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Original Research Article

Effect of Extraction Solvents on in vitro Antioxidant Activity of Costus speciosus Leaves

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Abstract

Costus speciosus (*C. speciosus*) has long been medicinally used in different systems of medicine. The plant has several pharmacological properties including antioxidant activity. Aim of the present work was to see the effects of extraction solvents on *in vitro* antioxidant activity of *C. speciosus* leaves. Leaves of *C. speciosus* were collected from the local market and identified by the taxonomist. Extracts of the leaves were prepared separately using acetone, methanol, ethanol, chloroform, ethyl acetate and petroleum ether. With the help of xanthine-xanthine oxidase assay, linoleic acid peroxidation assay and DPPH photometric assay, *in vitro* antioxidant activities of all extracts were checked. Total phenol, ascorbic acid, flavonoids and carotenoids contents of the extracts were also determined. Results showed that methanol extract of *C. speciosus* leaves had maximum *in vitro* antioxidant activity in comparison to other solvent extracts. This was due to presence of high amount of total phenol in methanol extract. Methanol extract of *C. speciosus* leaves, therefore, may be further investigated in search for natural antioxidant compounds.

Keywords: *Costus speciosus* leaves, Extraction solvents, *In vitro* antioxidant activity.

INTRODUCTION

C. speciosus (family, Costaceae) is an erect, perennial herb, found in tropical region of India along roadsides, streams and in wastelands [1]. The plant is also found in moist tropical evergreen forests, up to an altitude of 1200 m [2].

C. speciosus is commonly known as keu (Bengali , Hindi) though it has several other names like Kostam (Tamil), Kashmeeramu (Telegu), Channakoova (Malayalam), Kembuka (Sanskrit), Paskarmula (Guajarati), Tara (Assam), Spiral flag (English) etc [3].

In traditional medicine *C. speciosus* is used as purgative, anthelmintic, expectorant and stimulant. It is also used in treatments of rheumatism, dyspepsia, skin diseases, diarrhea, dysentery, cough and cold, fever, bronchial asthma, pneumonia, dropsy, urinary diseases, jaundice, eye and ear infections and snake bites [4].

Phytochemicals like dioscin, gracillin, methyl protodioscin, methylprotogracillin, protogracillin, 26-O-β-D-glucopyranosyl-(25R)-furost-5-ene-3β, diosgenin 3-O- β-Dglucopyranosyl (1→3)-β-D-glucopyranoside, 8-hydroxy triacontane-25-one, methyl triacontanoate, 5α-stigmast-9(11)-en-3β-ol, dihydrophytylplastoquinone, α-tocopherolquinone, 24-hydroxytriacontan-26-one, 24-hydroxytriacontan-27-one, 3-O-[β-D-glucopyranosyl(1→4)-β-D-glucopyranosyl]-26-O-(β-Dglucopyranosyl-22α-methoxy (25R) furost-5-en-3β,26-diol and its 22-hydroxy derivatives, 3-O-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]-26-O-[β-D-glucopyranosyl]- 22α-methoxy-(25R) furost-5-en-3β,26-diol,protodioscin, 3-O-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]-26-O-[β-Dglucopyranosyl]-22α-methoxy-(25R)furost-5-en-3β, diosgenin etc. were isolated from different parts of *C. speciosus* [5, 6].

C. speciosus showed different pharmacological activities. These include anti bacterial, anti fungal, anti-inflammatory, anti oxidant, anti cancer, anti diabetic, antipyretic, antifertility, anticholinestrase, and antihelminthic, hepatopretective, hypolipidemic, adaptogenic activities etc [7, 8].

Aim of the present work was to see effect of extraction solvents, if any, on *in vitro* antioxidant activity of *C. speciosus* leaves.

MATERIAL AND METHODS

Plant Material

Leaves of *C. speciosus* were collected from the local market and authenticated by the taxonomist of the department of Botany of the University of North Bengal, Siliguri. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of Sikkim Manipal University, Gangtok, Sikkim, India for future references.

***Costus Speciosus* Leaves**

Test Drug

Leaves of *C. speciosus* were washed thoroughly under tap followed by distilled water. Leaves were then shed dried and powered. The powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

Solvent Extraction

Test drug (100g) was extracted separately with 500 ml of methanol, ethanol, ethyl acetate, chloroform, petroleum ether and acetone in soxhlet at 37^{0}C for 15 minutes. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. This was applied separately for all extracts. Brown masses obtained were used for antioxidants assays as well as for determination of total phenol, ascorbic acid, flavonoids and carotenoids contents.

Antioxidant Assays

In vitro anti oxidant activity of *C. speciosus* leaves was assayed through superoxide anion generation by xanthine-/xanthine oxidase assay [9], linoleic acid peroxidation assay [10] and by DPPH photometric assay [11].

Determination of Anti Oxidant Chemicals

Anti oxidant chemicals viz. flavonoids, total phenols, ascorbic acid and total carotenoids present in *C. speciosus* leaves were determined by the methods of Chang *et al*., [12], McDonald *et al.*, [13], Cakmak and Marschner [14] and Jensen [15] respectively. .

Chemicals

Chemicals required for the study were purchased from Sigma Chemicals Co., USA; Merck, Germany; Himedia Lab and Loba Chem., India.

Statistical Analysis

All experiments were performed in triplicate. The results were expressed as mean ± SE. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison test. A p-value of <0.05 was considered statistically significant [16].

RESULTS

Results of *in vitro* antioxidant activity of different extracts of *C. speciosus* leaves through superoxide anion generation by xanthine-/xanthine oxidase, linoleic acid peroxidation and by DPPH photometric assays are given in Table-1. Results showed that all extracts (acetone, methanol, ethanol, chloroform, ethyl acetate and petroleum ether) of *C. speciosus* leaves had *in vitro* antioxidant activity but maximum activity was found in the methanol extract.

Inhibitions in xanthine oxidase, linoleic acid peroxidation and DPPH in methanol extract were 92%, 76% and 82% respectively. Results were statistically significant in comparison to that of other extracts. Results were also comparable with that of quercetin, a known antioxidant compound, where inhibition in

xanthine oxidase, linoleic acid and DPPH came 100%, 89% and 92% respectively.

Table-1: Inhibition in xanthine oxidation, linoleic acid peroxidation and DPPH scavenging capacity by the different solvent extracts of *C. speciosus* leaves

Powder obtained from extract of *C. speciosus* leaves	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Petroleum ether	14 ± 0.4	15 ± 0.2	11 ± 0.2
Ethyl acetate	22 ± 0.5	23 ± 0.4	21 ± 0.4
Ethanol	51 ± 0.7	41 ± 0.6	42 ± 0.3
methanol	92 ± 1.0*	76 ± 0.8*	82 ±0.7*
Acetone	43 ± 0.6	34 ± 0.5	45 ± 0.6
Chloroform	33 ± 0.5	25 ± 0.4	27 ± 0.4
Quercetin	100 ± 0.03	89 ±0.01	92 ± 0.01

Concentration used: 100 µg / ml. Results were a mean of triplicate experiments ± SE. *Significant

Table-2 shows that total phenol content of the methanol extract of *C. speciosus* leaves was 62 ± 0.6 mg/mg dry wt which was maximum in comparison to that of petroleum ether extract (10 ± 0.2 mg/mg dry wt), ethyl acetate extract (15 ± 0.5 mg/mg dry wt), ethanol extract (22 ± 0.4 mg/mg dry wt), acetone extract (24 ± 0.3 mg/mg dry wt) and chloroform extract (20 ± 0.2 mg/mg dry wt). The results were statistically significant. Flavonoids, ascorbic acid and carotenoid contents of different solvent extracts of *C. speciosus* leaves apparently showed difference in value but the results were not statistically significant.

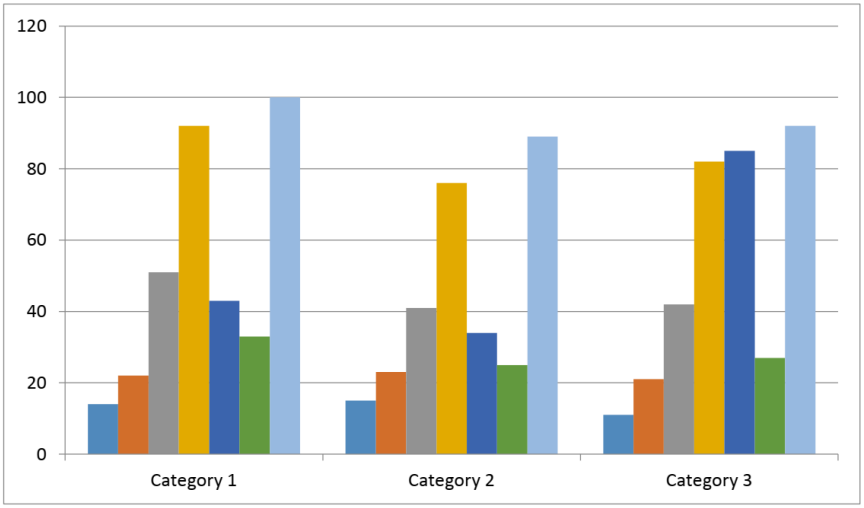
Table-2: Total phenol, flavonoids, ascorbic acid and carotenoid contents of different solvent extracts of *C. speciosus* leaves

Leaves of *C. speciosus*	Total phenol content (mg/mg dry wt)	Total flavonoids content (mg/mg dry wt)	Ascorbic acid content (mg/g dry wt)	Carotenoids content (mg/g dry wt)
Petroleum ether	10 ± 0.2	18 ± 0.4	19 ± 0.5	3.3 ± 0.8
Ethyl acetate	15 ± 0.5	22 ± 0.6	14 ± 0.7	4.1 ± 0.8
Ethanol	22 ± 0.4	21 ± 0.5	21 ± 0.8	4.4 ± 0.7
Methanol	62 ± 0.6*	24 ± 1.1	22 ±0.9	5.8 ± 1.5
Acetone	24 ± 0.3	21 ± 0.8	18 ± 0.6	4.3 ± 0.9
Chloroform	20 ± 0.2	22 ± 0.6	20 ± 0.7	3.9 ± 0.7

Results were a mean of triplicate experiments ± SE.
*Significant

DISCUSSION

Extraction solvents exert effect on the antioxidant activity of medicinal plant extracts [17]. Meena *et al.*, showed that methanolic extract of whole leaf powder of *Baccopa monnieri* exhibited significantly higher antioxidant activity than the other solvent extracts [18]. Dent *et al.*, used methanol, ethanol and acetone as extraction solvents of dry sage leaves and observed that ethanol extract had maximum antioxidant activity [19]. Acetone, ethanol, methanol, ethyl acetate, and hexane were used as extraction solvents for walnut green husk. It was observed that ethanol, acetone and methanol extracts exhibited stronger antioxidant activities, followed by ethyl-acetate and hexane extract [20]. In the present study *in vitro* anti oxidant activity of methanol extract of *C. speciosus* leaves was found maximum in comparison to that of other solvents viz. ethanol, chloroform, acetone, ethyl acetate and petroleum ether used for extraction purpose. Results were found statistically significant. There was maximum inhibitory effect of methanol extract of *C. speciosus* leaves in xanthine oxidation and linoleic acid peroxidation as well as scavenging capacity of DPPH which was comparable to that of quercetin, a standard antioxidant compound (Figure-1). Other investigator, however, showed the antioxidant activity of chloroform extract of *C. speciosus* leaves for its free radical scavenging activity [4].

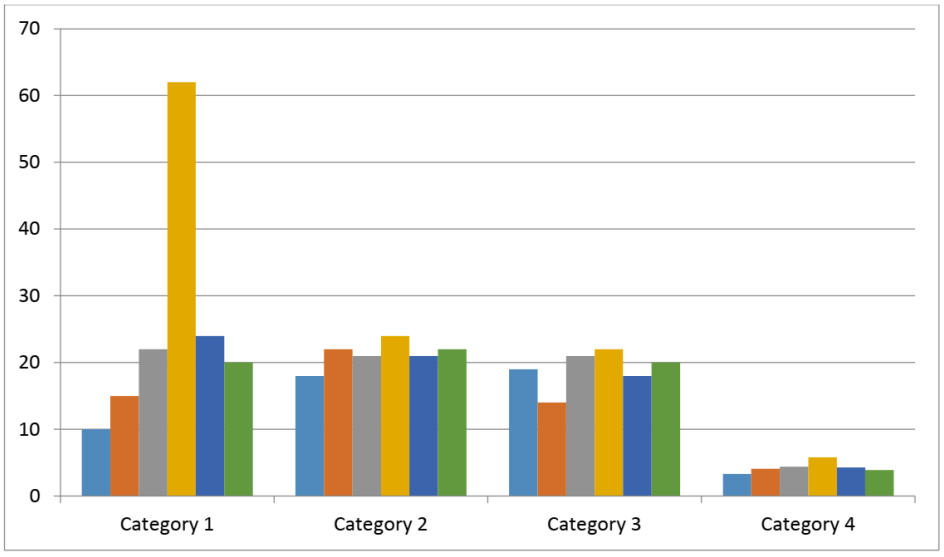


Category 1: Xanthine oxidase (% inhibition) Category 2 : Linoleic acid peroxidation (% inhibition) Category 3: DPPH (% inhibition)

Petroleum ether Ethyl acetate Ethanol Methanol
Acetone Chloroform Quercetin

Fig-1: Showing % inhibition of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by different solvent extract of *C. speciosus* leaves

It is reported that total phenol, flavonoids, ascorbic acid and carotenoids present in a plant are responsible for anti oxidant activity of the plant [21]. In the present study methanol extract of *C. speciosus* leaves showed presence of maximum amount of total phenol in comparison to that of ethanol, acetone, chloroform, petroleum ether and ethyl acetate extracts. Flavonoids, ascorbic acid and carotenoids contents of all the extracts of *C. speciosus* leaves, however, did not show any significant change (Figure-2). *In vitro* anti oxidant activity of *C. speciosus* leaves was therefore due to presence of high amount of total phenol in the leaves.



Category 1: Total phenol content Category 2: Total flavonoids content
Category 3: Ascorbic acid content Category 4: Carotenoids content

Petroleum ether Ethyl acetate Ethanol
Methanol Acetone Chloroform

Fig-2: Showing amounts (mg/g dry wt) of total phenol, flavonoid, ascorbic acid and carotenoid in different solvent extracts of *C. speciosus* leaves

It is known that season has significant effect on the synthesis of secondary metabolites in plants thereby changing their biologic activity [22, 23]. We are now studying seasonal effect on amounts of total phenol, flavonoids, ascorbic acid and carotenoids in *C. speciosus* leaves vis-à-vis its *in vitro* antioxidant activity.

CONCLUSION

Methanol extract of *C. speciosus* leaves may be further investigated in search for natural antioxidant compounds.

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Conflict of Interest: Nil

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