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IN VITRO ALPHA AMYLASE INHIBITORY ACTIVITY OF *COSTUS SPECIOSUS* LEAVES: EFFECT OF SEASON

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ABSTRACT
Costus speciosus (*C. speciosus*) is a medicinal plant. Since long the plant is being used in traditional medicine for treatment of diabetes. Recently we have observed that *C. speciosus* leaves of this region exert *in vitro* anti amylase inhibitory activity. As biological activity of a medicinal plant varies with season, aim of the present work was to see the seasonal effect on *in vitro* alpha amylase inhibitory activity of *C. speciosus* leaves. Leaves of *C. speciosus* were collected during summer, autumn, winter and rainy season from the local market and authenticated by the taxonomist. Leaves were extracted with ethanol and the extract was checked for *in vitro* alpha amylase inhibitory activity by the standard method. Acarbose, a standard alpha amylase inhibitor, was used as control. Results showed that *C. speciosus* leaves of summer (March – May) had maximum alpha amylase inhibitory activity. Result was comparable to that of acarbose. As inhibition in the activity of alpha amylase may keep post prandial blood glucose level of diabetic patient under control, leaves of *C. speciosus* of summer may be used in the treatment of diabetes.

KEYWORDS: *C. speciosus* leaves, alpha amylase inhibitory activity, seasonal variation, acarbose, diabetes.

1. INTRODUCTION
Several workers demonstrated variation of biological activity of plant with season. It is told that biological activities of plants are due to plant's secondary metabolites and amounts of secondary metabolites vary with season. As early as 1955 Fluck and Pharm noticed influence of climate on the active principles in medicinal plants.[1] Feeny in 1970 observed seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars.[2] In 1977 Gupta reported variation in morphological characters and active principle constituents of *Eclipta prostrata* Linn. under different seasonal and soil conditions.[3] Seasonal variations in the antioxidant activity of ground bamboo *Sasa argenteastriatus* leaves was studied by Qinxue *et al.* It was found out that the highest antioxidant activity appeared in December and the lowest was in May.[4] Antidiabetic activity of methanolic extract of *Loranthus micranthus* was found highest at the peak of the rainy season.[5] Antimicrobial activity of frequently used medicinal bulbous plants from South Africa was higher in spring and winter than in other seasons.[6] Dar and coworkers showed that anti-inflammatory activity of *Sargassum wightii* is most effective in winter.[7] We also reported that UV absorption property of *Amaranthus spinosus* was maximum during autumn.[8]

C. speciosus (family, Costaceae) is a medicinal plant. The plant has several pharmacological activities like antifungal, antioxidant, anticancer, anti-inflammatory, antidiabetic, diuretic, antipyretic, antifertility, hepatopretective, hypolipidemic, antibacterial, anticholinestrase, antihelminthic etc.[9,10] *C. speciosus* leaves are also known for alpha amylase inhibitory activity.[11,12] Recently we confirmed alpha amylase inhibitory activity of *C. speciosus* leaves of this region. Paper is under communication. Aim of the present work was to see the seasonal effect on *in vitro* alpha amylase inhibitory activity of *C. speciosus* leaves.

2. METHODOLOGY
2.1 Collection of plant materials
C. speciosus. leaves 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. Leaves of plants were washed thoroughly, shed dried and powered. The

powder, used as test drug, was stored desiccated at 4 ^{0}C until further use.

***Costus speciosus* leaves**

2.2 Solvent extraction

Test drug (75g) was extracted separately with 500 ml of ethanol in soxhlet at 37^{0}C for 20 minutes. Ethanol was chosen as solvent as in our earlier study we found that ethanol extract of *C. speciosus* leaves had maximum *in vitro* alpha amylase inhibitory activity. Results were under communication. The extract was filtered and the filtrate was evaporated to dryness *in vacuo* with rotary evaporator at 40 – 50 ^{0}C. Brown mass obtained was used for *in vitro* alpha amylase inhibition assay.

2.3 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.*[13] with slight modifications. 400 µl of 0.1 M sodium phosphate buffer (pH 7.0), 500 µl of 1% starch solution, 10 µg/ml, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml of all ethanol extracts separately dissolved in DMSO and 50 µl of pancreatic α-amylase (Sigma, St. Louis, USA) solution (2 U/ml) were mixed and incubated at 37 °C for 10 min. 3 ml of 3,5-dinitrosalicylic acid (DNS) color reagent was then added. The mixture was kept in a boiling water bath for 5 min and then diluted with 20 ml of distilled water. The absorbance was recorded at 540 nm. Control sample was prepared accordingly without test drug and acted as a negative control. Acarbose was used as positive control.

Inhibition capacity of test extract and acarbose were calculated as following:

Inhibition Percentage (%) = 1 − DO sample/DO control × 100.

All tests were done for five sample replications. IC_{50} value which is the concentration required to inhibit 50% of alpha amylase activity was calculated.

2.4 Statistical calculation

This was done by SPSS 20. The statistical significance of enzyme inhibitions between test drugs and acarbose, the known inhibitor, was evaluated with Duncan's multiple range test (DMRT). 5% was considered to be statistically significant.[14]

3. RESULTS

Results are summarized in Table -1. Acarbose, standard alpha amylase inhibitor, exerted 24.7±1.1, 29.5±1.0, 37.0±1.1, 64.2±1.3, 71.3±1.1 and 78.2±1.2 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC_{50} value came, 46.7±1.1µg/ml. On the other hand ethanol extract of *C. speciosus*. leaves of Summer (March – May) showed 26.1±0.9, 30.7±1.1, 39.0±1.2, 65.3±1.1, 72.6±1.2 and 80.2±1.4 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC_{50} value came 45.9±1.0 µg/ml.

Ethanol extract of *C. speciosus*. leaves during rainy season (June – August) in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed percent of inhibitions in alpha amylase activity as 20.4±0.7, 28.5±1.2, 37.2±1.3, 50.2±1.1, 60.5±1.3 and 68.4±1.1 respectively with IC_{50} value 59.7±1.1µg/ml. But ethanol extract of *C. speciosus*. leaves of Autumn (September – November) had 19.2±1.1, 24.5±1.0, 36.1±1.4, 47.2±1.2, 54.6±1.0 and 61.2±1.3 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and

100 µg/ml respectively. IC_{50} value found, 63.5±1.2 µg/ml. Ethanol extract of *C. speciosus*. leaves of Winter (December – February) had 11.3±1.1, 19.5±1.0, 30.3±1.2, 39.1±1.7, 50.9±1.1 and 59.7±1.2 percent of inhibitions in alpha amylase activity in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml respectively. IC_{50} value came, 76.7±1.3 µg/ml.

Table 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and ethanol extracts of *C. speciosus*. leaves of different seasons.

Drug/solvent extract	Concentration (µg/ml)	% of inhibition	IC_{50}Value (µg/ml)
Acarbose	**10** **20** **40** **60** **80** **100**	**24.7±1.1** **29.5±1.0** **37.0±1.1** **64.2±1.3** **71.3±1.1** **78.2±1.2**	**46.7±1.1**
Ethanol extract of *C. speciosus* leaves Summer (March – May)	**10** **20** **40** **60** **80** **100**	**26.1±0.9** **30.7±1.1** **39.0±1.2** **65.3±1.1** **72.6±1.2** **80.2±1.4**	**45.9±1.0***
Ethanol extract of *C. speciosus* leaves Rainy season (June – August)	**10** **20** **40** **60** **80** **100**	**20.4±0.7** **28.5±1.2** **37.2±1.3** **50.2±1.1** **60.5±1.3** **68.4±1.1**	**59.7±1.1**
Ethanol extract of *C. speciosus* leaves Autumn (September – November)	**10** **20** **40** **60** **80** **100**	**19.2±1.1** **24.5±1.0** **36.1±1.4** **47.2±1.2** **54.6±1.0** **61.2±1.3**	**63.5±1.2**
Ethanol extract of *C. speciosus* leaves Winter (December – February)	**10** **20** **40** **60** **80** **100**	**11.3±1.1** **19.5±1.0** **30.3±1.2** **39.1±1.7** **50.9±1.1** **59.7±1.2**	**76.7±1.3**

Values are mean ± SE *Significant

4. DISCUSSION

Incidence of diabetes mellitus in increasing rapidly. From the year 1980 to 2014 there was about four fold increase in the incidence of diabetes throughout the world. In India diabetes is spreading in such a way that experts comment…. *By 2020 India will be the diabetic capital of the world*. Due to this high incidence diabetes claims number of lives every year. Only in 2011 diabetes mellitus was the cause of death of about 3 million people worldwide.[15]

Diabetes mellitus specially Type – 2 diabetes mellitus is characterized by post prandial hyperglycemia. There are many therapeutic approaches to reduce post prandial hyperglycemia. One approach is through the inhibition of alpha amylase enzyme.[16] Alpha amylase is the carbohydrate splitting enzyme. Mode of action of alpha amylase enzyme is to hydrolyze complex carbohydrates of food to free sugars Therefore, by inhibiting alpha amylase enzyme post prandial hyperglycemia may be kept under control. Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of Type - 2 diabetes mellitus.[17] Still continuous effect is going on in search of alpha amylase inhibitors from different sources which even extended to the field of medicinal plants. Now-a-days, many medicinal plants like *Teucrium oliverianum*, *Gymnema sylvsetre*, *Coccinia grandis*, *Tinospora cordifolia*, *Azadirachta indica*, *Phyllanthus amarus*, *Aegel marmelos*, *Teucrium Orientale*, *Acalypha indica*, *Allium cepa*, *Allium sativum* etc. are known possessing alpha amylase inhibitory activity.[18]

In traditional medicine leaves of *C. speciosus* is being used in the treatment for diabetes since long.[10] Recently we found that *C. speciosus*. leaves of this region exert alpha amylase inhibitory activity. Since activity of medicinal plant varies with season[1-8] we undertook the present study to note the possible seasonal variation in alpha amylase inhibitory activity of *C. speciosus* leaves.

The present work showed that ethanol extract of *C. speciosus* leaves of summer, winter, autumn and rainy seasons in all concentrations (10, 20, 40, 60, 80 and 100 µg/ml) exerted alpha amylase inhibitory activity in *in vitro* experiments. The activity was comparable to that of acarbose, one standard alpha amylase inhibitor (Figure – 1). Maximum activity, however, was noted in *C. speciosus*. leaves of summer when compared with the alpha amylase inhibitory activity of the leaves extracts of other seasons and with acarbose (Figure – 2). Results were statistically significant. This is further evident when examined IC_{50} values of alpha amylase inhibitory activity of the plant leaves of different seasons (Figure – 3).

Isolation of alpha amylase inhibitor from *C. speciosus* leaves of summer is now needed. In our laboratory we are presently working in this direction.

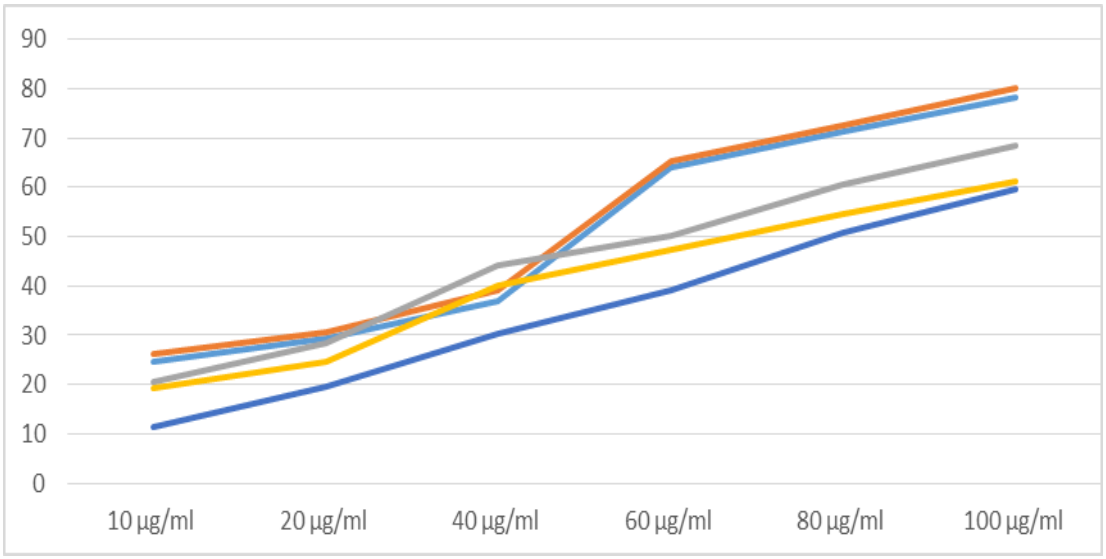

Figure 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and ethanol extracts of *C. speciosus* leaves of different seasons.

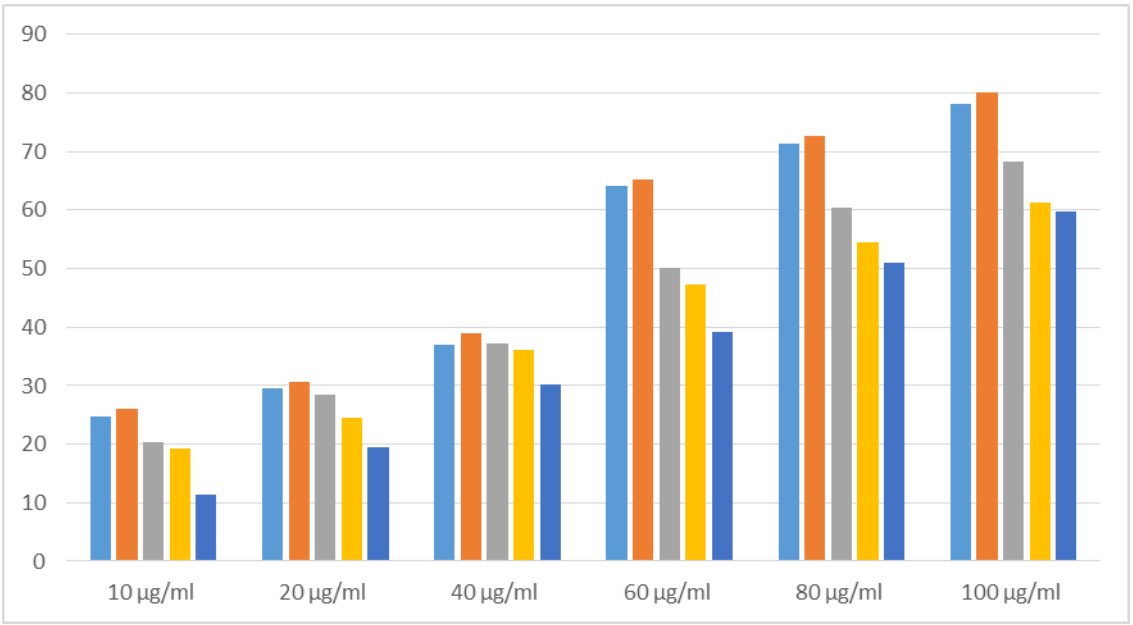

Figure 2: Alpha amylase inhibitory activity of acarbose and ethanol extracts of *C. speciosus* leaves of different seasons in varying concentrations.

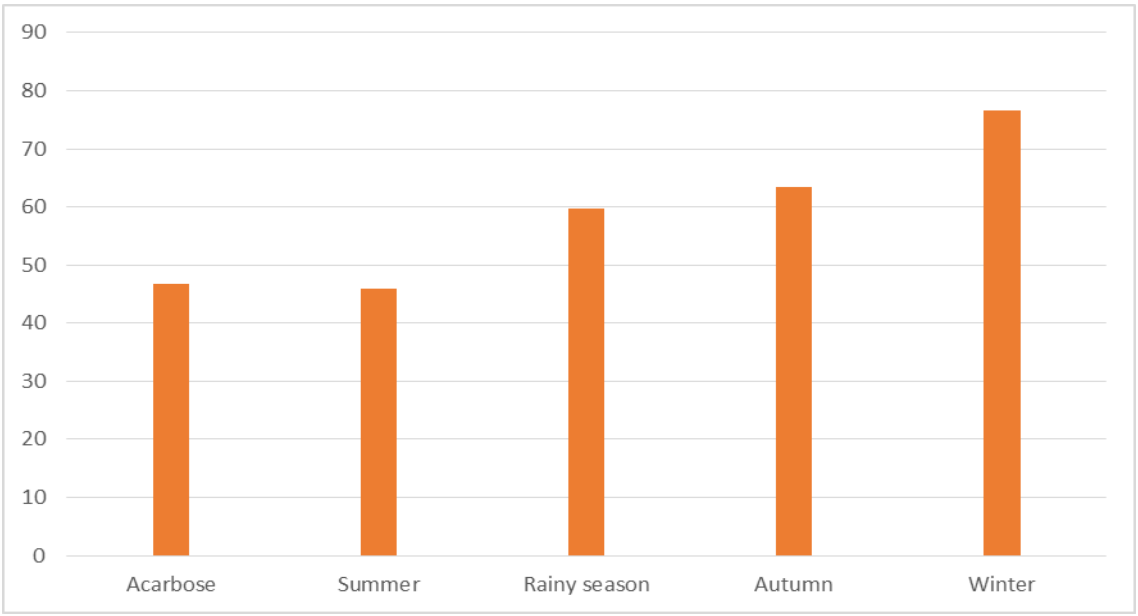

Figure 3: IC_{50} values (µg/ml) in alpha amylase inhibitory activity of acarbose and ethanol extracts of *C. speciosus* leaves of different seasons.

5. CONCLUSION

Agents having alpha amylase inhibitory activity can keep post prandial blood glucose level of Type – 2 diabetes mellitus patients under control. Hence search is going on for these agents. Under the circumstances the present work throws some light of getting alpha amylase inhibitor from *C. speciosus* leaves which may be used in future as a drug for Type-2 diabetes mellitus.

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