



Estimation of Antioxidant Activity in *Costus igneus* Leaf Extracts by Using Phosphomolybdenum Assay

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	Abstract <i>Costus igneus</i> , commonly known as the insulin plant, is a Spiral Flag member belongs to the family <i>Costaceae</i> . It usually grows as an ornamental plant in southern India, and its leaves are used as a dietary supplement in the treatment of diabetes mellitus. It has been traditionally reported for its anti-diabetic, anti-inflammatory, anti-proliferative, anti-urolithiasis, hypolipidemic, neuroprotective, and anti-microbial activity. The study was aimed to investigate the antioxidant activity in <i>Costus igneus</i> leaf extracts. Among the samples, the standard shows the highest rate of antioxidant activity followed by <i>Costus igneus</i> than other samples. The results obtained indicated a real good potential of antioxidant property in <i>C. igneus</i> which paved the way for newer useful drugs. Keywords: <i>Costus igneus</i> , antioxidant, Phosphomolybdenum assay.
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Introduction:

Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs and nutraceuticals (Sharma and Mujundar, 2003). According to Perumal and Gopalakrishnakone (2008) plant based drugs provide outstanding contribution to modern therapeutics. The revival of interest in plant-derived drugs is mainly due to the current widespread belief that the green medicine is safe and more dependable without side effects compared to the synthetic drugs (Sardesai *et al.*, 2014). In view of this, urge for extracting natural antioxidants and other pharmacologically significant compounds from plant sources have been showing interest among researchers in recent years (Singh *et al.*, 2004). Antioxidants are naturally occurring plant substances that protect the body from damage caused by harmful molecules called free radicals as they help to prevent oxidation, which can cause damage to cells and may contribute to aging (Kalpanakaloori *et al.*, 2021).

The main characteristic of an antioxidant is its ability to trap free radicals, improve immune function and perhaps lower the risk for infection, cardiovascular diseases, and cancer. The most dangerous free radicals are the atomic and molecular varieties of oxygen which is known as Reactive Oxygen Species (ROS). These

free radicals may oxidize nucleic acids, proteins, lipids and can initiate degenerative diseases. Though oxidation reactions are crucial for life, they can also be damaging (Mamta Pal *et al.*, 2014). Plants and animals have a complex system of multiple types of antioxidants, such as vitamin C and vitamin E, as well as enzymes, such as catalase (CAT), superoxide dismutase (SOD), and various peroxidases (Hamid *et al.*, 2010).

Costus igneus, a newly introduced plant in India from South and Central America. It is a perennial, upright, spreading plant reaching about 2 feet tall, with spirally leaves and attractive flowers. The Family *Costaceae* consists of four genera and approximately 200 species. The insulin plant has antimicrobial, antidiabetic, antiproliferative, hepatoprotective properties (Hegde *et al.*, 2014). In Ayurvedic system the rhizome of this plant is considered as bitter, astringent, acrid, cooling, aphrodisiac, purgative, anthelmintic, depurative, febrifuge, expectorant and useful in burning sensation, constipation, leprosy, worm infection, skin diseases, fever, asthma, bronchitis, inflammations and anaemia (Vishalakshi Devi and Asna Urooj, 2010). Having various health benefits of *Costus igneus*, the present study attempts to assess the antioxidant activity.

Materials and methods:

The plant selected for the study was *Costus igneus*. Fresh plant parts were washed thoroughly 2-3 times with running tap water and then with sterile water. Then it was shade dried for further evaluation of antioxidant activity. All chemicals and solvents used were of analytical grade.

Preparation of the Extract

The plant parts collected were cleaned off for extraneous matter and soil with tap water and later with distilled water thrice before drying. They were then surface sterilized with 60% alcohol followed by washing with distilled water, blotted with sterile blotting paper and dried at room temperature. The ground material was soaked in distilled water for 48 hour with stirring every ½ h using a sterilized glass rod. The final extract were passed through Whatman filter paper No.1

Phosphomolybdenum Assay (PM)

1ml each of 0.6 M sulphuric acid, 28 mm sodium phosphate and 4 mm ammonium molybdate were added in 20 ml of distilled water and made up volume to 50 ml by adding distilled water. 100 µl respective samples were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95°C for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Percentage of inhibition values from samples were calculated for each extract. Ascorbic acid (500µg/mL) was used as positive reference standard.

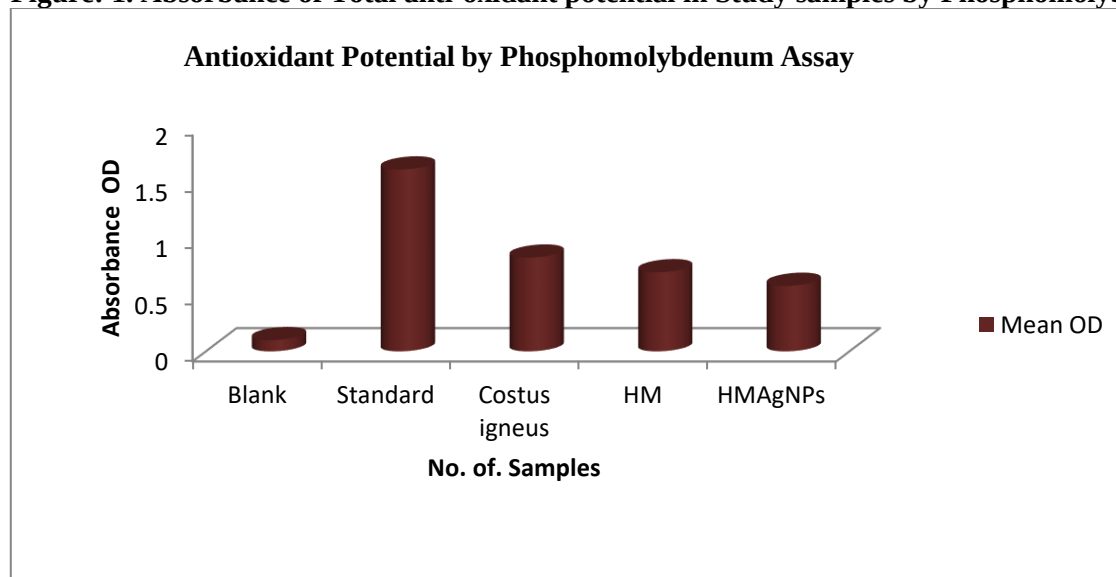
PM assay is based on the reduction of Phosphate-Mo (VI) to Phosphate Mo (V) by the sample and subsequent formation of a bluish green coloured phosphate/Mo (V) complex at acid pH. The Phosphomolybdenum method is routinely applied in the laboratory to evaluate the total antioxidant capacity of plant extracts.

Results and Discussion:

The analysis of antioxidant potential carried out by Phosphomolybdenum assay indicated the absorbance of total antioxidant potential at 695nm. The mean OD value for different samples by phosphomolybdenum assay is listed in the Table- 1; Figure -1. The table shows the mean OD value of 1.61 for standard (Ascorbic acid) followed by 0.83 for *Costus igneus*, 0.70 for HM sample, 0.58 for HMAgNPs and 0.10 for blank. The highest OD value is obtained in Standard (Ascorbic acid) with the OD value of 1.61 indicated that the extracts of *Costus igneus* have the ability to reduce the ferric ion, indicating the plant has antioxidant activity. Also the lowest OD value is noticed in HMAgNPs sample. The confined results confess, as the mean value increases, the antioxidant activity also increases. From the obtained results, it is concluded that the standard ascorbic acid have the highest antioxidant activity than other samples.

Table: 1. Absorbance of Total Anti-oxidant potential in Study samples by Phosphomolybdenum Assay

PM Absorbance at 695 nm					
S. No	Sample	OD I	OD II	OD III	Mean OD
1.	Blank	0.10	0.10	0.10	0.10
2.	Standard	1.61	1.59	1.62	1.61
3.	<i>Costus igneus</i>	0.86	0.82	0.82	0.83
4.	HM	0.70	0.71	0.70	0.70
5.	HMAgNPs	0.59	0.58	0.57	0.58

Figure: 1. Absorbance of Total anti-oxidant potential in Study samples by Phosphomolybdenum Assay

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 the *Costus* species produced marked and concentration-dependent increase in the reducing power. The results indicated a real good potential of antioxidant property. 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 (Halliwell, 1999).

The antioxidants neutralise their effect by reducing them as hydrogen donors, singlet oxygen quenchers, metal chelators (Shahidi and Wanasundara, 1992). 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 (Sunilkumar *et al.*, 2014). 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), peroxy (RO_2^-), superoxide (O_2^-), lipid peroxy (LOO) radicals and non-free radicals such as hydrogen peroxide (H_2O_2) and lipid peroxide (Yildirim and Mavi, 2000; Gulcin and Oktay, 2002).

Summary and Conclusion:

The present study exposed that the tested plant *Costus igneus* leaf extracts possess significant antioxidant potential. The results from Phosphomolybdenum assay revealed the highest rate of inhibition was noticed in Standard and the lowest rate of inhibition was noticed in HMAgNP samples. The results revealed the *C.igneus* leaf extracts has the ability to reduce the ferric ion indicating that the plant has antioxidant activity. The study also suggested that the standard ascorbic acid possess considerable antioxidant activity. Thus the *C.igneus* can be a source of newer useful drugs and of greater pharmacological importance as they neutralize the Reactive oxygen species.

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