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Phytochemical Analysis and Antioxidant Potential of *Costus speciosus L.*

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Authors' contributions

All the authors have contributed for this work. Author AB carried out the experimental analysis. Author RSD analyse the data and drafted the manuscript. Author SK design the work, protocol and made the data analysis. Authors PKJ and SKB proofread the work and manuscript.

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ABSTRACT

Costus speciosus L. is a tuberous plant commonly available in wetlands and near water bodies throughout Odisha state and used as food and medicinal purposes. The tribal communities of the state used 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 potentials and might be rich with diverse secondary metabolites. Keeping this in view an attempt has been made to evaluate the bioactive compounds present in the plant parts and antioxidant potentials in order to validate the tribal claims. Results revealed that the plant parts are rich with phenolic compounds and have antioxidant potential.

Keywords: Costus speciosus; bioactive compounds; antioxidant potentials; therapeutic values.

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1. 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. Phytochemical constituents exhibit a wide range of biological effects resulting in their protective or disease preventive properties [1]. Some possible actions are antioxidant activities, hormonal action, stimulation of enzymes, interference with DNA replication, anti-microbial effect and physical action [2,3]. It is necessary to identify the phytochemical components used in the treatment of diseases. *Costus 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 0.6 to 1.8 height, rhizome is tuberous and the leaves are spirally arranged with silky pubescent beneath. It is commonly known as “crepe ginger”. *Costus 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 [4]. The rural and aboriginals use the rhizome of *Costus speciosus* as raw vegetable. Rhizomes are also given in diseases as 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. [5,6]. The rhizomes are the major source of diosgenin [7]. The rural and tribal people use the leaves against diarrhoea, fever, headache, cough and rhizomes for skin diseases and snake bites [8]. The rhizome and leaves of *C. speciosus* have shown promising antifungal activity against many fungal strains [9]. 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.

2. MATERIALS AND METHODS

The aim of the present study is to investigate the phytoconstituents present in the leaf, rhizome, stem and seed of *Costus speciosus.* The phytochemicals present mightbe the reason behind various pharmacological properties of the plant. The study might lead to find appropriate solvent in which the compounds present could be extracted and furthermore 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 *Costus speciosus* rhizome extracts.

2.1 Ethnobotanical Data Collection

The field work was conducted with the tribal and rural communities of Mayurbhanj, Cuttack, Nayagarh, Puri and Khurdha. The methodological framework was followed as per standard technique of ethno-biological approaches of Christian and Brigitte [10]. Plant species was confirmed using standard flora book Saxena and Brahmam [2].

2.2 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 poly bags tagged with the botanical name as per standard sampling procedure and passport description [11,12,10]. The collected germplasm of experimental plant was propagated and grown in the field bank of Department of Botany, Ravenshaw University for further experimental work.

2.3 Extract Preparation

Soxhlet method was adopted [13] 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 *Costus speciosus* was extracted with hexane, petroleum ether, toluene, ethyl acetate, acetone, ethanol, methanol and distilled water respectively. Each extract was tested for presence of phytochemicals like tannin, flavonoids, alkaloids, phenolic compounds, glycosides, steroids, saponins using standard procedures to identify phytoconstituents as described by Harborne and Sofowora [14,15] [Fig. 1].

2.4 Phytochemical Assays

Phyto-chemical analysis of rhizome was carried out using standard procedure to identify the possible bioactive compound(s) [16,14,15].

Fig. 1. Phytochemical test of *Costus speciosus* (leaf, stem, rhizome, flower and seed) using different reagents

2.5 Test of Tannin

The powder was boiled in 10 mL of distilled water and filtered with 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.

2.6 Test for Saponin

The powder was boiled in 15 mL of distilled water and filtered with Whatman 42 filter paper. 5 mL of filtrate was mixed with 2 mL of normal distilled water and shaken vigorously. The stable persistent froth indicated the presence of saponins.

2.7 Test of Flavonoids

6 mL of dilute ammonium solution was added to portion of the aqueous filtrate of powder followed by addition of concentrated sulphuric acid. A yellow colouration indicated the presence of flavonoids.

2.8 Test of Terpenoids

Powder was mixed with 1 mL of methanol and 2.5 mL of chloroform and 3 mL of concentrated sulphuric acid was added. A reddish-brown colouration of interface indicated the presence of terpenoids.

2.9 Test of Glycosides

Powder was treated with 1% ferric chloride solution and was put into 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. Formation of rose-pink colour indicated the presence of glycosides.

2.10 Test of Phenolic Compounds

Powder was treated with 3-5 drops of 1% ferric chloride solution. Formation of bluish black colouration indicated the presence of phenolic compounds.

2.11 Test for Reducing Sugar

Powder was dissolved with 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 was obtained indicated the presence of reducing sugar.

2.12 Test for Steroids

Powder was dissolved in 2 mL of methanol and again dissolved in 5 mL chloroform and then 5 mL of concentrated sulphuric acid was added. Formation of 2 phases (upper red and lower yellow with green fluorescence) indicated the presence of steroids.

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

Extract→ Phytochemicals↓	Aqueous	Methanol	Methanol+water	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannin	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Flavonoid	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compound	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoid	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Steroid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycoside	+ve	+ve	+ve	+ve	+v	-ve	-ve	-ve	-ve
Reducing sugar	+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

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

Extract→ Phytochemicals↓	Aqueous	Methanol	Methanol+water	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannin	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoid	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compound	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoid	-ve-	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Steroid	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Glycoside	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve
Reducing sugar	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve
Colour	Light creamy	Light creamy	Light creamy	Very light creamy	Colourless	Colourless	Colourless	Colourless	Colourless

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

Extract→ Phytochemicals↓	Aqueous	Methanol	Methanol +water	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannin	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Flavonoid	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve
Phenolic compound	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Steroid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycoside	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Reducing sugar	+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

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

Extract → Phytochemicals↓	Aqueous	Methanol	Methanol +water	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannin	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Flavonoid	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve
Phenolic compound	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Terpenoid	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Steroid	-ve	-ve	-ve	+ve	+ve	-ve	-ve	-ve	-ve
Glycoside	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve
Reducing sugar	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve
Colour	Creamy	Creamy	Opaque white	Colourless	Opaque white	Light creamy	colourless	colourless	Light creamy

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

Extract → Phytochemicals↓	Aqueous	Methanol	Methanol +water	Ethanol	Acetone	Ethyl acetate	Toluene	Petroleum ether	Hexane
Tannin	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-ve
Flavonoid	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve
Phenolic compound	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve
Terpenoid	+ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
Steroid	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glycoside	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve
Reducing sugar	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Colour	Light yellow	Light yellow	Light orange	Very light yellow	Yellowish green	Colourless	Colourless	Colourless	Creamy

Table 6. Antioxidant activities of *Costus speciosus* extracts (100 µg/ mL)

Plant extract	DPPH scavenging activity (EC_{50} value)*		Metal chelating activity (EC_{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*

2.13 Test for Alkaloids

Powder mixed with 5 mL of 1% aqueous HCl on water bath and then filtered. 2-5 drops of dragendorff's reagent were added in the filtrate. The occurrence of orange-red precipitate indicated the presence of alkaloids in the sample extract.

2.14 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 was carried out followed by Cao et al. [17] and metal chelating activity was done using Gouda et al. [18]. The DPPH activity was expressed as EC_{50} values (effective concentration showing 50% of inhibition activity). DPPH was carried out (Table 6) using 5.0 mL of dilutions (100 µg/mL) of the experimental compounds and standard were mixed with 1 mL of a 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. All determination was performed in triplicate for better documentation. The Metal Chelating Activity of the plant extracts was determined using Gouda et al. [18]. About 1 ml of plant extract added to a solution of 0.5 mL ferrous chloride (0.2mM). Then about 0.2 ml. of Ferrozine (5mM) was added to it and incubated at room temperature for 10 minutes. The absorbency of the solution was then measured at 562 nm.

3. RESULTS AND DISCUSSION

The present study has designed as per the field work. The ethnobotanical survey has done in selected areas of Odisha and results revealed that plant parts are used as food, against skin infection, cough and as a birth control agent while mixed with *Dioscorea* species. The present study revealed the phytochemical constituents of aqueous, methanol, ethanol, acetone, ethyl acetate, toluene, petroleum ether and hexane extracts of *Costus speciosus* leaf, rhizome, stem, flower and seed respectively (Tables 1-5). All the extracts of the different parts of *Costus speciosus* showed different phytochemicals like tannins, flavonoids, phenolic compounds, saponins, terpenoids, glycosides, reducing sugar, etc. Among all the components tannin content was highest in all the selected plant parts followed by saponins, terpenoids, glycosides and reducing sugar. The amount of alkaloids and phenolic compounds was very low in the respective extracts. The bioactive compounds present suggest the pharmacological properties of the plant as a whole. The ethanol, methanol and water extracts showed good solubility of compounds in case of leaf extract of *Costus speciosus*. In case of rhizome extract ethanol and methanol extracts show good solubility. The stem extract shows good results in methanol: water (1:1) ratio. Aqueous extract of flower and seed show good solubility of compounds. There are lots of reports are available on its pharmacological values [19-21]. 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 show that the rhizome could be effective against joint pain and can inhibit bacterial growth.

4. CONCLUSION

The results of the present study revealed the phytochemical profile of leaf, rhizome, stem, flower and seed extracts of *Costus speciosus*. Qualitative investigation of the plant parts of *Costus speciosus* indicates the presence of different phytochemical constituents like tannin, flavonoid, terpenoid, saponins, glycosides, etc. & antioxidant potentials. Thus, this might be the reason for various pharmacological efficacy of the species. As *Costus speciosus* has been successfully used as a remedy in traditional systems 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.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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