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FREE RADICAL SCAVENGING ACTIVITY OF RHIZOME OF *COSTUS SPECIOSUS* (KOEN) J.E. SM

Gayatri Nahak and Rajani Kanta sahu*

B.J.B. Autonomous College, Botany Department, Bhubaneswar, Odisha, India

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For Correspondence:

Rajani Kanta Sahu

B.J.B Autonomous College, Bhubaneswar, Odisha, India

E-mail:

gayatri.bioteq@gmail.com
sahurajani.sahu@gmail.com

ABSTRACT

The Zingiberaceae plants are characterized by their tuberous or non-tuberous rhizomes, which have strong aromatic and medicinal properties. *Costus speciosus* (Koen) J.E. Sm. (Keu, Crape ginger) is the only species in the genus *Costus* that is medicinally important which possesses a diverse number of pharmacological activities. It is an Indian ornamental plant, has long been medicinally used in traditional systems of medicine. It has a wide numbers of phytochemical constituents which possesses activities like astringent, aphrodisiac, purgative, anthelmintic, depurative, febrifuge and expectorant. The present article gives an account of updated information on its phytochemical and free radical scavenging properties by DPPH assay. The assay revealed that ethanolic extract of *Costus speciosus* showed maximum level of free radical scavenging activity with 71.61±0.02% with IC_{50} value 25(µg/ml). In general, the ethanolic extracts were better free radical scavengers than the methanolic and aqueous extracts. Our findings also showed a strong correlation of antioxidant activity with the total phenolic content. The findings indicated promising antioxidant activity of crude extracts of the above plant needs further exploration for their effective use in both modern and traditional system of medicines.

INTRODUCTION

Zingiberaceae family constitutes a vital group of rhizomatous medicinal and aromatic plants characterised by the presence of volatile oils and oleoresins of export value. Generally, the rhizomes and fruits are aromatic, tonic and stimulant; occasionally they are nutritive. Some are used as food as they contain starch in large quantities while others yield an astringent and diaphoretic juice. The important genera coming under Zingiberaceae are Curcuma, Kaempferia, Hedychium, Amomum, Zingiber, Alpinia, Elettaria and *Costus*. In the genus Alpinia, A. galanga is the most important one, which finds varying uses in ayurvedic preparations such as "Rasnadi powder". *Costus speciosus* is the only species in the genus *Costus* that is medicinally important. This plant of Costaceae (*Zingiberaceae*) family is commonly known as keukand (hindi), Variegated Crepe Ginger (English). From the innumerable plants being researched since time immemorial, *Costus speciosus* (Koen) J.E.Sm. (Zingiberaceae) is an important one. It is an erect, succulent, perennial herb, up to 2.7 meters in height, arising from a horizontal rhizome. Rhizomes clothed with sheaths in the lower parts, leafy upwards, leaves elliptic to oblong or oblong-lancoelate, thick, spirally arranged, 15-35 cm X 6-10 cm, silky beneath, with stem clasping sheaths up to 4 cm, flowers large, white, in thick, cone-like terminal spikes, with bright red bracts, lip with yellowish throat; fruits globosely trigonous, red capsules, 2 cm in diameter, seeds black, with white aril[1]. The dried rhizome is curved or somewhat straight, cylindrical, branched piece, 10-30 cm in length and 3-5 cm in diameter in dried condition, upper surface marked with circular nodal scars with remnants of leaf bases, lower and lateral surfaces exhibit small circular scars of roots or few wiry rootlets fracture fibrous and fractured surface is yellowish brown. No characteristic taste or odor[12]. It has wide distribution in India, occurring throughout the sub-Himalayan tract from Himachal Pradesh to Assam, Vindhya and Satpura hills in central India and the western ghats of Maharashtra, Karnataka, Kerala[14]. Various medicinal properties are attributed to it, particularly in the treatment of asthma, fungal diseases, rheumatism, diabetes, hepatoprotective disorders[1].

The rhizomes and roots are ascribed to be bitter[4,6,14], astringent[4,6,14,21,25], acrid, cooling, aphrodisiac[1,4,21], purgative[4,6,14,21], anthelmintic[1,6,14,21,25], depurative[1,6,21,25] febrifuge, expectorant, tonic[1,14], improves digestion[4] and stimulant[4,14,25] herb that clears toxins. Juice of the rhizome is applied to head for cooling and relief from headache[14]. An alkaloid extract from *Costus speciosus* rhizomes had papaverine-like smooth muscle relaxant, antispasmodic activities in lab animals[3]. Rhizomes are given in pneumonia, rheumatism, dropsy, urinary diseases, jaundice and leaves are given in mental disorders. It is valued very much for its diosgenin content. *Costus* is one of the plants, which contains diosgenin in its rhizome. It is widely used as starting material in the commercial production of steroidal hormones. The rhizomes are useful in vitiated conditions of burning sensation, flatulence, constipation, helminthiasis, leprosy, skin diseases, fever, hiccough, asthma, bronchitis, inflammation and aneamia. It is used to make sexual hormones andcontraceptives[31].

The demand of neutraceuticals is increasing day by day so herbs can be a better option for the replacement of synthetic antioxidant agents. This paper deals with the comparative antioxidant activity of multi-solvent extracts of the rhizome of *Costus speciosus* based on their phenol contents.

MATERIALS AND METHODS

Plant Materials

The rhizomes of *Costus speciosus* was collected from the local tribal people of Jeypore, Koraput, Orissa. Fresh rhizomes were rinsed severally with clean tap water to make it dust and debris free. Then they were dried in the shady condition for 3to4 days until they become moisture free. Dried rhizomes were ground in electric chopper to get fine powder form for further use.

Preparation of plant extracts

50g of the dried and powdered form of rhizome of *Costus speciosus* was extracted successively with ethanol (each 400ml.) for 10-12 hrs, using a Soxhlet apparatus. Then collected solutions were filtered through Whatman No-4 filter paper. The extracts were evaporated to dryness under reduced pressure at 90^0C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at -18^0C until used for further analysis.

Phenolic Estimation

The total phenol content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described[22]. Reading samples on a UV-vis spectrophotometer at 650 nm. Results were expressed as Catechol equivalents (mg/g).

Qualitative Estimation of Phytochemicals

Phytochemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Trease *et al.*[9], Harborne[15] and Edeoga *et al.*[8].

Antioxidative activity

The evaluation of free radical scavenging activity (antioxidant activity) was conducted by the method of[5] with modifications. The following concentrations of extracts were prepared 40µg/mL, 80µg/mL, 120µg/mL, 160µg/mL and 200µg/mL. A stock solution of the sample (100mg/ml) was diluted for 5 concentrations. Each concentration was tested in triplicate. The portion of sample solution (0.5ml) was mixed with 3.0ml of 0.1mM 1,1-Diphenyl-2-2picrylhydrazyl (DPPH, in 95% distilled ethanol) and allowed to stand at room temperature for 30 minute under light protection. The absorbance was measured at 517nm.The scavenging activity of the samples at corresponded intensity of quenching DPPH. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as (%) scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1\text{-As/Ac}) \times 100$$

As is the absorbance of the sample at t =0 min.

Ac is the absorbance of the control at t=30 min.

In the DPPH test, antioxidants were typically characterized by their IC_{50} value (Inhibition Concentration of Sample required to scavenge 50% of DPPH radicals). The results were obtained by linear regression analysis of the dose response curve plotted using % inhibition and concentration.

RESULTS AND DISCUSSION

A medicinal herb can be viewed as a synthetic laboratory as it produces and contains a number of chemical compounds. These compounds responsible for phytochemical activities of the herb are secondary metabolites[7]. In the qualitative phytochemical screening, all the crude extracts have the main constituents. The pharmacological action of crude extract is determined by the nature of its constituents[24], such as alkaloids, terpenoids, flavonoids, glycosides, saponins, tannins, etc (Table-1). The difference in the purity and strength of the crude drugs may be due to quantitative and qualitative difference in the active principles or presence of compounds. Rhizomes of *Costus speciosus* contain diosgenin, 5 a-stigmast-9 (11)-en-3b-ol, sitosterol-β-D-glucoside, dioscin, prosapogenins A and B of dioscin, gracillin and quinones. Various saponins, many new aliphatic esters and acids are reported from its rhizomes, seeds and roots. Rhizomes possess antifertility, anticholinestrase, antiinflammatory, stimulant, depurative and anthelmintic activities[17]. Rhizomes yield diosgenin (2.12%) and tigogenin. Saponins showed estrogen like activity in albino rats similar to stilbestrol. Essential oil from rhizome showed antimicrobial activity[2]. Alkaloidal fraction from rhizomes showed papaverine like smooth muscle relaxant, antispasmodic, cardiotonic, hydrochloretic, diuretic and CNS depressant activities in laboratory animals. b-amyrin stearate, b-amyrin and lupeol palmitates from leaves[28].

Initially crude extracts were obtained by extractions with solvent of increasing polarity, viz. water, methanol and ethanol. The amounts of extracts in different solvent extracts are 2.26gm, 2.97gm and 3.12gm in *Costus speciosus*. The total phenolics in the extracts were determined spectrophotometrically by the Folin-ciocalteu method and the results were reported as catechol equivalents mg/g. As revealed by the data (Table-2), the total phenol content of ethanolic crude extract was higher than methanolic and aqueous extracts and they were as follow: 120mg/g, 109mg/g and 55mg/g respectively. In the present study, the values of ethanolic and methanolic extracts were higher than those of aqueous ones. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic component. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity[20].

Table-3 shows the results of the free radical (DPPH) scavenging activity in (%) inhibition in multi-solvent extracts of rhizome of *Costus speciosus*. The result revealed that the ethanol fraction of *Costus speciosus* rhizome exhibited the highest radical scavenging activity with 71.61±0.02% with IC_{50} value 210µg/ml followed by its methanol extract with 66.15±0.08% and aqueous extract with 60.13±0.07% at 200(µg/ml) (Fig-1). The Methanol and ethanol has been proven as effective solvent to extract phenolic compounds[30]. The chemical constituent tigogenin and diosgenin from rhizomes (2.6% diosgenin) and stems have been isolated. a-amyrin stearate, b-amyrin and lupeol which are the compounds for showing the strong antioxidant capacity shown by Joy *et al.*,[19].

Phenolic compounds are considered to be the most important antioxidative components of herbs and other plant materials, and a good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported[23,26]. Moreover, such a preparation consist more oxindole alkaloids and a larger spectrum of biologically active constituents. High percent of yield and high value of phenol content in ethanolic extracts show that phenolic constituents must be responsible for such properties. It is in agreement with the data of[11]. In our present study

we found that there is a positive correlation between total phenolic content and antioxidant activity in the multi-solvent rhizome extracts of *Costus speciosus*. Gupta *et al*.[13] isolated five new compounds (Oxo acids and branched fatty acids esters)–tetradecyl 13-methylpentadecanoate, tetra 11-methyltriadecanoate, 4-oxotriaconsanoic acid, 14–oxoheptacosanoic acid and 15-oxooctacosnoic acid from the rhizomes, which were also characterized[13] Methyl 3-(4-hydroxyphenyl)-2E-propenoate was also isolated from the rhizomes[1,29]. The rhizomes also contain saponins diosgenin[18], dioscin, gracillin and betasitosterol-β-D-glucoside[21,27]. The rhizomes yield an essential oil which contains pinocarveol (59.9%), cadinene (22.6%), cineol (10.7%), p-methoxybenzophenone (3.3%) and cavacrol (1.3%)[1]. Bis(2-ethylhexyl)phthalate was isolated from the rhizomes of *C.speciosus*[10]. Many studies have demonstrated a correlation between phenolic content and antioxidant activity[32]. The correlation between total phenolic content and antioxidant capacity in our plant samples is possible owing to the presence of following factors: the antioxidant activity observed in plant extracts may be due to the presence of phenolic compounds or polyphenols or flavonoids or tannins (Table-1 and 2).

Table-1: Preliminary Phytochemical screening of rhizome of *Costus speciosus*

Phytoconstituents	***Costus speciosus***
Alkaloids	+
Carbohydrates	+
Glycosides	+
Terpinoid	---
Protein & Amino acids	+
Fixed oils & fats	+
Tannins	+
Saponins	+
Steroids (Phytosterols)	+
Flavonoids	+
Phenols	+
Curcumin	---
Reducing sugar	---
Anthraquinones	+
+ denotes present and --- denotes absent	

Table-2: Crude extracts, Phenol content and IC_{50}Values in *Costus speciosus*

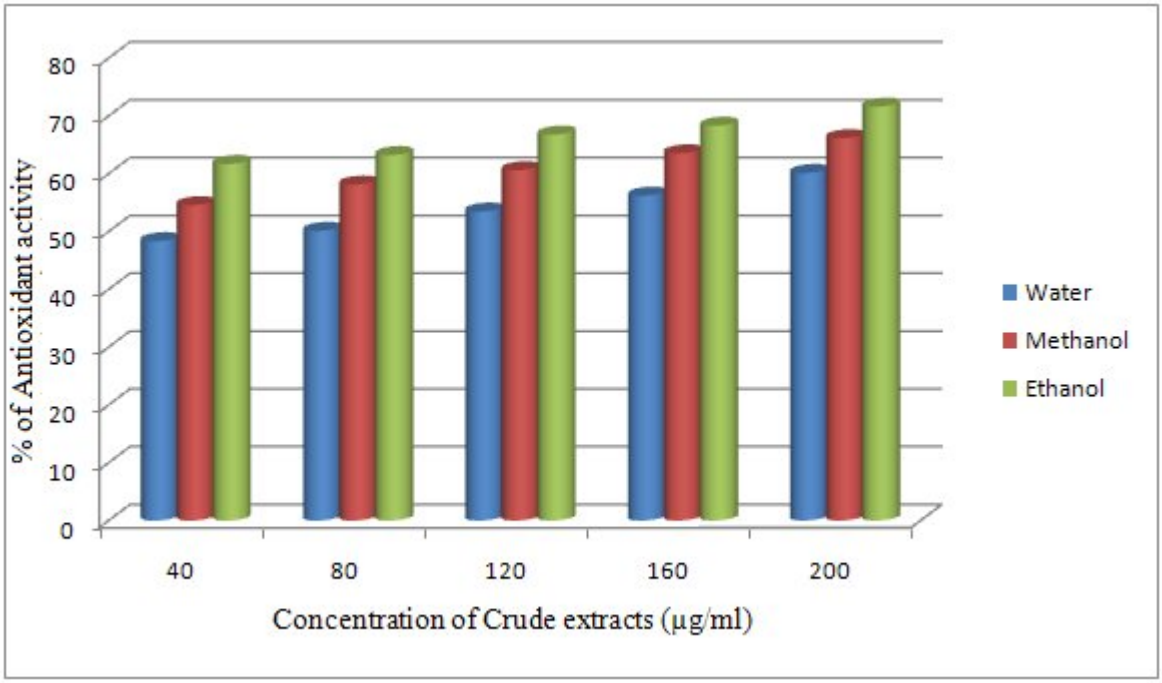
Parameters	**Water**	**Methanol**	**Ethanol**
Crude Extracts (gm)	2.26	2.97	3.12
Phenol content (mg/g)	55	109	120
IC_{50}Value (µg/ml)	80	38	25

Table-3: Antioxidant activity (%) of Multi-solvent extracts of *Costus speciosus*

Conc. of extracts (µg/ml)	Antioxidant activity (%)		
	Water	Methanol	Ethanol
40	48.33±0.01	54.56±0.02	61.69±0.04
80	50.10±0.05	58.12±0.06	63.23±0.05
120	53.45±0.06	60.61±0.03	66.76±0.04
160	56.20±0.02	63.53±0.05	68.30±0.03
200	60.13±0.07	66.15±0.08	71.61±0.02

Data are expressed as mean±S.D

Figure-1: DPPH free radical scavenging activity of *Costus speciosus*



CONCLUSION

As antioxidants, flavonoids and phenolics have been reported to be able to interfere with the biochemical pathways involved in the generation of reactive oxygen species (ROS), quenching free radicals, chelating transition metals and rendering them redox inactive in the Fenton reaction[16]. Therefore, the presence of high amount of flavonoids and their multifaceted actions make the aforementioned plant extract a good candidate for exploration of antioxidants. The experimental results shows the presence of polyphenolic compounds responsible for good antioxidant potential thus justifying its use in traditional and modern health care practices.

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REFERENCES

1. Anonymous, "The Wealth of India: First Supplement Series (Raw Materials)", National Institute of Science Communication and Information Resources, CSIR, 2007;2:211-213.
2. Asolkar L.V., Kakkar K.K., and Chakre O.J., "Second Supplement to Glossary of Indian Medicinal Plants with Active Principles Part I (A-K). (1965-81)", Publications and Information Directorate (CSIR), New Delhi. 1992;Pp-414.
3. Bhattacharya S.K., Parikh A.K., Debnath P.K., Pandey V.B., Neogy N,C., "Pharmacological studies with the alkaloids of *Costus speciosus*", Journal of Research in Indian Medicine", 1973;8(1), 10-19.
4. Bown Deni, "Encyclopaedia of Herbs, The Royal Horticulture Society, 2008;Pp-181.
5. Brand-Williams W., Cuvelier M.E. and Berset C., "Use of free radical method to evaluate antioxidant activity", Lebensmittel Wissenschaft and Technologie, 1995;28(1):25-30.
6. Chopra R.N., Nayar S.L., Chpora I.C., Glossary of Indian Medicinal Plants, National Institute of Science Communication and Information Resources, 2006;Pp-79.
7. Dubey N.R., Kumar R., and Tripathi P., "Global promotion of herbal medicine: India's opportunity", Current Science, 2004;86(1): 37-41.
8. Edeoga H.O., Okwu D.E. and Mbaebie B.O., "Mbaebie Phytochemical constituents of some Nigerian medicinal plants", African Journal of Biotechnology 2005;4 (7),685-688.
9. G. E. Trease, W. C. Evans; Trease and Evans Pharmacognosy, 15th Ed. W. B. Saunders Edinburgh London, New York, Philadelphia St. Louis Sydney Toronto, 2002;3-4, 528-533, 538-547.
10. Farooqui A.H.A. and Shukla Y.N., Indian Drugs, 1987;25(1), 37.
11. Gonçalves J.L.S., Lopes R.C., Oliveira D.B., Costa S.S., Miranda M.M.F.S., Romano M.T.V., Santos N.S.O., Wigg M.D., "In vitro anti-rotavirus activity of some medicinal plants used in Brazil against diarrhea", Journal of Ethnopharmacology, 2005;99 (3):403–407.
12. Gupta A.K., Tondon N., Sharma M., "Quality Standards of Indian Medicinal Plants", Medicinal Plants Unit, Published by Indian Council of Medical Research, 2008;Vol VII, 48.
13. Gupta M.M., Verma R.K., Akhila A., "Oxo acids and branched fatty acid esters from rhizomes of *Costus speciousus*", Phytochemistry, 1986;25(8), 1899-1902.
14. Gupta R.K., **"**Medicinal and Aromatic Plants with Colour Plates: Traditional and Commercial Uses Agrotechniques Biodiversity Conservation (HB)**",** CBS 2010;234-499.
15. Harborne J.B., "Phytochemical Methods, a guide to modern techniques of plant analysis", 3 edn. Springer (India) Private Limited, New Delhi, 1998.
16. Heim K.E., Tagliaferro A.R., & Bobilya D.J., "Flavonoid, antioxidants: chemistry, metabolism and structure–activity relationships. Journal of Nutritional Biochemistry, 2002;13,572–584.

17. Husain A., Virmani O.P., Popli S.P., Misra L.N., Gupta M.M., Srivastava G.N., Abraham Z., and Singh A.K., "Dictionary of Indian Medicinal Plants", CIMAP, Lucknow, India. 1992;Pp-546.
18. Srivastava S., Singh P., Mishra G., Jha K. K., Khosa R. L. "*Costus speciosus* (Keukand): A review", Pelagia Research Library, 2011, 2 (1): 118-128.
19. Joy P.P.J., Thomas S. Mathew, and Skaria B.P., "Zingiberaceous Medicinal and Aromatic Plants. Aromatic and Medicinal Plants Research Station, Odakkali, Asamannoor P.O., Kerala, India, 1998.
20. Karadeniz F.H.S., Burdurulu N., Koca and Soyer Y., "Antioxidant activity of selected fruits and vegetables grown in Turkey. Journal of Agriculture and Food Chemistry, 2005;29, 297-303.
21. Khare C.P., "Indian medicinal plants", Springer, 2007;181-182.
22. Kim K.T.K., Yoo M., Lee J.W., Eom S.H., Hwang I.K., Lee C.Y., "Protective effect of steamed American ginseng (*Panax quinquefolius* L.) on V79-4 cells induced by oxidative stress", J. Ethnopharm, 2007;111:443-445.
23. Madsen H.L, Nielsen B.R., Bertelsen G., and Skibsted L.H., "Screening of antioxidative activity of spices", Food Chemistry, 1996;57, 331-337.
24. Mukherjee P.K, "Quality Control of Herbal Drugs, an approach to evaluation of botanicals", 1st edn. Business Horizons, New Delhi, 2002.
25. Nadkarni K.M, "Indian Materia Medica", Bombay Popular Prakashan, 2009;1,385-386.
26. Pellegrini N., Simonetti P., Gardana C., Brenna O., "Brighenti activity of Vini Novelli (Young red wines)", Journal of Agriculture and Food Chemistry, 2000;48, 732-735.
27. Qiao C.F., Li Q.W., Dong H., Xu L.S., Wang Z.T., and Yao Za Zhi Z.Z., "Studies on Chemical. Constituents of two Plants from *Costus*", 2002;27(2), 123-5.
28. Rastogi R.P. and Mehrotra B.N., "Compendium of Indian Medicinal Plants", Vol. II. Central Drug Research Institute, Lucknow and Publications and Information Directorate, New Delhi. 1991;Pp-833.
29. Rastogi R.P., Mehrotra B.N., "Compendium of Indian Medicinal plants", Central Drug Research Institute, Lucknow and National Institute of Science Communication and Information Resources, New Delhi, 2004;4,224-225.
30. Siddhuraju P. and Becker K., "Antioxidant properties of various extracts of total phenolic constituents from three different agro-climatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves", Journal of Agriculture and Food Chemistry, 2003;51:2144-55.
31. Warrier P.K., Nambiar V.P.K., and Ramankutty C., "Indian Medicinal Plants", Orient Longman Ltd., Madras, 1993-1995;Vol.1-5.
32. Yang J.H., Lin H.C. and Mau J.L., "Antioxidant properties of several commercial mushrooms", Food Chem, 2002;77: 229-235.