



STUDIES ON THE ANTIOXIDANT ACTIVITY OF *Costus igneus* LEAF EXTRACT

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ABSTRACT

Plan: The antioxidant activity studies of the extract of *Costus igneus* leaves

Preface: *Costus igneus* commonly known as fiery costus or Spiral flag is a species of herbaceous plant. It is claimed to help build up insulin in human body and is sometimes referred to as insulin plant. *Costus igneus* (Fam: Zingiberaceae) is a tropical evergreen shrub with large, smooth, dark green leaves. The *Costus igneus* is valued mainly for its tonic, stimulant and antiseptic properties. It is said to be aphrodisiac and to be able to prevent the hair turning grey. Its root is anodyne, antibacterial, antispasmodic, aphrodisiac, carminative, stimulant, stomachic, tonic and vermifuge.

Methodology: In the present study the antioxidant activity of the leaf extract was studied using four methods viz. DPPH assay, reducing power assay, superoxide radical scavenging assay and Folin-ciocalteu assay.

Outcome: The studies have proved that extract possess antioxidant activity. In reducing power assay, the plant extract showed 75.43 % increase in reducing power compared to Ascorbic acid which showed 91.94%, at a concentration of 16µg/ml. In DPPH assay, plant extract produced 71.85% DPPH scavenging activity, compared to Ascorbic acid which produced 84.47% at a concentration of 160µg/ml. In super oxide scavenging activity, the plant extract produced 68.19% radical scavenging activity compared to the standard which showed 79.78% at a concentration of 800µg/ml. The IC₅₀ was found to be 177 and 367 µg/ml of the standard and plant extract respectively.

1. INTRODUCTION

Strong epidemiological evidence suggests that regular consumption of fruits and vegetables can reduce cancer risk. Towards this end, the past several decades have been an explosion of research focused on the role played by antioxidant nutrients in human cancer. Phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action, including of gene expression in cell proliferation, cell differentiation, oncogenes and tumour suppressor genes; induction of cell-cycle arrest and apoptosis, modulation of enzyme activities in detoxification, oxidation and reduction; stimulation of the immune system; regulation of hormone metabolism; as well as antibacterial and antiviral effects¹.

Naturally occurring antioxidants like ascorbic acid, carotenoids and phenolic compounds are highly effective biologically². They act by inhibiting lipid peroxidation (by inactivating lipoxygenase), scavenge free radicals and active oxygen species by propagating a reaction cycle and chelate heavy metal ions.³

Commonly *Costus igneus* is known as fiery costus or Spiral flag or insulin plant, and in literature, it is claimed to increase the levels of insulin⁴. *C. igneus* / *C. pictus* (Family: Zingiberaceae) is a tropical evergreen shrub with large, smooth, dark green leaves and grown in America and India due to its medicinal value. It is now accepted and used widely as an Ayurvedic medicinal herb. Allopathic doctors too recommend it for bringing blood sugar levels under control.⁵



Costus igneus plant

In traditional medicine *C. igneus* is appreciated mainly for its tonic, stimulant, antiseptic, anodyne, antibacterial, antispasmodic, aphrodisiac, carminative, stimulant, stomachic, tonic and vermifuge properties^{4, 5}. Therefore, the main objective of the present study was to evaluate the antioxidant activity of the leaves of this plant.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh leaves of the plant were collected from Puttur, Karnataka and authenticated by Dr. Noeline J. Pinto, Head of Botany Dept., St. Agnes College, at Mangalore. The Leaves were dried in shade and powdered for extraction.

2.2. Preparation of ethanolic extract

The leaves of *Costus igneus* were collected from Puttur, Karnataka, India during June 2009. The shade dried powdered leaves (5kg) were soaked in ethanol (95%) and kept aside for four days. After four days the ethanol layer was decanted off. The process was repeated four times. The solvent of total extract was distilled off and concentrate was evaporated on a water bath to a syrupy consistency and evaporated to dryness. The percentage yield was 0.6%.

2.3. Evaluation of Antioxidant activity by in-vitro Techniques:

2.3.1. Reducing power assay⁶

Different aliquots of the extracts and ascorbic acid were mixed with Phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml), the mixture was incubated at 50°C for 20 minute. TCA (2.5ml) was added and centrifuged at 6000 rpm for 10 minute. To the supernatant added 0.5 ml of ferric chloride. Absorbance was measured at 700 nm using Jasco spectrophotometer. A blank was prepared without adding extract. The experiment was repeated in triplicates and results were tabulated. Table 3 & Fig.2.

2.3.2. DPPH assay⁷

Different aliquots of extracts and standard solution were taken in different test tubes. To these 5 ml methanolic solution of 1, 1-diphenyl-2-picryl hydrazyl (DPPH) was added. The blanks were prepared as above without extract. The readings were noted at 515 nm using Jasco spectrophotometer, at 0, 1, 15 and 30 minutes (Table 1& Fig.1). The changes of absorbance of samples were measured and scavenging activity was expressed as:

$$\% \text{ Radical scavenging} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

2.3.3. Superoxide radical scavenging activity⁸

To different aliquots of sample was added 1 ml of sodium carbonate, 0.4 ml of NBT and 0.2 ml of EDTA. The zero minute reading is taken at 560 nm. To the above added 0.4 ml of hydroxylamine hydrochloride, incubated at 25°C for 15 minute. The final reading is taken at 560 nm again. The difference in the initial and final reading is taken for each sample, keeping suitable control samples. Readings are taken in triplicates. Table 2 and Fig.2.

$$\% \text{ Superoxide scavenging Activity} = 1 - \frac{\text{Difference in the absorbance of sample} \times 100}{\text{Difference in the absorbance of blank}}$$

2.3.4. Estimation of total Phenol content⁹

The extract samples (0.5 ml of different dilutions) were mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) for 5 min and aqueous Na₂CO₃ (4 ml, 1 M) were then added. The mixture was allowed to stand for 15 min and the phenols were determined by colorimetric method at 765 nm. The standard curve was prepared by 0, 50, 100, 150, 200, and 250 mg/ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent (mg/g of dry mass), which is a common reference compound.

2.3.5. Determination of total phenol content by Folin-Ciocalteu method

The total phenol content was found to be 78.89±3.94mg/g dry mass GAE.

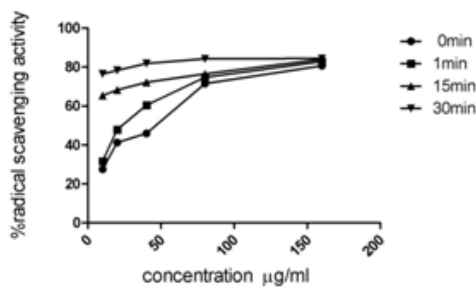


Fig.1: Ascorbic acid assay

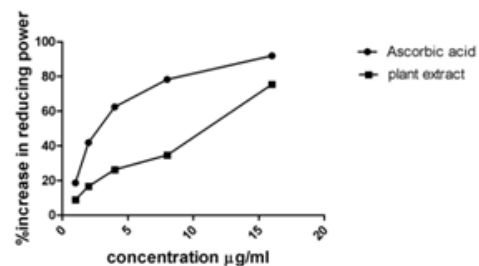


Fig.2: Reducing power assay

Table 1: In vitro Antioxidant Activity: DPPH Assay

sample	Conc. (µg/ml)	% DPPH radical scavenging activity			
		0min	1min	15min	30min
Ascorbic acid	10	27.53±0.01	31.72±0.01	65.42±	76.42±0.34
	20	41.25±0.009	47.94±0.002	68.32±0.001	78.36±0.005
	40	45.96±0.021	60.46±0.009	72.20±0.023	81.85±0.002
	80	71.53±0.032	74.78±0.030	76.54±0.024	84.32±0.004
	160	80.69±0.002	82.85±0.006	84.05±0.092	84.47±0.002
Plant extract	10	18.29±0.023	22.01±0.033	61.22±0.001	71.02±0.009
	20	29.25±0.003	36.80±0.050	63.15±0.025	71.25±0.001
	40	37.64±0.0057	50.01±0.006	66.3±0.006	71.58±0.020
	80	49.75±0.006	53.25±0.003	70.89±0.010	71.63±0.023
	160	60.80±0.031	67.77±0.004	70.16±0.007	71.85±0.004

All values are expressed as mean ±SEM, p<0.05 compared to standard. The data was analyzed by one way analysis of variance (ANOVA) followed by Post hoc Dunnett’s test by using the software Graph pad prism 5.

Table 2: Super oxide scavenging assay

Sample	Concentration µg/ml	Superoxide Radical scavenging activity (±SEM)	IC50 (µg/ml)
Gallic Acid	100	35.67±0.004	177
	200	52.31±0.035	
	400	63.78±0.006	
	600	71.22±0.021	
	800	79.78±0.004	
Plant extract	100	19.05±0.013	367
	200	41.63±0.045	
	400	50.44±0.005	
	600	58.84±0.009	
	800	68.19±0.032	

All values are expressed as mean ±SEM, p<0.05 compared to standard. The data was analyzed by one way analysis of variance (ANOVA) followed by Post hoc Dunnett’s test by using the software Graph pad prism 5.

Table 3: Reducing power assay

Tested Material	Concentrations (µg/ml)	Reducing Power in % (± SEM)	IC ₅₀ (µg/ml)
Ascorbic acid	1	18.64 + 0.011	2.25
	2	41.89± 0.012	
	4	62.48 ± 0.002	
	8	78.28 ± 0.004	
	16	91.94 ± 0.013	
Plant extract	1	8.96±0.041	4.40
	2	16.61±0.035	
	4	26.30±0.009	
	8	34.65±0.061	
	16	75.43±0.003	

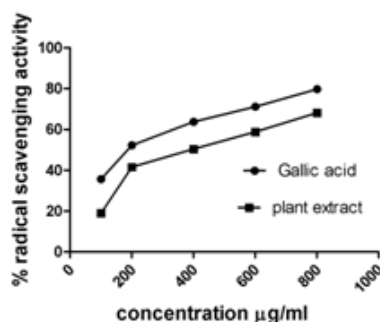


Fig.3: Super oxide scavenging assay

3. RESULTS

3.1. Reducing power assay

The reductive capabilities of the alcoholic extracts of *Costus igneus* were compared with ascorbic acid for the reduction of the $Fe^{3+} - Fe^{2+}$ transformation. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.

In this method the plant extract showed 75.43 % increase in reducing power compared to Ascorbic acid which showed 91.94%, at a concentration of 16µg/ml. The antioxidant activity may be attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity. The reducing power of alcoholic extract of *Costus igneus* increased with increasing amount of sample.

3.2. DPPH assay

The 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical widely used as the model system to investigate the scavenging activities of several natural compounds such as phenols or crude mixtures such as the ethanolic extract of plants. DPPH radical is scavenged by antioxidants through the donation of proton forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Radical scavenging activity increased with increasing percentage of the free radical inhibition.

DPPH is a relatively stable radical. The assay is based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH which reacts with suitable reducing agent. The electrons become paired off and solution loses color stoichiometrically depending on the number of electrons taken up. DPPH was used to determine the proton radical scavenging action of alcoholic extract of *Costus igneus*, because it possess a proton free radical and shows a characteristic absorbance at 517 nm. In this method, plant extract produced 71.85% DPPH scavenging activity, compared to Ascorbic acid which produced 84.47% at a concentration of 160µg/ml. From the present results, it may be postulated that *Costus igneus* has the potential to reduce the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principles.

3.3. Superoxide radical scavenging activity

Superoxides are produced from molecular oxygen due to oxidative enzymes of body as well as via non-enzymatic reaction such as auto oxidation of hydroxylamine hydrochloride. The scavenging activity towards the superoxide radical (O_2^-) is measured in terms of inhibition of generation of O_2^- . In the present study, superoxide radical reduces NBT to a formosan that is measured at 560 nm. In this method the plant extract produced 68.19% radical scavenging activity compared to the standard which showed 79.78% at a concentration of 800 μ g/ml. The IC₅₀ was found to be 177 and 367 μ g/ml of the standard and plant extract respectively. The result shows that alcoholic extract of *Costus igneus* has potent radical scavenging activity with increasing percentage inhibition. The probable mechanism of scavenging the superoxide anions may be due to the inhibitory effect of the extract towards generation of superoxides in the *in vitro* reaction mixture.

3.4. Determination of total phenol content by Folin- Ciocalteu method

The F-C assay has for many years been used as a measure of total phenolics in natural product, but mechanism of action is oxidation/ reduction reaction and as such considered another antioxidant method.

It is obvious that the total phenolic content measured by the Folin- Ciocalteu procedure does not give a full picture of the quantity or quality of the phenolic constituents in the extracts. In addition, there may be some interference rising from other chemical components present in the extract, such as sugars or ascorbic acid¹³. Similarly it must be noted that the efficiency of antioxidants depends strongly on the oxidation conditions and lipid substrate.

4. DISCUSSION

Pathology of many diseases such as inflammatory condition, cancer, diabetes and aging has been attributed to oxidative stress. Free radicals induced by peroxidation implicated in several pathological conditions such as atherosclerosis, ischemia, liver disorder, metal toxicity and pesticide toxicity.. Reactive oxygen species (ROS) such as superoxide anions (O_2^-), hydroxyl radicals (OH.) and nitric oxide (NO) damage enzymes and important cellular components causing injury through covalent binding and lipid peroxidation. Antioxidants may offer protection against oxidative stress by scavenging the free radicals, inhibiting the lipid peroxidation and by other mechanisms and thus prevent disease. Foods rich in antioxidants play an important role in the prevention of cardiovascular disease, cancer, neurodegenerative diseases, inflammation and problems caused by cell and cutaneous aging.

The results from the preliminary phytochemical screening showed presence of phenolic compounds, Flavonoides and tannins as phytochemical constituents which may be responsible for the antioxidant properties. In general, polyphenolic compounds are widely distributed in plant kingdom and possess strong antioxidant properties.¹⁰ Phenolic compounds are found in both edible and non edible plants, and known to have multiple biological effects, including antioxidant activity. Flavonoides and other plant phenolics, such as phenolic acids, stilbenes, tannins, lignans, and lignin, are especially common in leaves, flowering tissues, and woody parts such as stems and barks¹¹.

They are important in the plant for normal growth development and defence against infection and injury. Flavonoides also partly provide plant colours present in flowers, fruits, and leaves. They generally occur as glycosylated derivatives in plants, although conjugation with inorganic sulfate or organic acid is also known¹².

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