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AMARANTHUS SPINOSUS L. LEAVES - EXTRACTION, QUANTITATION & PURITY ESTIMATION OF GENOMIC DNA: EFFECT OF SEASON

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ABSTRACT
Amaranthus spinosus L.(*A. spinosus* L.) is an important medicinal plant having several pharmacological properties. In the present investigation attempts have been made to isolate pure DNA from *A. spinosus* L. leaves with the objective that extraction of DNA from the plant will help the genotype analysis leading to scientific identification of the plant. As season has effect on synthesis of chemicals in the plant parts, attempts have also been made to study the seasonal effect on extraction of pure DNA from *A. spinosus* L. leaves. Leaves of *A. spinosus* L. were collected during autumn, winter, summer and rainy seasons, identified by experts and the leaves were processed for extraction, quantitation & purity estimation of genomic DNA by the conventional methods. Results showed that pure DNA in high amount was extracted from *A. spinosus* L. leaves during winter (December – February). It is concluded that *A. spinosus* L. leaves of winter season may be used to get high amount of pure DNA.

KEYWORDS: *Amaranthus spinosus* L., DNA extraction, Purity estimation, Effect of season.

1. INTRODUCTION
Amaranthus spinosus L.(family, Amaranthaceae) is cultivated in Bangladesh, India, Sri Lanka, Japan, Indonesia, the Pacific islands and in Australia. The plant grows in cultivated areas as well as in waste places and is widely distributed in lower to middle hills (3000–5000 ft) of entire north eastern Himalayas.[1] The plant has several names, 'prickly amaranthus' in English, 'ban lure' or 'dhuti ghans' in Nepali, 'Kanta chaulai' and 'Kantanotya' in Hindi and Bengali respectively, 'Tanduliuyah' in Sankrit, 'Kantaneutia' and 'Chengkruk' in Oriya and Manipuri respectively.[2]

A. spinosus L. is a medicinal plant. As mentioned in Ayurvedic text the plant is used as diuretic, digestive, laxative and anti pyretic. It is also used to treat burning sensation, bronchitis, piles, blood diseases, anorexia, leprosy, and leucorrhoea.[3,4]

Pharmacological actions of *A. spinosus* L. have been explored which include antidiabetic[5] having anthelmentic and anti-inflammatory activity[6], anti-hyperlipidemic and spermatogenic effects[7], antidiarrhoeal and antiulcer activity[8], hepatoprotective and antioxidant property[9], antinociceptive activity[10], antibacterial effect[11], antitumor activity[12], antifertility property[13], antimalarial[14] and antitumor activity[15] etc. Reports from our laboratory also confirmed antibacterial[16], antiulcer[17] and anti diabetic activity[18] of *A. spinosus* L. leaves.

Phytochemical investigations of *A. spinosus* L. showed that the plant is a rich source of glycosides, linoleic acid, rutin, phenolic acids, alkaloids, flavonoids, steroids, saponin, betalain, amino acids, terpenoids, lipids, b-sitosterol, stigmasterol, catechuic tannins carotenoids etc.[19]

Objective of the present study was to isolate pure DNA from *A. spinosus* L. leaves with the idea that DNA of the plant will help the genotype analysis leading to scientific identification of the plant. As season has effect on synthesis of chemicals in the plant parts, attempts had also been made to study the seasonal effect on extraction of pure DNA from *A. spinosus* L. leaves.

2. METHODOLOGY
2.1 Collection of plant materials
A. spinosus 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 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.

***Amaranthus spinosus* L.**

2.2 DNA extraction

Extraction of genomic DNA from from the plant leaves was carried out by the method of Choudhary *et al.*[20] with slight modificatiion. Protocol was as under,

Leaves of *A. spinosus* L. were washed in running tap water followed by distilled water

↓

Leaves were blotted with filter paper to remove the water

↓

Leaves were cut into small pieces

↓

Plant Leaves (2 gm) were placed in clean, dry and cold porcelain pestle and mortar

↓

The material was grand completely, 8 ml of 2 –ME / CTAB extraction solution was added while grinding

↓

With the help of spatula, the material was transferred to small glass beaker

↓

Incubation was done for 10 to 60 mins at 65^0 C with occasional mixing

↓

The homogenate was extracted with an equal volume of 24:1 chloroform/iso amyl alcohol. The extraction was mixed well by inversion.

↓

The extraction was centrifuged for 5 mins at 10000 rpm at 4^0 C

↓

Top aqueous phase was removed. 1/10 volume of CTAB / NaCl solution (hold to temperate 65^0 C) was added to the recovered aqueous phase.

↓

The aqueous phase was mixed well by inversion
↓
It was extracted with equal volume of chloroform / iso amyl alcohol
↓
Extracted material was mixed well. The top aqueous phase was recovered.
↓
1 volume of CTAB precipitation solution was added.
↓
Solution was mixed well by inversion. The mixture was incubated 30 min at 65^0 C.
↓
The mixture was centrifuged for 5 min at 3000 rpm
↓
Remove the supernatant was removed and the pellet was re suspended in high salt TE buffer (0.5 to 1 ml per gram of the starting plant material). Incubate the mixture for 30 min at 65^0C.
↓
DNA was precipitated by adding 0.6 volume of isopropanol.
↓
Solution was mixed well
↓
The mixture was centrifuged for 15 min at 10000 rpm
↓
The pallets obtained were washed with 80% ethanol. Pallets were dried and re-suspended 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.[21]

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.[22]

2.5 Reagents / Chemicals

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

2.6 Statistical analysis

The values were expressed as mean ± SEM and were 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 $p < 0.05$.

3. RESULTS

3.1 Effect of seasons on extraction of DNA

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

Result showed that mean amount of the substance recovered after extraction of DNA from *A. spinosus* L. leaves was highest in rainy season (38.3 µg/g of leaves) followed by summer (38.1 µg/g of leaves) and then winter (37.0 µg/g of leaves). In autumn, however, mean amount of the substance recovered after extraction of DNA from *A. spinosus* L. leaves was lowest (36.3 µg/g of leaves).

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

Season	Amount of obtained substance (µg/g of leaves)	Mean amount of the substance (µg/g of leaves)
Autumn	36.3 36.4 36.4 36.5 36.1	36.3
Winter	37.2 37.1 37.1 36.5 37.3	37.0
Summer	37.9 38.1 38.5 38.0 38.2	38.1
Rainy season	38.5 38.1 38.3 38.2 38.4	38.3

3.2 Effect of seasons on amount of DNA

Result related to effect of season on amount of DNA in extracting samples from *A. spinosus* L. leaves was given in Table – 2.

3.3 Effect of seasons on purity of DNA

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

Table 2: Showing effect of season on amount of DNA in extracting samples from *Amaranthus spinosus* L. leaves.

Season	Amount of DNA after extraction from *Amaranthus spinosus* L. leaves (µg/g of leaves) ± SEM
Autumn	35.5 ± 1.0
Winter	38.2 ± 1.2
Summer	37.0 ± 1.3
Rainy season	38.0 ± 1.4

Results are mean of five experiments.

Result showed that amount of DNA recovered from *A. spinosus* L. leaves was highest in winter (38.2 ± 1.2 µg/g of leaves), followed by rainy season (38.0 ± 1.4 µg/g of leaves) and then summer (37.0 ± 1.3 µg/g of leaves). In autumn amount of DNA recovered from *A. spinosus* L. leaves was found lowest (35.5 ± 1.0 µg/g of leaves). The results, however, were not statistically significant.

Table 3: Showing purity of the extracted DNA samples from *Amaranthus spinosus* L. leaves in different seasons.

Season	OD values 260 nm(A)	OD values 280 nm(A)	Ratio (A 260/ A 280)
Autumn	0.88	0.45	1.90
Winter	0.89	0.46	1.80
Summer	0.85	0.45	1.82
Rainy season	0.87	0.48	1.85

Results are mean of five experiments.

Results showed that extracted DNA sample from *A. spinosus* L. leaves was more pure in rainy season in comparison to other seasons. Ratio of A 260 and A 280 came 1.80 for extracted DNA samples of winter. But the same ratios were 1.82, 1.85 and 1.90 for extracted DNA samples of summer, rainy season and autumn respectively.

4. DISCUSSION

Plant produces many compounds such as proteins, polyphenols, polysaccharides, flavonoids, carotenoids, tannin etc. These compounds as secondary metabolites interfere with DNA isolation. DNA-based experiments are also interfered by these secondary metabolites.[23,24] Therefore, in spite of having lots of DNA isolation protocols DNA isolation from plant species is still a challenging job. Further, metabolites in plant leaves change with many factors specially season.[25] However, up to now, reports are scanty about the effect of the season on DNA isolation of plant species. In the present investigation we have extracted the genomic DNA from *A. spinosus* L. leaves of different seasons and found that the amount of material obtained in course of extraction of DNA from *A. spinosus* L. leaves was maximum in rainy season followed by summer, winter and autumn (Fig – 2). Zhang et al noted that season, environment stress and refrigerated storage had a big effect on genomic DNA isolation of tung tree.[26]

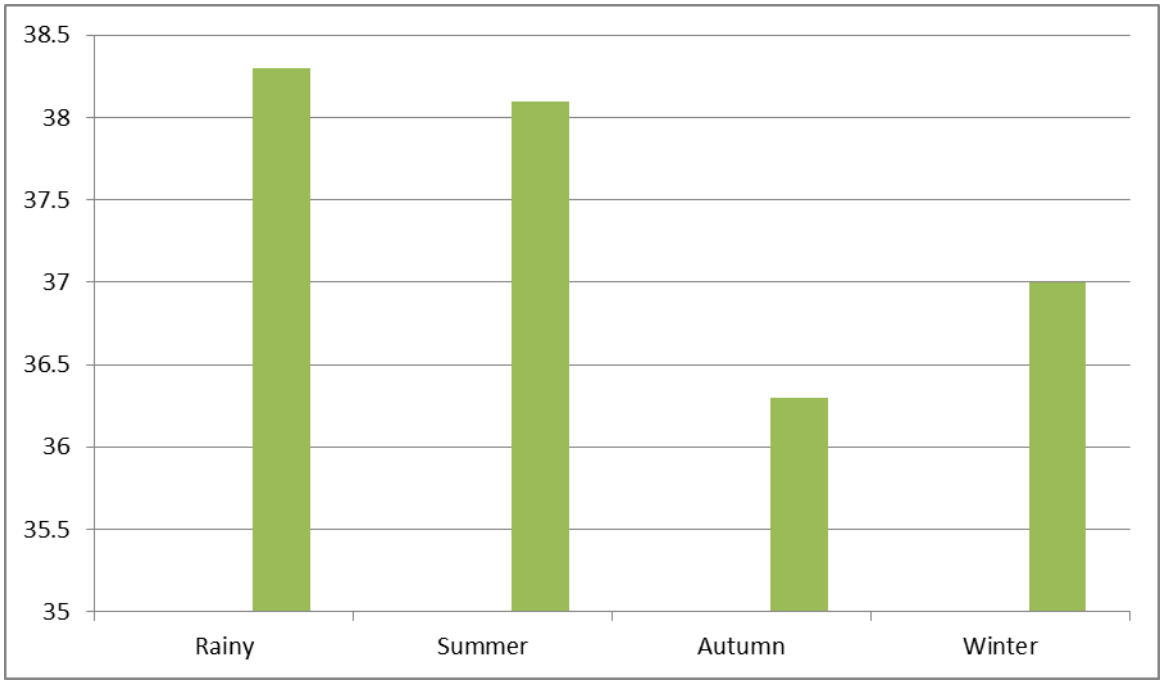

Figure 2: Effect of season on amount of material obtained during extraction of DNA from *Amaranthus spinosus* L. leave

Amount of the substance was in µg/g of Amaranthus spinosus L. leaves.
Result was mean of 5 experiments.

In the present study material obtained during extraction of DNA from *A. spinosus* L. leave was estimated for quantitation of DNA. Results showed that amount of DNA was maximum in winter followed by rainy season, summer and autumn but the results were not statistically significant (Fig – 3). Robbins et al also noted seasonal variations in the nucleic acid content and RNA:DNA ratio of the gonad of the scallop *Pecten maximus*.[27] Knight and Ackerly showed that ecologically diverse California flora with small 2C-DNA values predominate in all environments, but species with large 2C-DNA values occur at intermediate July maximum temperatures.[28]

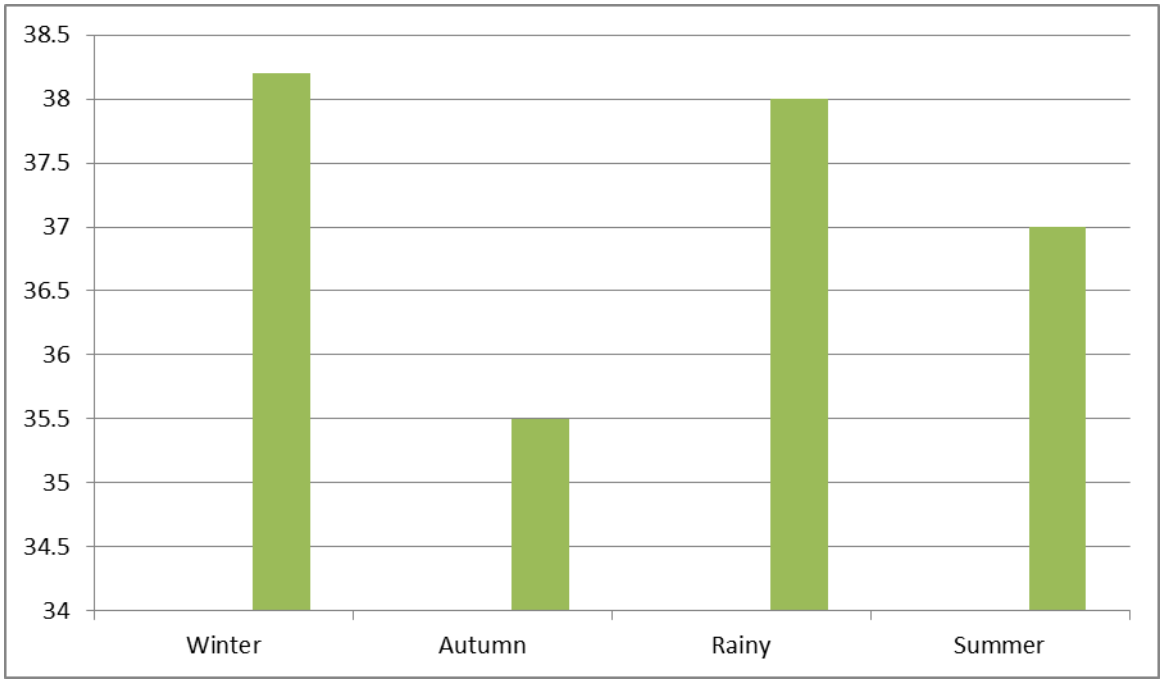

Figure 3: Effect of season on concentration of extracted DNA from *Amaranthus spinosus* L. leaves.
Concentration of DNA was in µg/g of leaves. Result was mean of 5 experiments

We have also studied purity of the extracted DNA sample from *A. spinosus* L. leaves by noting the ratio of A 260 / A 280. Results showed that isolated DNA sample from the leaves of *A. spinosus* L. of winter was comparatively pure (1.80) than those DNA samples isolated from *A. spinosus* L. leaves during summer followed by rainy season and autumn (Fig. - 4). This is in accordance with the findings of Jansena et al. who showed that UV-B, a minor component of sunlight and

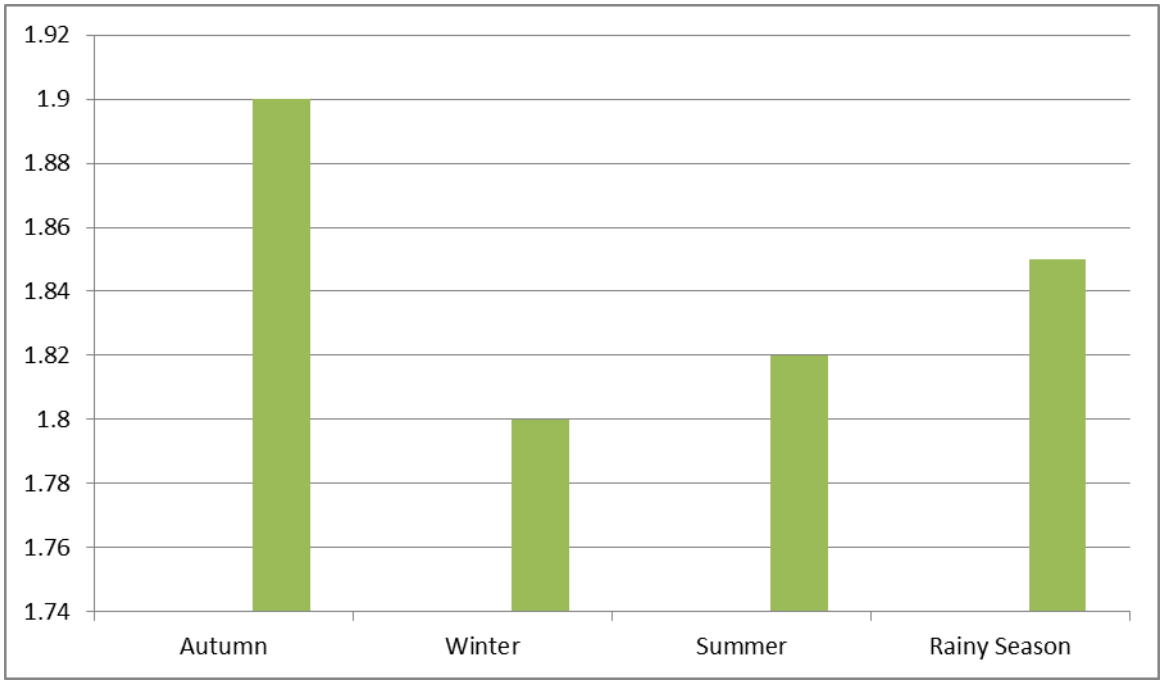

Figure 4: Effect of season on purity of extracted DNA from *Amaranthus spinosus* L. leaves
Purity was assessed by the ratio of A 260 nm / A 280 nm. DNA is pure if the ratio is 1.8

Intensity of which varies with season, has a disproportionately damaging effect on higher plants specially on DNA, proteins and membranes.[29]

5. CONCLUSION

In the present study pure DNA was isolated in high amount from *A. spinosus* L. leaves during winter (December – February). It is, therefore, concluded that *A.*

spinosus L. leaves of winter may be used to get high amount of pure DNA.

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

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