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SEASONAL EFFECT ON EXTRACTION, QUANTITATION & PURITY ESTIMATION OF GENOMIC DNA FROM *COSTUS SPECIOSUS* LINN. LEAVES

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ABSTRACT
Costus speciosus Linn. (*C. speciosus* L.), family Costaceae, is a medicinal plant of several pharmacological activities. Such an important plant needs genetic study to ascertain both breeding and genetic diversity. For genetic study isolation of pure deoxy ribonucleic acid (DNA) is essential. It is known that amount of pure bioactive compound (secondary metabolite) present in a plant varies with season which, in turn, could cause change in biological activity of the plant. Aim of the present work was to isolate genomic DNA from leaves of *C. speciosus* L., to know purity of the isolated DNA, and to study seasonal effect on extraction, quantitation & purity estimation of the isolated DNA. *C. speciosus* L. leaves were collected from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India during autumn (September – November), winter (December - February), summer (March – May) and rainy seasons (June – August). Leaves were authenticated by the experts of the department of Botany of the said University. Genomic DNA was isolated from the collected *C. speciosus* L. leaves of four seasons by conventional method. All DNA samples were quantitated by colorimetric method. Purity estimation of genomic DNA was done by spectrophotometer. Amount of DNA isolated from *C. speciosus* L. leaves of autumn (September – November), was found maximum in comparison to the amount of DNA isolated from the plant leaves in other seasons though the value was not statistically significant. DNA isolated from *C. speciosus* L. leaves of autumn was pure as evidenced by ratio of A260/A280 which came 1.82. But the ratio of A260/A280 of DNA samples isolated from *C. speciosus* L. leaves during winter, summer and rainy season were 1.98, 2.16 and 2.12 respectively meaning thereby the samples were mixed with either protein or polysaccharides or both. It is concluded that *C. speciosus* L. leaves of autumn should be used to get maximum amount of pure DNA from the plant leaves.

KEYWORDS: *Costus speciosus* Linn, DNA extraction, Purity estimation, Effect of seaso.

INTRODUCTION
Plant exerts biological activities due to secondary metabolites. Secondary metabolites are chemicals produced by plants which have no direct role in growth but are responsible for different activities in order to defend themselves against exogenous biotic / abiotic constraints. There are mainly four classes of secondary metabolites in plants. These are phenolic compounds, alkaloids, terpenoids and sulphur containing compounds. These chemicals exert pharmacological effects of plants such as antimicrobial, antidiabetic, antioxidant, anti inflammatory, anti allergic, anticancer, anti gastric ulcer etc.[1]

Amount of secondary metabolite present in a plant varies with season. This causes change in biological activity of the plant.[2] 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.[3] 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.[4] 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.[5] 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 season was most effective in reducing carrageenan-induced edema in rats.[6] 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.[7] Report from our

laboratory showed that *Cassia alata* leaves during the period of May – June had maximum hepato protective effect and UV absorption property of *Amaranthus spinosus* was maximum during autumn.[8,9]

It is known that genetic study is required to ascertain both breeding and genetic diversity of plants and for genetic study isolation of deoxy ribonucleic acid (DNA) is essential.[10] Aim of the present dissertation was therefore to study seasonal effect on extraction, quantitation & purity estimation of genomic DNA from *Costus speciosus* Linn. leaves.

2. METHODOLOGY

2.1 Collection of plant materials

C. speciosus L. leaves were collected from the medicinal plants garden of the University of North Bengal, Dist. Darjeeling, West Bengal, India during Autumn (September – November), Winter (December – February), Summer (March - May) and rainy season (June – August) in between 9 and 10 am. Leaves were authenticated by the experts of the department of Botany of the said university. A voucher specimen (No. SM-MB-011/19) 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.

Fig. 1 *Costus speciosus* L. leaves.

2.2 DNA extraction

Extraction of genomic DNA from from the plant leaves was carried out by the method of Choudhary *et al*[11] with our slide modification.[12] Protocol was as under,

Wash the plant leaves in running tap water followed by distilled water

↓

Blot the plant leaves with filter paper to remove the water

↓

Cut plant leaves into small pieces

↓

Weigh 2gm of plant leaves and place it in clean, dry and cold porcelain pestle and mortar

↓

Grind the material completely

↓

Add 8ml of 2 –ME/CTAB extraction solution, while grinding

↓

With the help of spatula, transfer the material to small glass beaker

↓

Incubate for 10 to 60 mins at 65^0 C with occasional mixing

↓

Extract the homogenate with an equal volume of 24:1 chloroform/iso amyl alcohol

↓

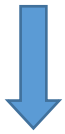
Mix well by inversion

↓

Centrifuge for 5 mins at 10000 rpm at 4^0 C

↓

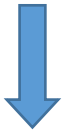
Remove the top aqueous phase. Add 1/10 volume of CTAB/NaCl solution (hold to temperate 65^0 C) to the recovered aqueous phase



Mix well by inversion

Extract with equal volume of chloroform / iso amyl alcohol

Mix and recover the top aqueous phase

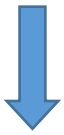


Add 1 volume of CTAB precipitation solution

Mix well by inversion. Incubate the mixture 30 min at 65^0 C.

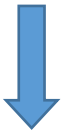
Centrifuge for 5 min at 3000 rp

Remove the supernatant and re-suspend the pellet in high salt TE buffer (0.5 to 1 ml per gram of the starting plant material). Incubate for 30 min at 65^0C.

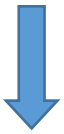


Precipitate DNA by adding 0.6 volume of isopropanol.

Mix well



Centrifuge for 15 min at 10000 rpm



Wash the pallet with 80% ethanol, dry and re-suspend in a minimal volume of TE buffer (0.1 to 0.5 ml per starting material).

2.3 DNA estimation

DNA estimation was done by the method of Gendimenico et al.[13]

2.4 Estimation of the purity of the DNA

Purity of DNA is estimated by taking UV absorptions at 260 nm and 280 nm. It is considered that pure sample of DNA has the ratio of the absorbance at 260 nm and 280 nm (A260/A280) at 1.8. The ratio less or more than 1.8 indicated that the preparation is contaminated either with proteins or with phenol or other compounds.[14]

2.5 Reagents / Chemicals

All reagents / chemicals were used from the kits of Bioera.

2.6 Statistical analysis

All values were expressed as mean ± SEM and analysed using one-way analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS). Differences between means were tested employing Duncan's multiple comparison test and significance was set at $p < 0.05$.[15]

3. RESULTS

3.1 Effect of seasons on extraction of DNA

As per the protocol of plant DNA extractions discussed in the methodology section extractions of DNA from *C. speciosus* L. leave samples of autumn, winter, summer and rainy season were carried out. Each experiment was done for five times. Result on the effect of season on the amount of material obtained after extraction of DNA from *C. speciosus* L. leaves was given in Table – 1.

Result showed that mean amount of the substance recovered after extraction of DNA from *C. speciosus* L. leaves was highest in autumn (36.56 µg/g of leaves) followed by winter (34.56 µg/g of leaves) and then summer (31.62 µg/g of leaves). In rainy season, however, mean amount of the substance recovered after extraction of DNA from *C. speciosus* L. leaves was lowest (30.44 µg/g of leaves).

3.2 Effect of seasons on amount of DNA

Result related to effect of season on amount of DNA in extracting samples from *C. speciosus* L. leaves was given in Table – 2. Result showed that amount of DNA recovered from *C. speciosus* L. leaves was highest in autumn (30.1± 1.1 µg/g of leaves), followed by winter (27.9± 1.5 µg/g of leaves) and then in summer (26.2±1.3 µg/g of leaves). In rainy season amount of DNA recovered from *C. speciosus* L. leaves was found lowest (26.0± 1.0 µg/g of leaves). The results, however, were not statistically significant.

Table – 1: Showing effect of season on the amount of material obtained after extraction of DNA from *C. speciosus* L. leaves.

Season	Amount of obtained substance (µg/g of leaves)	Mean amount of the substance (µg/g of leaves)
Autumn	36.6 36.9 37.1 36.2 36.0	36.56
Winter	34.2 34.7 35.1 34.6 34.2	34.56
Summer	31.8 32.1 31.5 31.7 31.0	31.62
Rainy	31.2 30.0 30.1 30.4 30.5	30.44

Table – 2: Showing effect of season on amount of DNA in extracting samples from *C. speciosus* L. leaves.

Season	Amount of DNA after extraction from *C. Speciosus* L. leaves (µg/g of leaves) ± SEM
Autumn	30.1± 1.1
Winter	27.9± 1.5
Summer	26.2± 1.3
Rainy	26.0± 1.0

Results are mean of five experiments.

3.3 Effect of seasons on purity of DNA

Result related to effect of seasons on purity of DNA samples extracted from *C. speciosus* L. leaves was given in Table – 3.

Table – 3: Showing purity of the extracted DNA samples from *C. speciosus* L. leaves in different seasons

Season	OD values 260 nm(A)	OD values 280 nm(A)	Ratio (A 260/ A 280)
Autumn	0.78	0.428	1.82
Winter	0.82	0.414	1.98
Summer	0.84	0.388	2.16
Rainy	0.83	0.391	2.12

Results showed that extracted DNA sample from *C. speciosus* L. leaves was more pure in autumn in comparison to other seasons. Ratio of A 260 and A 280 came 1.82 for extracted DNA samples of autumn. But the same ratios were 1.98, 2.16 and 2.12 for extracted DNA samples of winter, summer and rainy season respectively.

4. DISCUSSION

Since time immemorial plants are being used for therapeutic purpose. Plants are utilized in traditional medicines as well as in Ayurveda, Homeopathy and Unani and Siddha systems of medicine. This is as plants are easily available, comparatively cheaper and safe. As per World Health Organization (WHO) about 80% of the world population are dependent on medicinal plants as medicines for their primary healthcare.[16,19]

India has an officially recorded list of 45,000 plant species out of which 7500 species of plants are currently being used in medicinal purpose.[20] *Gymnema sylvsetre, Coccinia grandis, Tinospora cordifolia, Musa sapientum, Mangifera indica, Ocimum sanctum, Phyllanthus amarus, Teucrium polium, Teucrium oliverianum, Phyllanthus emblica,Azadirachta indica, Phyllanthus amarus, Aegel marmelos, Teucrium orientale, Acalypha indica, Allium cepa, Allium sativum* etc. are used in the treatment of diabetes.[21] *Moringa*

oleifera, Mentha piperita, Ananas comosus, Amaranthus gangeticus, Artemisia absinthium,Berberis integerrima, Berberis vulgaris Bacopa monnieri, Piper betle, Sida retusa, Curcuma longa, Ficus bengalensis, Foenciculum vulgare, Justicia adhatoda, Melissa officinalis, Salvia officinalis, Terminalia chebula, Vitex negundo etc.act as natural antioxidant.[22,23]. *Olax zeylanica, Ophiorrhiza mungos, Atalantia ceylanica, Hibiscus furcatus, Leucas zeylanica, Mollugo cerviana,* and many others are using as anti solar plants.[24] Likewise, *Abies wabbiana, Acontium heterophyllum, Aegle marmelos* etc. exert anti gastric ulcer activity; *Ageratum conyzoides, Allium sativum, Aphanamixis polystachya* etc. act as antimicrobial agent; *Acorus calamus, Albizzia procera, Allemanda cathartica* etc. have anti cancer activity.[25,26]

Such important medicinal plants need genetic study. Plant genetics is the study of genes**,** genetic variation, and heredity in plants. This study is also important for plant breeding and diversity. There is urgency to plant breeding due to tremendous increase in population.

Ultimate goal of plant breeding is to develop improved plants which can easily adapt to a specific region, have resistance towards disease and pest, and have tolerance to extreme environmental conditions like cold, heat, drought, salinity etc. Genetic diversity, on the other hand, is one of the important constituents of biological diversity. For genetic study of plant, isolation of deoxyribonucleic acid (DNA) is essential. Most of the medicinal plants contain high amount of secondary metabolites like polyphenolics, tanins, alkaloids and other metabolites. Examples are, tea (*Camellia sinensis*), pokeweed (*Phytolacca dodecandra*), broad bean (*Vicia faba* L.) etc. Under the condition it is very difficult to isolate genomic DNA as well as to obtain high quality DNA with good maintenance time from the medicinal plants. By an optimised protocol Haque *et al.* isolated genomic DNA from gum containing plants [27]. Using a rapid efficient method Dehestani and Kazemi Tabar isolated DNA from plants with high levels of secondary metabolites.[28] Kim *et al* isolated high quality genomic DNA from fruit trees and conifers using PVP [29].Friar isolated DNA from plants with large amounts of secondary metabolites [30]. Other workers also isolated DNA from different plants.[31,32]

Amount of chemical substance present in plant vary with season. Scientists came to this conclusion after several work in this direction. As early as 1955 Fluck and Pharm showed that climate has influence on the active principles in medicinal plants.[33] Feeny showed that amount of oak leaf tannins and nutrients changes with season.[34] It was observed that amount of active principle constituents of *Eclipta prostrata* L. varies under different seasonal conditions and was maximum during summer [35]. That leaf quality of two northern hardwoods tree species varies with season was reported by Schultz *et al*.[36] Vasicine contents in *Adhatoda vasica* was found maximum in autumn.[37] In 1989 Mauffette and Oechel found that leaf chemistry of the coast live oak *Quercus agrifolia* changes with season.[38] Drossopoulos *et al.* reported that mineral nutrients and carbohydrates in walnut tree leaves were maximum in rainy season.[39] In 1997 Coli *et al*. investigated seasonal and vertical variation in activity of pear thrips (Thysanoptera: Thripidae) within stands of sugar maple and noted maximum activity in autumn.[40] Fernnandez De Sim *et al*. found that phenolic compounds of Spanish oak wood were more in rainy season.[41] Accumulation of bacoside A in *Bacopa monniera* dependson season and was maximum in summer was the observation of Ganjewala and his co-workers.[42] Mitra in 2014 noted that concentration of anti gastric ulcer compound in *Astilbe rivularis* (saxifragaceae) leaves was maximum during July & August.[43] Mitra also suggested that in the leaves of titeypati (*Artemisia vulgaris* L.) anti bacterial compound was present in maximum amount in September & Octobe.[44]

Aim of the present study was, therefore, to extract genomic DNA from *C. speciosus* L. leaves and to see the seasonal effect, if any, on extraction, quantitation & purity estimation of genomic DNA from *C. speciosus* L. leaves.

We successfully extracted, probably for the first time, the genomic DNA from *C. speciosus* L. leaves in different seasons and found that the amount of material obtained after extraction of DNA from *C. speciosus* L. leaves was maximum in autumn followed by winter, summer and rainy season (Fig – 1).

Amount of DNA after extraction from *C. speciosus* L leaves was quantitated. It appeared that DNA was present in maximum amount in autumn followed by winter, summer and rainy season but the results were not statistically significant (Fig – 2).

We also studied purity of the extracted DNA samples from *C. speciosus* L. leaves by noting the ratio of A 260 / A 280.Our study showed that isolated DNA sample from the leaves of *C. speciosus* L. of autumn was comparatively pure than those DNA samples isolated from *C. speciosus*L. leaves during winter, summer and rainy season (Fig. - 3)

Zhang *et al.* noted that season, environment stress and refrigerated storage had a big effect on genomic DNA isolation of tung tree [45].Reports from our laboratory also suggested that amount and purity of genomic DNA extracted from different plants vary with season.[46,50]

5. CONCLUSION

The present study has shown that pure DNA in good amount may be extracted from *C. speciosus* L. leaves during autumn. DNA extraction from *C. speciosus*L. leaves during other seasons like winter, summer and rainy season will neither yield good amount of DNA nor

produce pure DNA. Therefore, in need of extraction of DNA from *C. speciosus* L.leaves, leaves of *C. speciosus* L.of autumn may be utilized.

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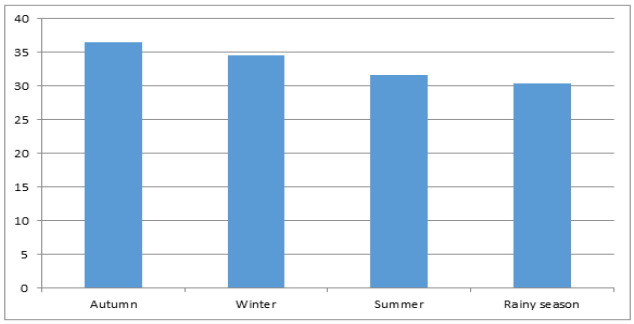
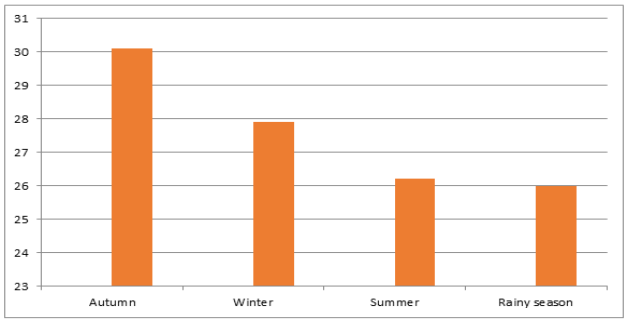
Conflict of interest: The authors declare that they have no conflict of interest.



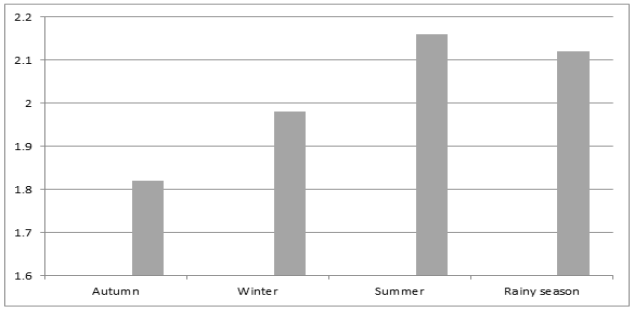
Amount of the substance was in µg/g of *C. speciosus* L.leaves.

Figure -2: Effect of season on amount of material obtained after extraction of DNA from *C. speciosus* L. leaves.



Concentration of DNA was in µg/g of leaves. Results were mean of 5 experiments.

Figure -3: Effect of season on concentration of extracted DNA from *C. speciosus* L. Leaves.



Purity was assessed by the ratio of A 260 nm / A 280nm. DNA is pure if the ratio is 1.8(All results were mean of 5 experiments)

Figure -4: Effect of season on purity of extracted DNA from leaves of *C. speciosus* L.

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