

Seasonal Effect on In Vitro Antioxidant Activity of *Costus speciosus* Leaves

Prasenjit Mitra¹, Tanaya Ghosh², Prasanta Kumar Mitra^{3*}

¹Department of Biochemistry, All India Institute of Medical Sciences (AIIMS), Jodhpur, India

²Department of Medical Biotechnology, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India

³Professor & Head, Department of Medical Biotechnology, Sikkim Manipal University, Sikkim Manipal Institute of Medical Sciences, Gangtok, Sikkim, India

*Corresponding author: Prasanta Kumar Mitra

| Received: 19.05.2019 | Accepted: 25.05.2019 | Published: 30.05.2019

DOI: [10.21276/haya.2019.4.4.7](https://doi.org/10.21276/haya.2019.4.4.7)

Abstract

Effect of season on *in vitro* antioxidant activity of *Costus speciosus* (*C. speciosus*) leaves was studied. Leaves of *C. speciosus* of different seasons were collected from the local market and authenticated by the taxonomist. *In vitro* antioxidant activity of the leaves was measured by superoxide anion generation with the help of xanthine-xanthine oxidase assay, linoleic acid peroxidation assay and DPPH photometric assay. Amount of total phenols present in the leaves of different seasons was also estimated. Results showed that *in vitro* antioxidant activity of the leaves of *C. speciosus* was maximum during summer (March – May) in comparison to other seasons of the year. Amount of total phenols present in the leaves was also found maximum in summer. *In vitro* antioxidant activity of *C. speciosus* leaves, therefore, was due high amount of total phenols in the leaves. Leaves of *C. speciosus* of summer may be further investigated to get natural antioxidant compound.

Keywords: *Costus speciosus* leaves, *In vitro* antioxidant activity; Total phenols, Effect of season.

Copyright @ 2019: This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

INTRODUCTION

It is reported that biological activities of plants vary with seasons of the year. Osadebe *et al.* worked on seasonal variation for the antidiabetic activity of methanolic extract of *Loranthus micranthus* and noted that the activity is highest at the peak of the rainy season [1]. Effect of seasonal variation on the antineoplastic activity of *Alstonias cholaris* R. Br. in HeLa cells was studied by Jagetia and Baliga. Highest cell killing effect was observed by the plant of summer collection [2]. Ncube *et al.*, studied seasonal variation in antimicrobial activity of frequently used medicinal bulbous plants from South Africa and noted that the activity was higher in spring and winter than in other seasons [3]. Effect of seasonal variation on the anti-inflammatory activity of *Sargassum wightii* was studied by Dar and coworkers. They found that the plant collected during winter was most effective in reducing carrageenan-induced edema in rats [4]. Report from our laboratory showed that *Cassia alata* leaves during the period of May – June had maximum protective effect on anti tubercular drugs induced hepatotoxicity in rats [5]. We also reported that UV absorption property of *Amaranthus spinosus* was maximum during autumn in comparison to other seasons of the year [6].

C. speciosus (family, Costaceae) commonly known as ‘keu’ is a medicinal plant of varying pharmacological activities such as anti cancer, anti diabetic, antipyretic, anti oxidant, antifertility, anticholinestrase, anti bacterial, anti fungal, anti-inflammatory, antihelminthic, hepatoprotective, hypolipidemic, adaptogenic activities etc [7]. Recently we have seen that methanol extract of *C. speciosus* leaves could exert maximum *in vitro* antioxidant activity (results are under communication). The aim of the present work was to see effect of season on *in vitro* antioxidant activity of *C. speciosus* Leaves.

MATERIAL AND METHODS

Plant Material

Leaves of *C. speciosus* were collected from the local market during summer (March – May), rainy season (June – August), Autumn (September – November) and Winter (December – February). Leaves were authenticated by the experts of the department of Botany of the University of North Bengal, Dist. Darjeeling, West Bengal, India. A voucher specimen was kept in the department of Medical Biotechnology, Sikkim Manipal Institute of Medical Sciences of the Sikkim Manipal University, Gangtok, Sikkim, India for future references.

Test Drug

Leaves of *C. speciosus* of different seasons were washed thoroughly under tap followed by distilled water. Leaves were then separately shed dried and powdered. The powder, used as test drug, was stored desiccated at 4 °C until further use.

Extraction of the Test Drug

Test drugs (100g each) obtained from *C. speciosus* leaves of different seasons were separately

extracted with 500 ml of methanol in soxhlet at 37°C for 15 minutes. Methanol was used as solvent because we have noted earlier (results are under communication) that methanol extract of *C. speciosus* leaves had maximum *in vitro* antioxidant activity. Extracts obtained were filtered and the solvents of all extracts were evaporated separately to dryness *in vacuo* with rotary evaporator at 40 – 50 °C. Obtained brown mass was used for antioxidant assays as well as for the determination of total phenols.



Costus speciosus leaves

Antioxidant Assays

In vitro antioxidant activity of powdered leave extracts of *C. speciosus* of different seasons was assayed through superoxide anion generation by xanthine-xanthine oxidase assay [8], linoleic acid peroxidation assay [9] and by DPPH photometric assays [10].

Determination of Total Phenols

Total phenols were determined following the method of McDonald *et al.*, [11].

Chemicals

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

Statistical Analysis

All experiments were performed triplicate. The results were expressed as mean \pm 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 [12].

RESULTS

Effect of seasons on inhibitions of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by leave extracts of *C. speciosus* was shown in Table-1. Results show that extracts of leaves of *C. speciosus* of different seasons had *in vitro* antioxidant activity but maximum activity was found during Summer (March – May). Inhibitions of xanthine oxidase, linoleic acid peroxidation and DPPH scavenging capacity of summer sample were 94%, 77% and 85% respectively. Values were statistically significant in comparison to the values obtained for leave extracts of autumn, winter and rainy seasons. Results were also comparable to that of quercetin, a known antioxidant compound, where inhibition in xanthine oxidase, linoleic acid peroxidation and DPPH came 100%, 87% and 96% respectively.

Table-1: Effect of seasons on inhibitions of xanthine oxidation, linoleic acid peroxidation and scavenging capacity of DPPH by leaves extracts of *C. speciosus*

Powdered leaves of <i>C. speciosus</i> of different seasons	Xanthine oxidase (% inhibition)	Linoleic acid peroxidation (% inhibition)	DPPH (% inhibition)
Summer (March – May)	94 ± 1.2*	77 ± 0.9*	85 ± 0.7*
Rainy season (June - August)	23 ± 1.3	18 ± 0.8	16 ± 0.5
Autumn (September - November)	17 ± 1.0	29 ± 1.2	26 ± 1.0
Winter (December – February)	20 ± 1.6	20 ± 1.5	20 ± 0.7
Quercetin	100 ± 0.01	87 ± 0.8	96 ± 0.02

Concentration used: 100 µg / ml. Dose was fixed based on our earlier report [13]. Results were mean of triplicate experiments ± SE.

*Significant

Table-2 showed effect of season on total phenols content of the powdered leaves extracts of *C. speciosus*. Leaves extract of summer sample of *C. speciosus* contained maximum amount of total phenols (68 mg/mg dry wt.) while extracts of *C. speciosus*

leaves for autumn, winter and rainy seasons contained 28 mg/mg dry wt., 33 mg/mg dry wt. and 22 mg/mg dry wt. of total phenols respectively. Results were found statistically significant.

Table-2: Effect of season on total phenols content of the leaves extracts of *C. speciosus*

Powdered leaves of <i>C. speciosus</i> of different seasons	Total phenol content (mg/mg dry wt.)
Summer (March – May)	68 ± 0.7*
Rainy season (June - August)	22 ± 0.5
Autumn (September - November)	28 ± 0.6
Winter (December – February)	33 ± 0.5

Results were a mean of triplicate experiments ± SE

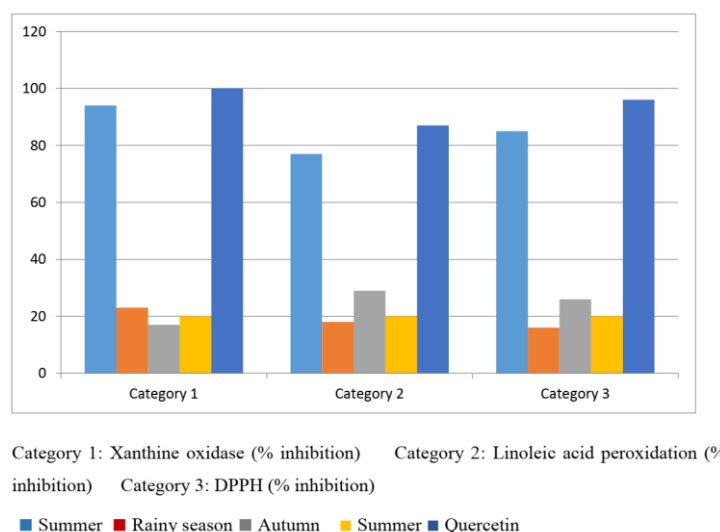
*Significant

DISCUSSION

Seasonal changes in antioxidant activity of medicinal plants are known in literature. Ercisli *et al.* noted that antioxidant activity of tea leaves was higher in July in comparison to other months of the year [14]. Antioxidant activity of *Baccharis dracunculifolia* collected monthly over a period of one year revealed considerable variation [15]. Sivaci and Duman studied seasonal antioxidant activity in stems and leaves of some almond (*Prunus amygdalus* L.) varieties and noted their maximum antioxidant activity during summer [16]. Seasonal variation in antioxidant activity of *Laurus nobilis* was studied by Bahmanzadegan *et al.* They found that

the plant exerted maximum antioxidant activity during spring [17]. We also studied effect of season on *in vitro* antioxidant activity of *Syzygium cumini* L. leaves. Results showed that antioxidant activity of the plant leaves was maximum during summer in comparison to other seasons of the year [18].

In the present study effect of season on *in vitro* antioxidant activity of *C. speciosus* leaves was studied. Antioxidant activity of the plant leaves, measured by inhibitions in xanthine oxidase, linoleic acid peroxidation and scavenging capacity of DPPH, was found maximum during summer ie, March – May (Figure-1).

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

It is known that phenolic compounds are responsible for antioxidant activity thereby exert multiple biological effects like free radical scavenging abilities, anti inflammatory, anti carcinogenic anti diabetic, anti gastric ulcer activities etc. [19]. We estimated total phenols content in *C. speciosus* leaves in

different seasons of the year. Amount of total phenols in the leave extracts was found maximum in summer (Figure-2). Antioxidant activity of *C. speciosus* leaves during summer was, therefore, due to maximum accumulation of total phenols in the plant leaves.

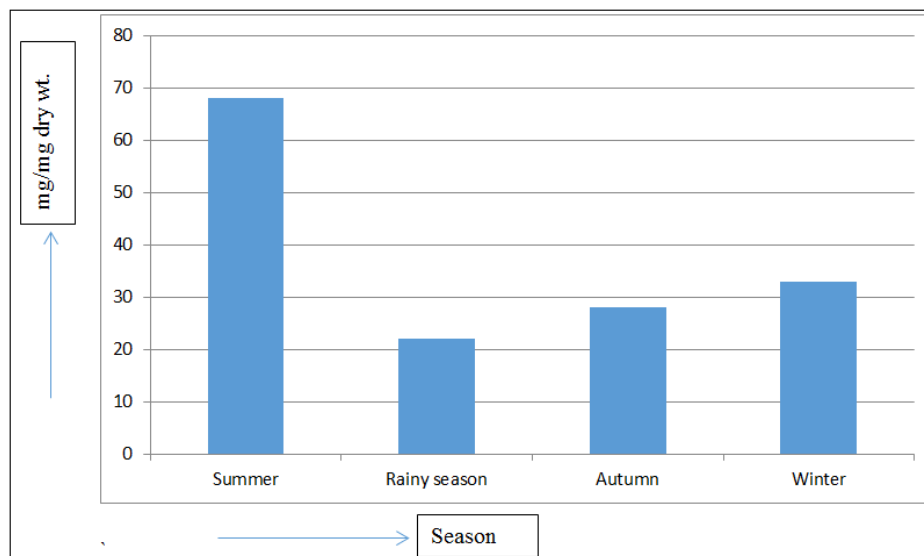


Fig-2: Amount of phenolic compounds in *C. speciosus* leaves: Effect of season

There is high demand of natural antioxidant as synthetic antioxidant such as butylated hydroxyanisole and butylated hydroxytoluene, though commercially available, are not safe [19]. As *C. speciosus* leaves of summer showed maximum *in vitro* antioxidant activity we are now planning to isolate natural antioxidant compound from the summer sample of *C. speciosus* leaves. Work in this direction is presently in progress in our laboratory.

CONCLUSION

In the present study summer variety of *C. speciosus* leaves showed maximum *in vitro* antioxidant activity. These leaves, therefore, may be used as natural antioxidant.

ACKNOWLEDGEMENT

Identification of *C. speciosus* leaves by the taxonomists of the department of Botany, University of North Bengal, Siliguri, Dist. Darjeeling, West Bengal is gratefully acknowledged.

Conflict of Interest: Nil

REFERENCE

- Osadebe, P. O., Omeje, E. O., Uzor, P. F., David, E. K., & Obiorah, D. C. (2010). Seasonal variation for the antidiabetic activity of *Loranthus micranthus* methanol extract. *Asian Pacific Journal of Tropical Medicine*, 3(3), 196-199.
- Jagetia, G. C., & Baliga, M. S. (2005). The effect of seasonal variation on the antineoplastic activity of *Alstoniascholaris* R. Br. in HeLa cells. *Journal of ethnopharmacology*, 96(1-2), 37-42.
- Ncube, B., Finnie, J. F., & Van Staden, J. (2011). Seasonal variation in antimicrobial and phytochemical properties of frequently used medicinal bulbous plants from South Africa. *South African Journal of Botany*, 77(2), 387-396.
- Dar, A., Baig, H. S., Saifullah, S. M., Ahmad, V. U., Yasmeen, S., & Nizamuddin, M. (2007). Effect of seasonal variation on the anti-inflammatory activity of *Sargassum wightii* growing on the N. Arabian Sea coast of Pakistan. *Journal of Experimental Marine Biology and Ecology*, 351(1-2), 1-9.
- Mitra, P., Ghosh, T., & Mitra, P. K. (2013). Seasonal variation in hepatoprotective activity of *Cassia alata* linn. leaves on antitubercular drugs induced hepatotoxicity in rats. *International Journal of Pharmacy Practice & Drug Research*, 3(1), 76-86.
- Ghosh, T., Mitra, P., & Mitra, P. K. (2019). Seasonal effect on UV absorption property of *Amaranthus spinosus* L. leaves. *European Journal of Biomedical and Pharmaceutical Sciences*, 6(5), 430-435.
- El-Far, A. H., Shaheen, H. M., Alsenosy, A. W., El-Sayed, Y. S., Al Jaouni, S. K., & Mousa, S. A. (2018). *Costus speciosus*: Traditional uses, phytochemistry, and therapeutic potentials. *Pharmacognosy Reviews*, 12(23), 120-127.
- Chang, W. S., Chang, Y. H., Lu, F. J., & Chiang, H. C. (1994). Inhibitory effects of phenolics on

- xanthine oxidase. *Anticancer research*, 14(2A), 501-506.
9. Choi, C. W., Kim, S. C., Hwang, S. S., Choi, B. K., Ahn, H. J., Lee, M. Y., ... & Kim, S. K. (2002). Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant science*, 163(6), 1161-1168.
 10. Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. D., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research*, 15(2), 127-130.
 11. McDonald, S., Prenzler, P. D., Autolovich, M., & Robards, K. (2001). Phenolic content and antioxidant activity of olive extracts. *Food Chemistry*, 73, 73-84.
 12. Bliss, C. I. (1967). Statistics in biology, *Statistical methods for research in the natural sciences*, Vol. 1, McGraw Hill Book Company, NY, 558.
 13. Mitra, P. K., Ghosh, T., & Mitra, P. (2016). In vitro anti oxidant activity of chromatographically separated fractions from the leaves of *Aastilbe rivularis* buch. – Ham. Ex D. Don. *SMU Medical Journal*, 3(2): 226-239.
 14. Ercisli, S., Orhan, E., Ozdemir, O., Sengul, M., & Gungor, N. (2008). Seasonal variation of total phenolic, antioxidant activity, plant nutritional elements, and fatty acids in tea leaves (*Camellia sinensis* var. *sinensis* clone Derepazari 7) grown in Turkey. *Pharmaceutical Biology*, 46(10-11), 683-687.
 15. Teixeira, E. W., Negri, G., Salatino, A., & Stringheta, P. C. (2010). Seasonal variation, chemical composition and antioxidant activity of Brazilian propolis samples. *Evidence-Based Complementary and Alternative Medicine*, 7(3), 307-315.
 16. Sivaci, A., & Duman, S. (2014). Evaluation of seasonal antioxidant activity and total phenolic compounds in stems and leaves of some almond (*Prunus amygdalus* L.) varieties. *Biological research*, 47(1), 1-5.
 17. Bahmanzadegan, A., Rowshan, V., Zareian, F., Alizaden, R., & Bahmanzadegan, M. (2015). Seasonal variation in volatile oil, polyphenol content and antioxidant activity in extract of *Laurus nobilis* grown in Iran. *J. Pharm. Pharmacol*, 3, 223-231.
 18. Mitra, P., Mitra, P. K., & Ghosh, T. (2018). Effect of Season on *In Vitro* Anti Oxidant Activity of *Syzygium cumini* L. Leaves. *Global Journal of Pharmacy & Pharmaceutical Sciences*, 5(5), 1-5.
 19. Brannen, A. L. (1975). Synthetic anti oxidant. *Journal of American Oil Chemist Society*, 52, 59-63.