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EFFECT OF EXTRACTION SOLVENTS ON *IN VITRO* ALPHA AMYLASE INHIBITORY ACTIVITY OF *COSTUS SPECIOSUS* LEAVES

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ABSTRACT
Alpha amylase inhibitory activity of *Costus speciosus* (*C. speciosus*) leaves is known in literature. But conflicting reports are available on alpha amylase inhibitory activity of different solvent extracts of *C. speciosus* leaves. Aim of the present work was to see the effect of extraction solvents on *in vitro* alpha amylase inhibitory activity of *C. speciosus* leaves. Leaves of *C. speciosus* were collected from the local market and identified by the taxonomist. Solvent extractions of the leaves were made separately by using chloroform, petroleum ether, ethanol, isopropanol and hexane. Extracts were separately dried and processed for *in vitro* alpha amylase inhibitory activity by standard method. Acarbose, an alpha amylase inhibitor, was used as control. Results showed that ethanol extract of *C. speciosus* leaves had maximum alpha amylase inhibitory activity in comparison to that of other solvent extracts. *In vitro* alpha amylase inhibitory activity of ethanol extract of *C. speciosus* leaves (IC_{50} value 43.3±1.0 µg/mL) was comparable to that of acarbose (IC_{50} value 47.2±1.0 µg/mL). This study therefore suggests uses of ethanol extract of *C. speciosus* leaves in the management of diabetes.

KEYWORDS: *Costus speciosus* leaves; extraction solvent extractions; alpha amylase inhibitory activity, acarbose.

1. INTRODUCTION
C. speciosus (family, Costaceae) is a medicinal plant. It is an erect, perennial herb and native to the Malay Peninsula of Southeast Asia. In India the plant is found in Sub-Himalayan tract, up to an altitude of 1200 m, in moist tropical evergreen forests, along roadsides, streams and in wastelands of many places of central India and in the Western Ghats of Karnataka, Maharashtra and Kerala. There are many species of the genus Costus but the cultivated species are mainly *C. cuspidatus, C. giganteus, C.barbatus, C.chartaceus, C. spectabilis, C. igneus, C. osae* etc.[1,2]

Taxonomic classification of *C. speciosus* is as under:
Kingdom – Plantae, Subkingdom – Tracheobinota, Super Division – Spermatophyta, Division – Mangoliophyta, Class – Liliopsida, Sub Class – Zingiberidae, Order – Zingiberales, Family – Costaceae, Genus – Costus, Species – Speciosus. *C. speciosus* is commonly known as keu (Bengali, Hindi) though the plant has several other names like Kembuka (Sanskrit), Tara (Assam), Kostam (Tamil), Kashmeeramu (Telegu), Paskarmula (Guajarati), Channakoova (Malayalam), Spiral flag (English) etc.[3]

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

Many phytochemicals like α-tocopherolquinone, 24-hydroxytriacontan-26-one, dioscin, gracillin, methyl protodioscin, methylprotogracillin, protogracillin, 26-O-β-D-glucopyranosyl-(25R)-furost-5-ene-3β, diosgenin, diosgenin 3-O- β-Dglucopyranosyl (1→3)-β-D-glucopyranoside, 8-hydroxy triacontane-25-one, methyl triacontanoate, 26-diol, protodioscin, 5α-stigmast-9(11)-en-3β-ol, dihydrophytylplastoquinone, 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, 3-O-[α-L-rhamnopyranosyl(1→2)-β-D-glucopyranosyl]-26-O-[β-Dglucopyranosyl]-22α-methoxy-(25R)furost-5-

en-3β etc. were found present in different parts of *C. speciosus.*[5,6]

C. speciosus exerts different pharmacological activities. These include anti-inflammatory, antibacterial, antidiabetic, diuretic, antipyretic, antifungal, antioxidant, anticancer, antifertility, anticholinestrase, and antihelminthic, hepatopretective, hypolipidemic, adaptogenic activities etc.[7,8] *C. speciosus* leaves also exert alpha amylase inhibitory activity.[9,10]

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

2. METHODOLOGY

2.1 Collection of plant materials

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**

2.2 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.

2.3 Solvent extraction

Test drug (75 g) was extracted separately with 500 ml of chloroform, petroleum ether, ethanol, isopropanol and hexane 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 *in vitro* alpha amylase inhibition assay.

2.4 Alpha amylase inhibition assay

Alpha amylase inhibition assay of the test drug was carried out by the method described by Deguchi *et al.*[11] 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 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 drug 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 in each case.

2.5 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.[12]

3. RESULTS

Results are summarized in Table -1.

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

Drug/solvent extract	Concentration (µg/ml)	% of inhibition	IC_{50} Value (µg/ml)
Acarbose	**10** **20** **40** **60** **80** **100**	**22.1±1.1** **28.9±1.0** **49.3±1.1** **63.5±1.2** **69.1±1.1** **76.2±1.2**	**47.2±1.0**
Chloroform extract of *C. speciosus* leaves	**10** **20** **40** **60** **80** **100**	**10.2±0.8** **19.9±1.0** **33.1±1.1** **44.8±1.2** **58.3±1.1** **67.5±1.2**	**66.9±1.1**
Petroleum ether extract of *C. speciosus* leaves	**10** **20** **40** **60** **80** **100**	**19.1±0.7** **26.5±1.0** **40.9±1.2** **52.1±1.1** **58.8±1.2** **67.4±1.3**	**57.5±1.1**
Ethanol extract of *C. speciosus* leaves	**10** **20** **40** **60** **80** **100**	**28.5±1.0** **32.9±1.1** **59.7±1.1** **69.2±1.2** **76.2±1.3** **80.2±1.2**	**43.3±1.0***
Isopropanol extract of *C. speciosus* leaves	**10** **20** **40** **60** **80** **100**	**22.5±1.0** **31.8±1.1** **46.2±1.2** **50.9±1.3** **65.7±1.1** **71.7±1.0**	**58.9±1.0**
Hexane extract of *C. speciosus* leaves	**10** **20** **40** **60** **80** **100**	**12.6±1.0** **23.9±1.0** **41.5±1.2** **48.8±1.1** **56.9±1.2** **68.7±1.2**	**61.4±1.2**

Values are mean ± SE *Significant

Acarbose, standard alpha amylase inhibitor, in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 22.1±1.1, 28.9±1.0, 49.3±1.1, 63.5±1.2, 69.1±1.1 and 76.2±1.2 percent of inhibitions in alpha amylase activity respectively with IC_{50} value 47.2±1.0 µg/ml. Chloroform extract of *C. speciosus* leaves, however, showed 10.2±0.8, 19.9±1.0, 33.1±1.1, 44.8±1.2, 58.3±1.1 and 67.5±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 66.9±1.1 µg/ml.

Petroleum ether extract of *C. speciosus* leaves in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 19.1±0.7, 26.5±1.0, 40.9±1.2, 52.1±1.1, 58.8±1.2 and 67.4±1.3 percent of inhibitions in alpha amylase activity respectively with IC_{50} value 57.5±1.1 µg/ml. Ethanol extract of *C. speciosus* leaves, on the other hand, showed 28.5±1.0, 32.9±1.1, 59.7±1.1, 69.2±1.2, 76.2±1.3 and 80.2±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 43.3±1.0 µg/ml.

Isopropanol extract of *C. speciosus* leaves in the concentrations of 10, 20, 40, 60, 80 and 100 µg/ml showed 22.5±1.0, 31.8±1.1, 46.2±1.2, 50.9±1.3, 65.7±1.1 and 71.7±1.0 percent of inhibitions in alpha amylase activity respectively with IC_{50} value 58.9±1.0 µg/ml. Hexane extract of *C. speciosus* leaves, however,

showed 12.6±1.0, 23.9±1.0, 41.5±1.2, 48.8±1.1, 56.9±1.2 and 68.7±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 61.4±1.2 µg/ml.

4. DISCUSSION

Diabetes mellitus, a chronic metabolic non-communicable disease, is now considered as one of the killer diseases. The disease took several lives till today. Only in 2015 diabetes mellitus was the cause of death for 5 million people worldwide.[13] Incidence of diabetes mellitus is continuous increasing throughout the world. There were approximately 108 million diabetic patients in the world in 1980 but in 2014 the number has been increased to 422 million. Presently highest population of diabetics are found in China, India, USA, Brazil, Mexico and Indonesia. In India diabetes is increasing so fast that the number of adults with diabetes is expected to reach 87 million by the year 2030. As per report of National urban diabetic survey, the incidence of diabetes in few cities of India is, Bangaluru – 12.4%, Chenai – 13.5%, Delhi – 11.6%, Hyderabad – 16.6%, Kolkata – 11.7%, Mumbai – 9.3%. Of all these diabetics about 80% are suffering from Type – 2 diabetes.[14]

Diabetes mellitus particularly Type – 2 diabetes mellitus is characterized by postprandial hyperglycemia. One of the therapeutic approaches, therefore, is to reduce postprandial hyperglycemia.[15] This can be done by inhibiting carbohydrate splitting enzymes. One such enzyme is alpha amylase which hydrolyses complex carbohydrates of food to free sugars. Inhibition of alpha amylase reduces hydrolysis of complex carbohydrate thereby postprandial hyperglycemia is checked.[16] Acarbose, one alpha amylase inhibitor, has already been included in the list of drugs of Type - 2 diabetes mellitus.[17] In this context medicinal plants were investigated for alpha amylase inhibitory activity and many medicinal plants viz. *Musa sapientum*, *Mangifera indica*, *Ocimum sanctum*, *Phyllanthus amarus*, *Teucrium polium*, *Teucrium oliverianum*, *Gymnema sylvsetre*, *Coccinia grandis*, *Tinospora cordifolia*, *Phyllanthus emblica*, *Aegel marmelos*, *Teucrium Orientale*, *Acalypha indica*, *Allium cepa*, *Allium sativum*, *Azadirachta indica*, *Phyllanthus amarus* etc.were found having alpha amylase inhibitory activity.[18]

The present work showed that all solvent extracts (Ethanol, isopropanol, petroleum ether, hexane and chloroform) of *C. speciosus* leaves had alpha amylase inhibitory activity in *in vitro* experiments. The activity was comparable to that of acarbose, standard alpha amylase inhibitor (Figure – 1). Maximum activity, however, was noted in ethanol extract. This was evident when examined alpha amylase inhibitory activity in all doses (10, 20, 40, 60, 80 and 100 µg/ml) of ethanol extract as well as acarbose and chloroform, petroleum ether, isopropanol and hexane extracts (Figure – 2). This was further evident when examined IC_{50} value in alpha amylase inhibitory activity of ethanol extract of *C. speciosus* leaves (43.3±1.0 µg/ml) and the IC_{50} values of acarbose (47.2±1.0 µg/ml), isopropanol extract (58.9±1.0 µg/ml), petroleum ether extract (57.5±1.1 µg/ml), hexane extract (61.4±1.2 µg/ml) and chloroform extract (66.9±1.1 µg/ml) of *C. speciosus* leaves (Figure – 3). Results were statistically significant. Pizon *et al.* also observed that ethanol extract of *C. speciosus* had maximum percentage inhibition on alpha amylase activity[9] while Irani *et al.* noted that methanol extract from *C. speciosus* leaves hadµ maximum percentage inhibition on alpha amylase activity.[10] The present study therefore advocates use of ethanol extract of *C. speciosus* leaves in Type – 2 diabetes mellitus to keep postprandial blood glucose level under control.

It is known that biological activity of medicinal plants depends on season.[19,20] We are now working on seasonal variation in alpha amylase inhibitory activity of *C. speciosus* leaves.

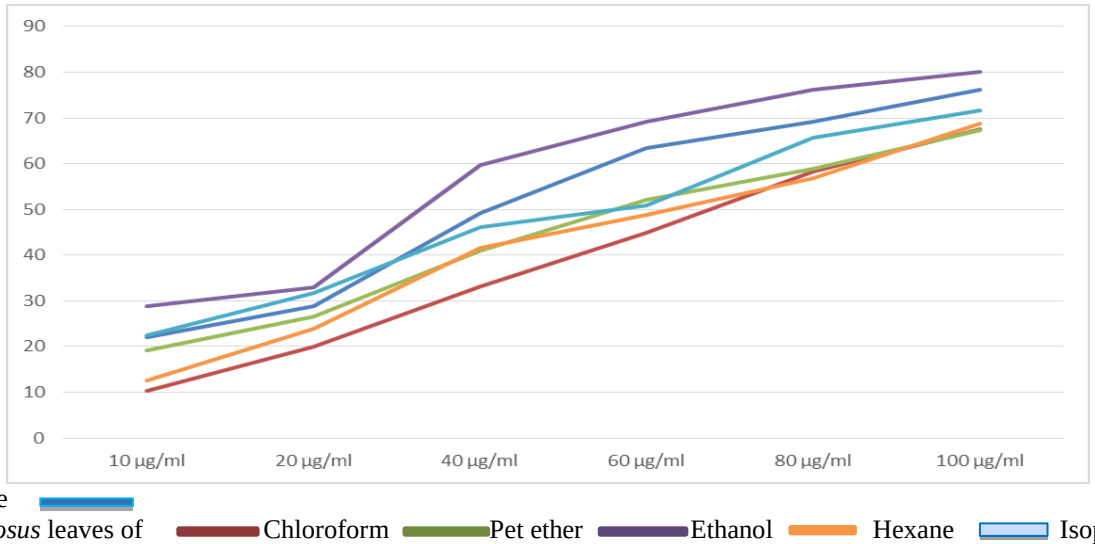

Figure 1: Alpha amylase inhibitory activity of acarbose (standard alpha amylase inhibitor) and different solvent extracts of *C. speciosus* leaves.

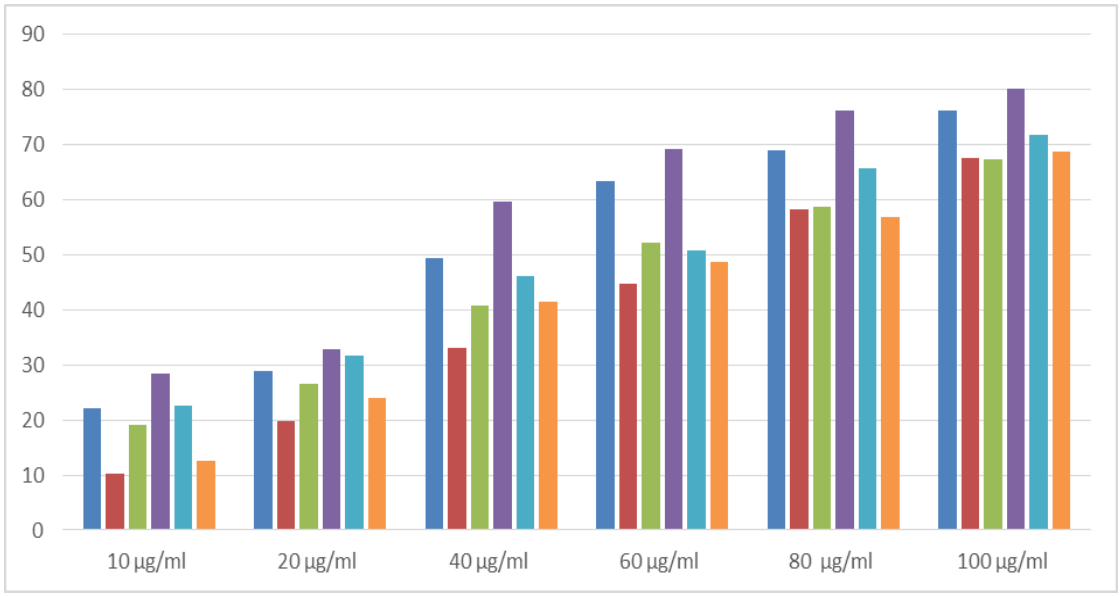

Figure 2: Alpha amylase inhibitory activity in different doses of acarbose and various solvent extracts of *C. speciosus* leaves in the same doses.

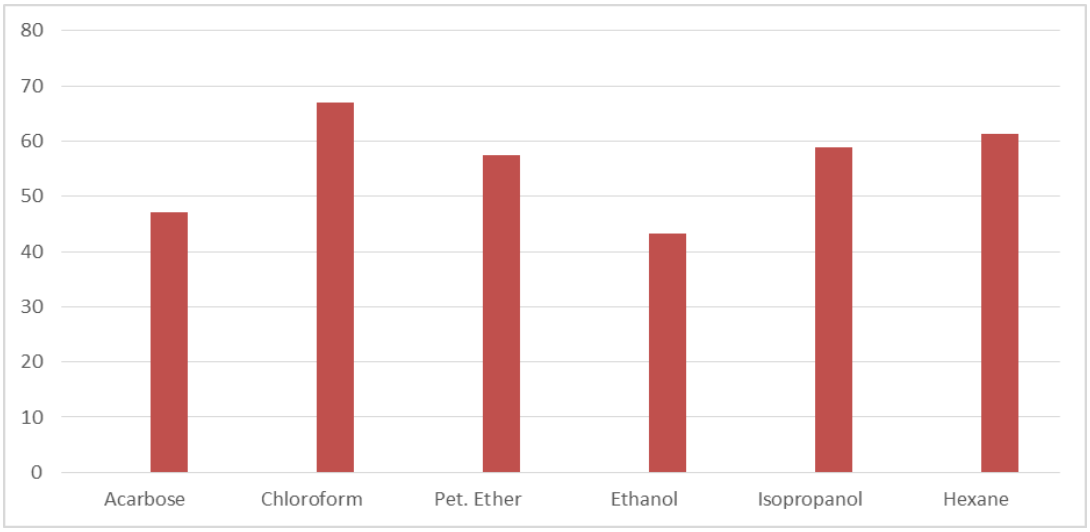

Figure 3: IC_{50} values (µg/ml) in alpha amylase inhibitory activity of acarbose and different solvent extracts of *C. speciosus* leaves.

5. CONCLUSION

Based on the present work compound responsible for alpha amylase inhibitory activity may be isolated from the ethanol extract of *C. speciosus* leaves which, in turn, may be used in future as antidiabetic substance.

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