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Phytochemical Analysis and Antioxidant Potential of Insulin Plant

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ABSTRACT

Costus speciosus (J.Koenig) Sm. is a tuberous plant commonly available in wetlands and near water bodies throughout Odisha state which is used as food and for medicinal purposes. The tribal communities of state use the rhizome to cure joint pain, skin infections and consume as nutraceutical. The above claims are supportive of the fact that the rhizome might have antioxidant potential and rich in diverse secondary metabolites. Keeping this in view an attempt has been made to evaluate the bioactive compounds present in the plant parts and antioxidant potential in order to validate the tribal claims. Results revealed that the plant parts are rich in phenolic compounds and have antioxidant potential.

Key words: *Costus speciosus*, Bioactive compounds, Antioxidant potential, Therapeutic values

Introduction

According to Ayurveda, the rhizomes of *Costus speciosus* are bitter, astringent, acrid, cooling, aphrodisiac, purgative, antihelminthic, depurative, febrifuge, expectorant, improve digestion and are stimulants that clear toxins. The plants are rich in varieties of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides, saponins and volatile oils which are important in combating different diseases. *C. speciosus* exhibits significant antidiabetic properties and is given the name "insulin plant" as a very important antidiabetic agent Diosgenin is found in them (Sulakshana and Rani, 2013). Phytochemical constituents exhibit a wide range of biological effects

resulting in their protective or disease preventive properties (Akpan *et al.*, 2012). Some possible actions are antioxidant activities, hormonal action, stimulation of enzymes, interference with DNA replication, antimicrobial effects, and physical action (Okwu and Okwu, 2004). It is necessary to identify the phytochemical components used in the treatment of diseases. *C. speciosus* is an important medicinal plant and is the only species of family Costaceae found in Odisha. It is a straight long leafy stem that grows about 1.5 to 2.5m height, rhizome is tuberous and the leaves are spirally arranged with silky pubescence beneath. It is commonly known as "crepe ginger". *C. speciosus* is found in humid tropics of both the hemispheres. The plant is not only useful in providing a food source but also used in traditional

health care remedy. (Behera *et al.*, 2016). The rural and aboriginals use the rhizome of *C. speciosus* as raw vegetables. Rhizomes are used in treating diseases like pneumonia, rheumatism, dropsy, urinary diseases, jaundice, etc. The leaves are reported to possess antidiabetic properties, used against skin infections, dysentery, aid in mental disorders, etc. (Duraipandian *et al.*, 2012). The rhizomes are major source of diosgenin. The rural and tribal people use the leaves against diarrhoea, fever, headache, cough and rhizomes for skin diseases and snake bites (Ariharan *et al.*, 2012). The rhizome and leaves of *C. speciosus* have shown promising antifungal activity against many fungal strains (Duraipandian and Ignacimuthu, 2011). Keeping all the above-mentioned potentials of the plant species, an attempt has been made to document its ethnobotanical values and to validate scientifically the tribal claims.

Materials and Methods

Aim of the present study is to investigate the phytoconstituents present in the leaf, rhizome, stem, and seed of *C. speciosus*. The phytochemicals present might be the reason behind various pharmacological properties of the plant. The study might lead to find an appropriate solvent in which the compounds present could be extracted and furthermore to find out which part of the plant extract could be more effective. Ethnobotanical survey data was collected from various tribal groups and validated with the antioxidant activity of *C. speciosus* rhizome extracts.

Ethnobotanical data collection

The field work was conducted with the tribal and rural communities of Mayurbhanj, Cuttack, Puri and Khurdha. The methodological framework was followed as per standard technique of ethnobiological approaches of Christian and Brigitte, 2004. Plant species were confirmed by Dr. Sanjeet Kumar, APRF, Odisha.

Selection and collection of experimental plant

The experimental plant was collected from the Khurdha district, in the peripheral area of Chandaka-Dampara Wildlife Sanctuary and was kept in polybags tagged with the botanical name as per standard sampling procedure and passport description (Hawkes 1980, Christian and Brigitte, 2004). The collected germplasm of experimental plant was propagated and grown in the field bank

of Department of Botany, Ravenshaw University for further experimental work.

Extract preparation

Soxhlet method was adopted (Tiwari *et al.*, 2011) to obtain the extracts of experimental plant parts. The residue was collected and left for air drying and dried crude extract was stored in refrigerator for experimentation. The plant material was collected from the garden of Botany Department of Ravenshaw University. Then it was thoroughly washed, dried and grounded. 100 gm of powdered leaf, rhizome, stem and seed of *C. speciosus* was extracted with hexane, petroleum ether, toluene, ethyl acetate, acetone, ethanol, methanol and distilled water respectively. Each extract was tested for the presence of phytochemicals like tannins, flavonoids, alkaloids, phenolic compounds, glycosides, steroids, saponins using standard procedures to identify phytoconstituents as described by standard methods (Kumar *et al.*, 2017).

Phytochemical analysis

Phytochemical analysis of rhizome was carried out using standard procedure to identify the possible bioactive compounds.

Test of Tannins: 0.5 g powder was boiled in 10 ml of distilled water and filtered through whatman 42 filter paper. 2 ml of filtrate was taken in a test tube and 3-5 drops of 0.1 % ferric chloride solution were added. The brownish green or blue-black colouration indicated the presence of tannins.

Test for Saponins: 0.5 g powder was boiled in 15 ml of distilled water and filtered through Whatman 42 filter paper. 5 ml of filtrate was taken in a test tube and mixed with 2 ml of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

Test of Flavonoids: 6 ml of dilute ammonium solution was added to a portion of the aqueous filtrate of 0.5 g powder taken in the test tube followed by the addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

Test of Terpenoids: 0.5 g powder taken in the test tube was mixed with 1 ml of methanol and 2.5 ml of chloroform. Then to this 3 ml of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

Test of Glycosides: 0.5 g powder taken in the test tube was treated with 1 % ferric chloride solution

and was placed in water bath for 5 minutes at 100 °C. The mixture was cooled and equal volume of benzene was added. The benzene layer was separated and 5 ml of ammonia solution was added to it. Formation of rose-pink colour indicated the presence of glycosides.

Test of Phenolic compounds: 0.5 g powder was treated with 3-5 drops of 1 % ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

Test for Reducing sugars: 0.5 g powder was dissolved in distilled water and filtered. The filtrate was boiled with 2 drops of Fehling's solution A and B for 5 minutes. An orange-red precipitate obtained indicated the presence of reducing sugar.

Test for Steroids: 0.5 g powder was dissolved in 2 ml of methanol and again dissolved in 5 ml chloroform followed by addition of 5 ml concentrated sulphuric acid. Formation of 2 phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

Test for Alkaloids: 0.5 g powder was mixed with 5 ml of 1% aqueous HCl on water bath and then filtered. 2-5 drops of Dragendorff's reagent were added to the filtrate. The formation of orange-red precipitate indicated the presence of alkaloids in the sample extract.

Estimation of antioxidant activity

In order to study the antioxidant activity of experimental plant extracts, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and metal chelating (MC) activity were evaluated. The standard methods were adopted for the said scavenging activity. DPPH assay was carried out followed by Cao *et al.*, 1997 and

metal chelating activity was done followed by Gouda *et al.*, 2014. The DPPH activity was expressed as IC₅₀ values (effective concentration showing 50 % of inhibition activity). DPPH test was carried out using 5.0 ml of dilutions (100 µg/ml) of the experimental compounds and standard were mixed with 1 ml of 0.001 % ethanolic solution of DPPH. DPPH solution was freshly prepared in each experiment and was stored in dark at 4± 2 °C. The compounds were incubated for 20-30 minutes in the dark at 30±2 °C. After incubation, Spectrophotometer readings were taken at 517 nm. The experiments were performed in triplicate for better documentation. The Metal Chelating Activity of the plant extracts was determined followed by Gouda *et al.* (2014).

Results and Discussion

The present study revealed the phytochemical constituents of aqueous, methanol, ethanol, acetone, ethyl acetate, toluene, petroleum ether and hexane extracts of *C. speciosus* leaf, rhizome, stem, flower, and seed respectively (Tables 1-5). All the extracts of the different parts of *C. speciosus* showed different phytochemicals like tannins, flavonoids, phenolic compounds, saponins, steroids, terpenoids, glycosides, reducing sugars, etc. The bioactive compounds present suggest the pharmacological properties of the plant. The ethanol, methanol and water extracts showed good solubility of compounds in case of leaf extract of *C. speciosus*. In case of rhizome extract, ethanol and methanol extracts showed good solubility. The stem extract showed good results in methanol:water (1:1) ratio. Aqueous extract of flower and seed showed good solubility of compounds. Khayyat and AL-Kattan (2017), have re-

Table 1. Phytochemical screening in different leaf extracts of *C. speciosus*

Extract → Phytochemicals ↓	Aqueous	Methanol	Methanol: water(1:1)	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannins	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Steroids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycosides	+ve	+ve	+ve	+ve	+v	-ve	-ve	-ve	-ve
Reducing sugars	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
Colour	Straw yellow	Dark green	Yellowish green	Dark green	Dark green	Blackish green	Light green	Greenish yellow	Light green

ported the presence of tannins, saponins, steroids and flavonoids in the rhizome of *C. speciosus*.

The most common free radicals that all aerobic organisms produce are reactive oxygen species (ROS). Biological molecules like DNA, proteins, lipids, and lipoproteins are vulnerable targets for them as ROS can react very easily with them. If the DNA repair system fails to reverse the damages to DNA, this leads to harmful mutations and more likely to develop into cancer (Beckman *et al.*, 1994). Similarly, when the target molecules of these harmful oxidants are proteins, they inhibit the enzymes, and degrade the proteins. Oxidative injury is caused by the peroxidation of lipids and causes pathophysiological disorders like diabetes, cirrhosis, atherosclerosis, arthritis, cancer, inflammation, diabetes, and genotoxicity (Sunitha, 2016). Antioxidants derived by the plants are the most efficient solution to the pathologies driven by free radical attacks (Girgis *et al.*, 2015). For this, plants show effective counterac-

tion mechanisms. Medicinal plants contain flavonoids and phenolics that possess property to protect living organisms against the hazardous effect of ROS as they have strong free radical scavenging potential and metal ions chelation properties (Govindarajan *et al.*, 2005). *C. speciosus* is a very rich source of major antioxidant compounds such as phenolic acids, xanthenes, tannins, lignans, flavones, ascorbic acid, β -carotene, α -tocopherol, glutathione, and flavonoids (Devi and Urooj, 2010). Maji *et al.*, (2020) have reported that the presence of phenolic contents present in different parts of the plant is responsible for antioxidant activity. The antioxidant activity of rhizome extract showed good scavenging activity in methanol and acetone extracts in both DPPH assay & Metal chelating activity. The presence of phenolic compounds and antioxidant potential showed that the rhizome could be effective against joint pain and can inhibit bacterial growth. One of the reasons behind the antioxidative poten-

Table 2. Phytochemical screening in different rhizome extracts of *C. speciosus*

Extract → Phytochemicals ↓	Aqueous	Methanol	Methanol : water(1:1)	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannins	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoids	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	-ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Steroids	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
Reducing sugars	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Colour	Light creamy	Light creamy	Light creamy	Very light creamy	Colour- less	Colour- less	Colour- less	Colour- less	Colour- less

Table 3. Phytochemical screening in different stem extracts of *C. speciosus*

Extract → Phytochemicals ↓	Aqueous	Methanol	Methanol: water(1:1)	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannins	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoids	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve
Phenolic compounds	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Steroids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycosides	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Reducing sugars	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
Colour	Light pale yellow	Light green	Light green	Light green	Light green	Light yellow	Fluorescent green	Very light green	Very light green

tial of the methanolic extract is the presence of flavonoids, triterpenoids, glycosides, steroids, and tannins in *C. speciosus* (El-Far *et al.* 2018). Spiral ginger plant shows diverse pharmacological effects due to the presence of a variety of bioactive phytochemicals. But the compounds like Diosgenin, Costunolide and Eremanthin are highly specific which exhibit effective antidiabetic, anticancerous, and antioxidative properties through different mechanisms. Hence, *C. speciosus* can be exploited as an economically sustainable source of effective drug

against deadly disease like diabetes with less or no side effects. (Sohrab *et al.*, 2021).

The results of the present study revealed the phytochemical profile of leaf, rhizome, stem, flower, and seed extracts of *C. speciosus*. Qualitative investigation of the plant parts of *C. speciosus* indicated the presence of different phytochemical constituents like tannins, flavonoids, saponins, phenolics, terpenoids, steroids, glycosides. The extracts of rhizome showed antioxidant potential. Thus, this might be the reason for various pharmacological efficacy of

Table 4. Phytochemical screening in different flower extracts of *C. speciosus*

Extract → Phytochemicals ↓	Aqueous	Methanol	Methanol : water(1:1)	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannins	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Steroids	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Glycosides	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve
Reducing sugars	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Colour	Creamy	Creamy	Opaque white	Colourless	Opaque white	Light creamy	colourless	colourless	Light cream

Table 5. Phytochemical screening in different seed extracts of *C. speciosus*

Extract → Phytochemicals ↓	Aqueous	Methanol	Methanol : water(1:1)	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannins	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoids	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Alkaloids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponins	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compounds	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Steroids	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycosides	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve
Reducing sugars	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Colour	Light yellow	Light yellow	Light orange	Very light yellow	Yellowish green	Colour less	Colour less	Colour less	Creamy

Table 6. Antioxidant activities of *C. speciosus* extract

Plant extract (100 µg/ mL)	DPPH scavenging activity (IC ₅₀ value) *		Metal chelating activity (IC ₅₀ value) *	
	Methanol extract	Acetone extract	Methanol extract	Acetone extract
Rhizome	62.05± 0.03	69.21 ± 0.30	61.40 ± 0.26	66.20 ± 0.50
Leaves	53.05± 0.05	46.20 ± 0.10	43.40 ± 0.50	48.20 ± 0.50
Stem	50.05± 0.03	44.20 ± 0.20	41.40 ± 0.50	42.20 ± 0.50
BHT	132.86 ± 0.20	97.92	± 0.62	

*Values in µg/mL

the species. As *C. speciosus* has been successfully used as a remedy in traditional system of cure for quite a long time it provides a wide area of interest for the researchers in development of new drug molecules. The beneficial prospective can also be seen in combination with other curative agents.

Conflict of Interest

Authors declare no conflict of interest.

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