as spiral ginger, an ornamental plant which has its origin in Mexico, but cultivated in Indian state of Kerala. *C. speciosus* could be collected from its natural habitat, but due to ruthless and indiscriminate collection it is endangered. These species are used in many of the indigenous preparation, mainly known for anti-diabetic and anti-hyperlipidaemic properties. The current study is an attempt to compare the phytochemical and antioxidant potential of the two species. ***Methods*:** Phytochemical test was carried out in order to figure out the constituents present in the two species of *Costus*. Further antioxidant activity was carried out in ethanolic extracts by DPPH radical scavenging assay and reducing power assay *in vitro* by standardised chemical methods using ascorbic acid as standard. Preliminary phytochemical tests and WHO recommended parameters of standardisation were performed. HPTLC fingerprinting profile of the leaf was also been carried out. ***Results*:** Antioxidant potential of *C. speciosus* and *C. pictus* is attributed to mainly phytoconstituents such as tannins, phenols, flavonoids. Both the species showed equally good antioxidant potential, *C. speciosus* was better radical scavenger in DPPH assay while *C. pictus* had a better reducing power. ***Conclusion*:** The study has provided evidence of good antioxidant in the species of *Costus* investigated.

KEYWORDS

Ascorbic acid, Free radicals, insulin plant, spiral ginger

Received: 06.03.2017 **Accepted:** 23.03.2017 **DOI:** 10.5530/jams.2017.2.2

Costus speciosus (Koen. Ex Retz.) J. E. Smith, Fam. Costaceae (Zingiberaceae) is a succulent perennial herb with tuberous, horizontal rhizome growing up to 1 to 3 m height with spirally arranged leaves. It is found commonly along roadsides, streams and wastelands throughout the country in most tropical evergreen forests, up to an altitude of 1200 m.[1] The plant is commercially important for the chemical compound called diosgenin which is a precursor for steroids. The plant is used as anthelmentic and in filariasis. The bruised leaves are applied in fevers,[2] in scabies and on wounds;[3] in mumps;[4] cough, asthma[5] and for hair growth.[6] Phytoconstituents isolated from this species include diosgenin,[7] furostanolsaponins-costusosides I and J,[8] β-sitosterol-β-D-glucoside, prosapogenins A and B of dioscin, gracillin, dihydrophytylplastoquinone, α-tocopherolquinone and 5α-stigmastero-9(11)en-3β-ol; methyl hexadecanoate, methyl octadecanoate and tetracosanyl octadecanoate,[8] 24-hydroxytriacontan- 26-one and 24-hydroxytriacontan-27-one.[8] Other components identified are 31-norcycloartanone, cyloartanol, cycloartenol and cycloalaudenol.[9]

Costus pictus D. Don. is an ornamental plant which is newly introduced to India. It is an erect herb growing up to 3 metres tall, having stem horizontally striped at base; leaves narrowly lanceolate, dark green above, lighter green below; small leaves are present on the basal part; bracts green, with outer margin coloured maroon. Flowers yellow; lip with maroon striations, darker yellow stripe down the middle region; anther cream coloured. *C. pictus* species is used in the treatment of renal disorders and as hypoglycaemic.[10-11]

In this study, we have put an effort to compare the two species by their phytochemical nature, qualitative HPTLC fingerprinting profile and their chemical nature screening for antioxidant activity *in vitro*.

MATERIAL AND METHODS

Collection of plant material and extraction

Leaves of *C. speciosus* and *C. pictus* were collected from SDM College of Ayurveda, Udyavar, which was grown in our medicinal plant garden. The plant was identified, authenticated and a museum sample with number 610/15050902 for *C. speciosus* and 610/15050903 for *C. pictus* were deposited in SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi 574118. (Figure 1) Specimen was dried in shade and stored in air tight container at 25^0 C for further study. Cold percolation method was followed for ethanolic extract preparation. The air dried leaf was kept below 45^0 C and it was then powdered. The powdered material weighing about 100g was kept with 2 liters of ethanol in a percolator for 24 hrs followed by filtration; the filtrates were taken in a pre-weighed china dish. The extracts were concentrated and residue were then dried and kept in desiccators. The extract thus prepared was used for phytochemical tests and *in vitro* antioxidant activity.

Figure 1. Macroscopy of *Costus speciosus* and *C. pictus*

1.1 Fresh leaves of *C. pictus* and *C. speciosus*

1.2 Dried leaves of *C. pictus*

1.3 Dried leaves of *C. speciosus*

Standardization

The powdered plant material was standardized for pharmacopoeial constants as per method.[12]

Phytochemical screening

Total ethanol extract was tested for the presence of different phytoconstituents like alkaloid, steroid, flavonoid, tannin, glycoside etc.[13]

Thin-layer chromatography/HPTLC

Sample preparation

Dried plant powder (1g) was extracted with 10 ml of ethanol (90%) and filtered. The filtrates were made up to 10 ml and used further for application.

Development and documentation

Eight micro liters each of ethanolic extract of *C. pictus* and *C. speciosus* were applied on aluminium plate pre-coated with silica gel 60 F_{254} of 0.2 mm thickness (Merck, Germany) using CAMAG LINOMAT 5 applicator.[14] The plate was developed in CAMAG glass twin trough chamber previously saturated with mobile phase toluene: ethyl acetate (8.0:1.0). The plate was derivatised using vanillin- sulphuric acid (VS), and heated at 105 °C till the spots appeared.[15-16] The developed plates were visualized in CAMAG visualizing chamber under short UV and long UV and scanned in CAMAG SCANNER 4 under 254 nm, 366 nm, 540 nm (pre-derivatisation) with the help of CAMAG WinCATS software. R_f values and densitograms were recorded.

In vitro anti-oxidant activity of ethanolic extract

Antioxidant properties of the species were carried out by DPPH radical scavenging assay[17] and reducing power assay.[18] DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) was purchased from Sigma, USA. All the other chemicals used were of analytical grade. DPPH which is a free radical is purple in colour and if the extract is a radical scavenger purple colour turns to yellow colour. 1ml extract (in different concentration 6.4, 10, 20, 40, 60, 80 and 100 µg/ml) and 1ml of 0.002% of DPPH in methanol were prepared and incubated at room temperature for 30 min. The absorbance of sample was measured at 517 nm with double beam UV-Visible spectrophotometer (SYSTRONICS 2201).

$$\% \text{ Inhibition} = \frac{A0 - A1}{A0} X\ 100$$

A_0 = Absorbance of control

A_1 =Absorbance of sample

Reducing power assay was carried out according to the method of Oyaizu (1986), 0.75 ml of each of different conc. of extract (6.4, 10, 20, 40, 60, 80 and 100 µg/ml) was mixed with 0.75 ml of phosphate buffer (0.2M pH 6.6) and potassium ferricyanide (1%V/V) followed by incubation at 50^0C for 20 min. Reaction was stopped with the addition of 0.75 ml of 10% trichloroacetic acid. Centrifuged at 800 rpm for 10 min.1.5 ml of supernatant was mixed with 1.5ml of distilled water and 0.1ml of ferric chloride (0.1%). Followed by 10 min incubation and absorbance was measured at 700 nm with double beam UV-Visible spectrophotometer (SYSTRONICS 2201). Higher the absorbance of reaction mixture greater is the reducing power.

RESULTS AND DISCUSSION

Standardisation is one of those essential parameters in assessing quality and safety of herbal drugs. The herbs were free of foreign matter. The leaf part was selected for the study. Loss on drying reveals the moisture content, the sample *C. speciosus* had 7.12% and *C. pictus* had 7.26% of moisture; total ash indicates amount of total inorganic content, total ash in case of *C. speciosus* was 14.04% where as 15.12% in C. *pictus*; Acid insoluble ash, which is the acid-insoluble part of total ash, mainly silica was 4.0% in case of *C. speciosus* and 1.39% in *C. pictus*; water soluble ash, which is the water-soluble part of total ash indicating inorganic content without water-insoluble inorganic salts such as silica was 12.2 and 11.0% respectively for *C. speciosus* and *C. Pictus*; alcohol soluble extractive value indicative of the percentage of active constituents soluble in ethanol was found to be 4.61% for *C. speciosus* and 2.10% for *C. pictus*; water-soluble extractive value indicative of the percentage of active constituents soluble in water was 4.97% in *C. speciosus and* 4.56% in *C. pictus* (Table 1).

Table 1. Results of physico-chemical tests of *Costus speciosus* and *C. pictus*

Parameter	Results n=3 %w/w	
	C. speciosus	*C. pictus*
Loss on drying at 110^{0}C	7.12	7.26
Total ash	14.04	15.12
Acid insoluble ash	4.00	1.39
Water soluble ash	12.20	11.00
Alcohol soluble extractive	4.61	2.10
Water soluble extractive	4.9735	4.563

Phytochemical tests carried out in ethanolic extract showed the presence of alkaloids, carbohydrates, flavonoids, saponins and tannins except for phenols which happen to be present in *C. speciosus* (Table 2).

Table 2. Results of preliminary phytochemical tests of *Costus speciosus* and *C. pictus*

Test	*C. speciosusus*	*C. pictus*
Alkaloids	+	+
Carbohydrate	+	+
Carboxylic acid	-	-
Coumarins	-	-
Flavanoids	+	+
Phenol	+	-
Quinone	-	-
Resins	-	-
Steroid	-	-
Saponins	+	+
Tannin	+	+
Terpenoid	-	-

+ present; - absent

Phytochemical investigation is one of the tools for qualitative assessment which includes preliminary phytochemical screening where comparison of the two species can be done; HPTLC which is both qualitative and semi quantitative the quantitative emerged as tool for comparative study for phytochemical analysis of herbal drugs and formulations. HPTLC finger printing profile shows different colored bands which corresponds to different R_f values. At 254nm, 2 spots in *C. speciosus* with R_f of 0.50, 0.58 (all green) and 3 spots in *C. pictus* 0.38, 0.50, 0.58 (all green); at 366 nm *C. speciosus* shows 9 spots having R_f values 0.31, 0.38, 0.50, 0.58, 0.62, 0.76, 0.90 (all fluorescent red) except 2 spots having R_f values 0.46, 0.84 (fluorescent blue), in *C. pictus* 8 spots having R_f of 0.31, 0.38, 0.50, 0.58, 0.62, 0.90 (all fluorescent red) except 0.46, 0.84 (Fluorescent blue); under white light in *C. speciosus* three spots were evident with R_f of 0.34 (L. yellow), 0.50 (L. green), 0.58 (D. green) but in *C. pictus* four spots were observed with R_f values of 0.31, 0.38, 0.50, 0.58 (All green). Following post derivatisation in *C. speciosus* 4 spots were evident with R_f 0.34, 0.46, 0.50, 0.54 (All purple) except 0.58 (green) and in *C. pictus* 5 spots were present at R_f 0.34, 0.46, 0.50, 0.54 (All

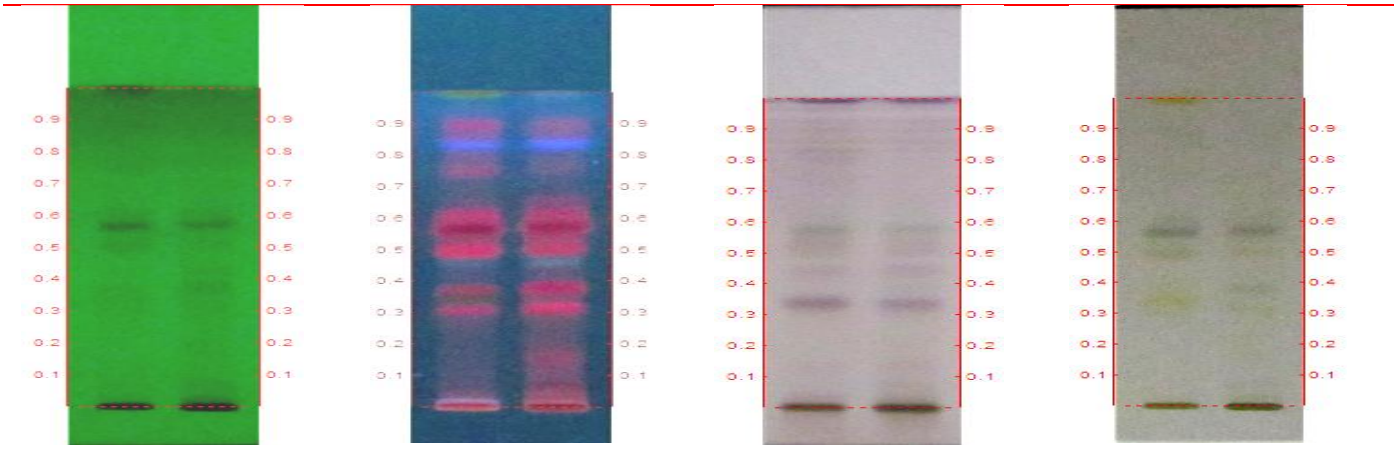
purple) except 0.58 (green) (Table 3 and Figure 2). R_f values by densitometric scan are mentioned in (Table 4). 3-D superimposable densitograms at 254 nm, 366 nm and 540 nm (pre-derivatisation) showed the similarity and differences in the chemical fingerprint (Figure 3).

Table 3. R_f values of ethanolic extract of *Costus speciosus* and *C. pictus*

Short UV		Long UV		Post derivatization		White light	
C. speciosus	*C. pictus*	*C. speciosus*	*C. pictus*	*C. speciosus*	*C. pictus*	*C. speciosus*	*C. pictus*
-	-	0.31(FD.red)	0.31(FD.red)	-	-	-	0.31(L.green)
-	-	-	-	0.34(D.purple)	0.34(D.purple)	0.34(L.yellow)	-
-	0.38(L.green)	0.38(FD.red)	0.38(FD.red)	-	-	-	0.38(L.green)
-	-	0.46(FL.blue)	0.46(FL.blue)	0.46(L.purple)	0.46(L.purple)	-	-
0.50(L.green)	0.50(L.green)	0.50(FD.red)	0.50(FD.red)	-	0.50(L.purple)	0.50(L.green)	0.50(L.green)
-	-	-	-	0.54(L.purple)	0.54(L.purple)	-	-
0.58(D.green)	0.58(D.green)	0.58(FD.red)	0.58(FD.red)	0.58(L.green)	0.58(L.green)	0.58(D.green)	0.58(L.green)
-	-	0.62(FD.red)	0.62(FD.red)	-	-	-	-
-	-	0.76(FD.red)	-	-	-	-	-
-	-	0.84(FD.blue)	0.84(FD.blue)	-	-	-	-
-	-	0.90(FD.red)	0.90(FD.red)	-	-	-	-

F-Fluorescent; D-Dark; L-Light

Figure 2. TLC photodocumentation of ethanolic extract of *Costus speciosus* and *C. pictus*



2.1 Short UV 2.2 Long UV 2.3 Post derivatisation 2.4 White light

Solvent system - Toluene : Ethyl acetate (8:1)

Track 1- Ethanolic extract of *C. speciosus* (8 µl); 2 - Ethanolic extract of *C. pictus* (8 µl)

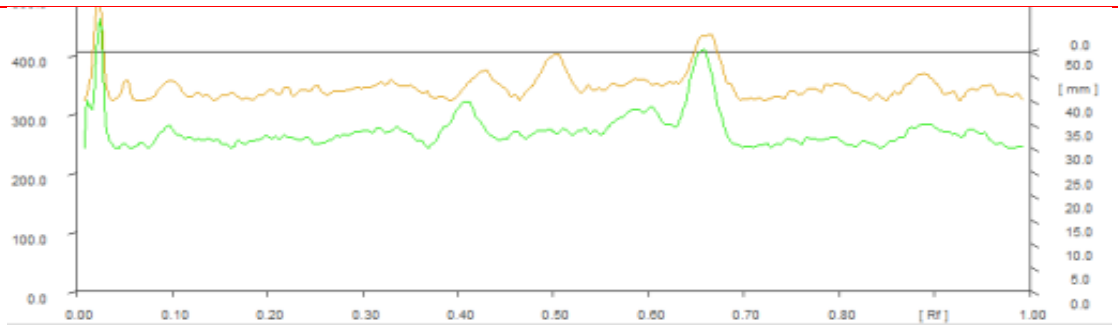
Table 4. R_f values by densitometric scan of *Costus speciosus* and *C. pictus*

R_f values	Peaks at 254 nm		Peaks at 366 nm		Peaks at 540 nm	
	C. speciosus	*C. pictus*	*C. speciosus*	*C. pictus*	*C. speciosus*	*C. pictus*
0.02	11.37	9.58		0.79	30.68	19.92
0.05	-	1.55	-	-	-	1.65
0.10	5.53	4.00	-	10.22	-	3.39
0.12	-	-	16.53	-	-	-
0.13	-	0.86	-	-	-	-
0.15	-	-	-	8.10	-	-
0.16		1.06	11.83	-	-	-
019	-	-	-	-	-	2.28
0.20	1.85	1.40	-	-	-	-

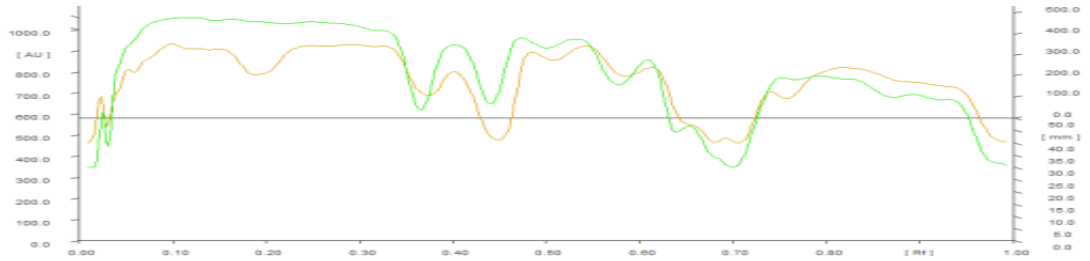
0.22	-	1.19	-	-	-	-
0.25	-	2.67	20.27	12.30	-	-
0.30	4.54	-	-	-	-	-
0.32	-	-	-	8.48	-	-
0.33	-	7.09	-	-	-	-
0.34	3.85	-	-	-	-	-
0.36	-	-	-	-	4.12	-
0.37	-	-	-	-	-	6.56
0.40	-	-	7.88	6.06	-	-
0.41	12.97	-	-	-	-	-
0.43	-	8.62	-	-	4.63	-
0.45	-	-	-	-	-	12.21
0.46	2.15	-	-	-	-	-
0.47	-	-	7.12	-	-	-
0.48	-	-	-	7.26	-	-
0.50	4.01	13.75	-	-	-	-
0.53	-	-	9.46	-	-	-
0.54	-	2.13	-	12.05	-	-
0.56	-	2.05	-	-	-	-
0.58	9.52	-	-	-	14.78	8.81
0.59	-	4.92	-	-	-	-
0.60	6.29	-	-	-	-	-
0.61	-	-	5.61	7.53	-	-
0.65	-	-	1.37	-	-	-
0.66	23.99	20.27	-	-	37.43	40.55
0.69	-	-	-	0.10	-	-
0.70	-	-	-	-	8.36	-
0.74	-	-	-	3.19	-	-
0.75	1.08	1.26	4.00	-	-	-
0.76	-	-	-	-	-	4.64
0.77	-	1.82	-	-	-	-
0.79	1.75	-	9.60	-	-	-
0.80	-	4.42	-	-	-	-
	-	-	-	23.92	-	-
0.83	0.71	-	-	-	-	-
0.89	-	7.36	3.75	-	-	-
0.90	6.74	-	-	-	-	-
0.93	-	-	2.58	-	-	-
0.94	3.64	-	-	-	-	-
0.96	-	3.97	-	-	-	-

Values in % area; highlighted values are compounds with same R_f

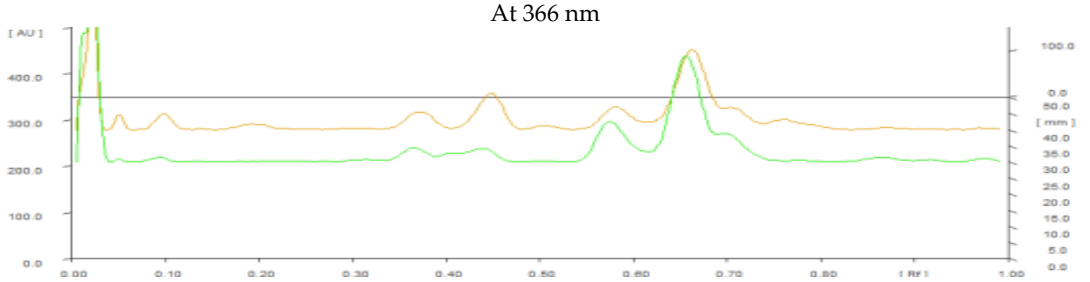
Figure 3. 3-D Chromatogram of ethanolic extract of *Costus speciosus* and *C. pictus*



At 254 nm



At 366 nm



At 540 nm

DPPH assay is measure of radical quenching activity of antioxidant present in an extract. DPPH radical scavenging assay of *C. speciosus* and *C. pictus* was concentration dependent and the analysis of the results obtained was an indication of remarkable and concentration dependent reducing activity. The IC_{50} of Ascorbic acid (Std) was 2.53 µg/ml while that of *C. specious* and *C. pictus* was 63.70 µg/ml and 111.55 µg/ml respectively this was very much manifested through the assay for *C. speciosus* (Table 5) and *C. pictus* (Table 6).

Table 5. DPPH assay for antioxidant evaluation activity of *Costus speciosus* and *C. pictus*

Concentration	*C. speciosus*		*C. pictus*	
	Mean ± SEM	% Inhibition	Mean ± SEM	% Inhibition
Control	0.388 ± 0.00622	0	0.525 ± 0.00584	0
6.4 µg/ml	0.363 ± 0.00166*	6.44	0.512 ± 0.00088^{ns}	2.48
10 µg/ml	0.352 ± 0.00185**	9.28	0.507 ± 0.00033 ^{ns}	3.43
20 µg/ml	0.310 ± 0.00484***	20.10	0.469 ± 0.00057***	10.67
40 µg/ml	0.252 ± 0.00566***	35.05	0.427 ± 0.00450***	18.67
60 µg/ml	0.204 ± 0.00348***	47.42	0.367 ± 0.00664***	30.10
80 µg/ml	0.146 ± 0.00832***	62.37	0.334 ± 0.00650***	36.38
100 µg/ml	0.161 ± 0.00057***	58.50	0.293 ± 0.00536***	44.19

*P<0.05 in comparison to control; **P<0.01 in comparison to control; ***P<0.001 in comparison to control

Table 6. Reducing power assay of *Costus speciosus* and *C. pictus*

Concentration	Mean ± SEM	
	C. speciosus	*C. pictus*
Control	0.086 ± 0.00066	0.143 ± 0.00233
6.4 µg/ml	0.0933 ± 0.00133 ^{ns}	0.158 ± 0.00115^{ns}
10 µg/ml	0.099 ± 0.000 ^{ns}	0.163 ± 0.00033 ^{ns}
20 µg/ml	0.119 ± 0.00821 ^{ns}	0.195 ± 0.00057***
40 µg/ml	0.138 ± 0.00120 ^{ns}	0.267 ± 0.01041***
60 µg/ml	0.169 ± 0.00425**	0.333 ± 0.00200***
80 µg/ml	0.197 ± 0.00366***	0.374 ± 0.00977***
100 µg/ml	0.231 ± 0.0033***	0.430 ± 0.01159***

ns: non significant, P>0.05 in comparison to control;**p<0.01in comparison to control
***p<0.001in comparison to control

The result of antioxidant assay carried out at various concentrations ranging from 6.4 to 100µg/ml of the alcoholic extract of *C. speciosus* and *C. pictus* showed that free radical scavenging effect of the tested extract were concentration dependent (Table 6).
In reducing power assay substances, which have reduction potential, react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.The ethanolic extract of both the species produced marked and concentration-dependent increase in the reducing power. The results obtained indicated a real good potential of antioxidant property in *C. pictus* than in *C. speciosus*. However it should

be ascertained using *in vivo* systems, whether it could mimic the same in intact animals is the query to be answered. The current investigation based upon phytochemical composition of *C. pictus* and *C. speciosus* are found to contain phenols and flavanoids which are rich source of free radical scavengers and has attracted a great deal of research interest to screen them out for antioxidant potential. Results obtained for reducing power assay is represented in Table 6. The ethanolic extract produced marked and concentration-dependent increase in the reducing power. In scientific term antioxidants can be broadly defined as any substance, which when present at low concentration compared to that of an oxidizable substrate, significantly prevents or delays oxidation of that substrate.[19]

HPTLC is an important phytochemical analytical technique to differentiate these species from the related species *Costus igneus* using the fingerprint reported earlier.[20] Evaluation of acknowledged drugs, especially plant products are considered of due importance for antioxidant activity. It is initially screened *in vitro* followed by *in vivo* conditions. ROS are generated in different organs at different tissue under different circumstances and are of different types. Antioxidants as the name suggest it protects the body from oxidative injury and it prevents further damage to the tissue there by protecting the organ. De facto the body itself strives to keep the balance between ROS and its endogenous antioxidant system. Reactive oxygen species (ROS) these are produced in the body in due course of metabolism, stress, toxins, environmental pollutants, fried and spicy food etc. These cause cell death, hypoxia and ischemic reperfusion injury and tissue damage. Substrate can be living tissue, protein, carbohydrate and DNA.

The antioxidants neutralise their effect by reducing them as hydrogen donors, singlet oxygen quenchers, metal chelators.[21] The body for its defence has developed endogenous antioxidant system, which can be divided into two groups, the enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants Superoxide dismutase (SOD), Catalase, Glutathione peroxidise (GSH-Px) and non enzymatic antioxidants are lipid soluble vitamins (Vitamin E, Vitamin A, Provitamin A i.e. β-carotene), water soluble vitamin C, Glutathione etc.[22] The generation of ROS initiates the process of defence mechanism, wide variety of enzymes like SOD, Glutathione, Catalase, Flavonoids, α-tocopherol, ascorbic acid and several others come to combat. The significance of these antioxidants depends on the kind of antioxidant generated, time and which tissue or organ is the target of damage. Different kind of ROS are nitric oxide (NO), peroxyl (RO_2-), superoxide (O_2^-), lipid peroxyl (LOO) radicals and non-free radicals such as hydrogen peroxide (H_2O_2) and lipid peroxide.[23-25] Herbs having antioxidant property are said to possess anti-inflammatory, wound healing, anti-ulcer, anti-atherosclerotic, antitumor, anti-mutagenic, antibacterial properties too.[26-27] Antioxidants from natural source are of importance because of their lesser side effects and lower price. The natural antioxidants are vegetables, fruits and many more dietary supplements.[28]

CONCLUSION

The study thus carried out unfolded the antioxidant potential of *C. speciosus* and *C. pictus* which is acknowledged to tannins, phenols and flavonoids. In DPPH radical scavenging assay *C. speciosus* was a better antioxidant than its counterpart *C. pictus* but on the other hand *C. pictus* had a superior reducing power in comparison with *C. speciosus*.

CONFLICT OF INTEREST

Nil

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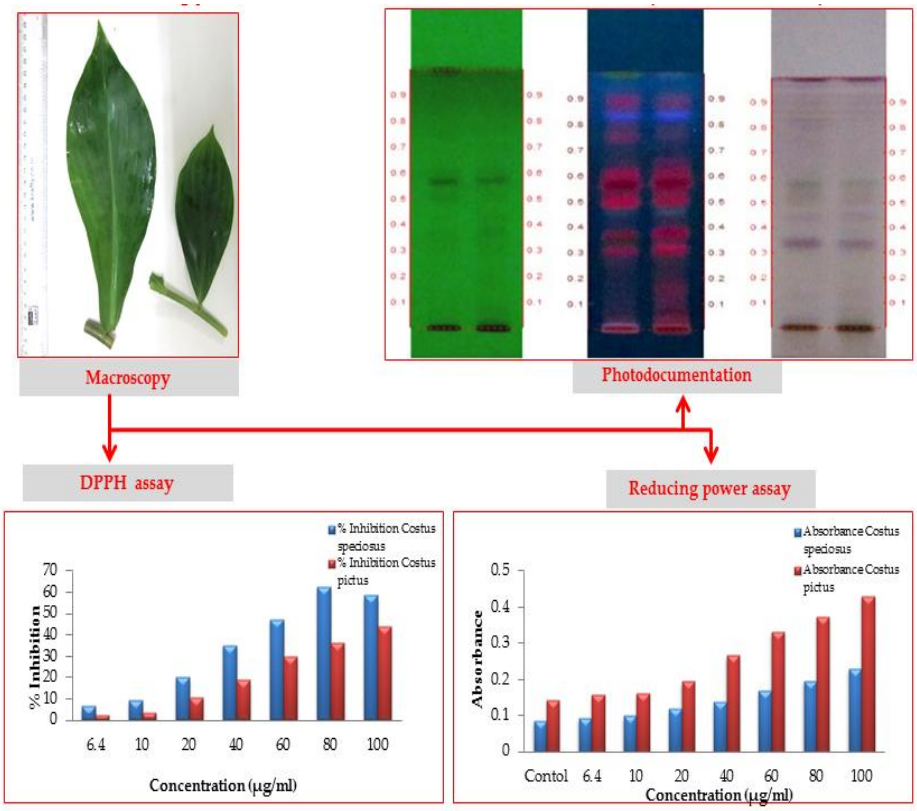
GRAPHICAL ABSTRACT



Cite this article as: Suchitra Prabhu, Puneeth, Priyadarshini Rao, Sunil Kumar Koppala Narayana, Ravishankar Basaviah. Comparative Phytochemical and Antioxidant Properties of *Costus pictus* and *C. speciosus*. J Ayu Med Sci 2017;2(1):121-8. DOI: 10.5530/jams.2017.2.2