SEASONAL EFFECT ON EXTRACTION, QUANTITATION & PURITY ESTIMATION OF GENOMIC DNA FROM CASSIA ALATA L. LEAVES

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ABSTRACT
Identification of a plant scientifically has its own importance. This is done by genetic study for which isolation of pure DNA from the plant source is essential. Cassia alata L.(C. alata L.) is a medicinal plant having several pharmacological properties. For its scientific identification isolation of genomic DNA from the plant is required. Objective of the present work was, therefore, isolation-quantitation and purity estimation of the genomic DNA from C. alata L. leaves. As season has effect on synthesis of chemicals in the plant parts, objective was also to study the seasonal effect on concentration of pure DNA in C. alata L. leaves. Leaves of C. alata 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 DNA was isolated from C. alata L. leaves in high amount during summer season (March – May). Isolated DNA was found pure. It is concluded that C. alata L. leaves of summer season may be used to get high amount of pure DNA.

KEYWORDS: Seasonal effect, Cassia alata L., DNA extraction, Quantitation, Purity estimation.
1. INTRODUCTION

*Cassia alata* L. (family, Caesalpiniaceae), a medicinal plant native to Ghana and Brazil, is now widely distributed throughout the world.\(^1\) Even in India the plant is found in Sikkim and Darjeeling Himalayas. It is an erect tropical annual herb with leather compounded leaves. *C. alata* L. is widely known in the name of wild senna. The plant has other names too like cakramard in Sanskrit, ringworm weed in English and dadmari in Hindi Its therapeutic values are mentioned in Ayurvedic text.\(^2,3\) Leaves of *C. alata* L. are anti parasitic, used in eczema, asthma, ringworm, bronchitis and in poisonous insect bites. Bark of the plant is used to treat skin diseases and extract of aerial parts of the plant is diuretic, CNS depressant, and anti inflammatory. Traditionally the plant is used as antihelminthic, in infection and in uterus disorder.\(^4,5\) Modern researchers advocated the use of *C. alata* L. for treatments of blennorrhagia, haemorrhoids, constipation syphilis, inguinal hernia diabetes and intestinal parasitosis.\(^6-8\)

Antimicrobial activity of *C. alata* L. was confirmed by several workers.\(^9,10\) We also noted that leaves of *C. alata* L. could inhibit growth of *Staphylococcus aureus*.\(^11,12\) At the same time we have noted that *C. alata* L. leaves could inhibit growth of growing albino rats and the effect was maximum for the leaves of *C. alata* L. of July and August.\(^13\)

*C. alata* L. is a source of 5,7,4'-trihydroflavanone, kaempferol-3-O-β-Dglucopyranoside, quercetin, chrysoeriol, kaempferol, n-dotriacontanol, stearic acid, palmitic acid, n-triacontanol, kaempferol-3-O-β-D-glucopyranosyl-(1->6)-β-D-glucopyranoside, palmitic acid ceryl ester.17-hydrotetraatriacontane etc.\(^14\)

Objective of the present study was to isolate genomic DNA from leaves of such an important medicinal plant, *C. alata* L. Seasonal effect on extraction, quantitation & purity estimation of genomic DNA from *C. alata* L. leaves was also undertaken as it is known that synthesis of chemicals in plant parts varies with season.\(^15,16\)

2. METHODOLOGY

2.1 Collection of plant materials

*C. alata* 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) at about 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.

**Fig – 1: Cassia alata Linn.**

### 2.2 DNA extraction

Extraction of genomic DNA from the plant leaves was carried out by the method of Choudhary *et al.*[^17] with slight modification. Protocol was as under,

- Leaves of *C. alata* 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\(^0\) C.

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.\textsuperscript{[18]}
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.[19]

2.5 Reagents / Chemicals
All reagents / chemicals were used from the kits of Biorad

2.6 Statistical analysis
The values were expressed as mean ± SEM and were analyzed 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 C. alata 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 C. alata L. leaves was tabulated in Table – 1.

Result showed that mean amount of the substance recovered after extraction of DNA from C. alata L. leaves was highest in autumn (38.2 μg/g of leaves), followed by rainy season (37.5 μg/g of leaves) and then winter (35.9 μg/g of leaves). In summer, however, mean amount of the substance recovered after extraction of DNA from C. alata L. leaves was lowest (34.5 μg/g of leaves).
Table 1: Showing effect of season on the amount of material obtained after extraction of DNA from *Cassia alata* L. leaves.

<table>
<thead>
<tr>
<th>Season</th>
<th>Amount of obtained substance (μg/g of leaves)</th>
<th>Mean amount of the substance (μg/g of leaves)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>38.7 38.2 39.7 37.6 37.2</td>
<td>38.2</td>
</tr>
<tr>
<td>Winter</td>
<td>37.9 37.4 37.3 30.2 36.8</td>
<td>35.9</td>
</tr>
<tr>
<td>Summer</td>
<td>35.2 33.3 34.5 35.0 34.7</td>
<td>34.5</td>
</tr>
<tr>
<td>Rainy</td>
<td>37.2 38.7 37.0 37.9 36.8</td>
<td>37.5</td>
</tr>
</tbody>
</table>

3.2 Effect of seasons on amount of DNA

Result related to effect of season on amount of DNA in extracting samples from *C. alata* 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 *C. alata*. leaves was given in Table – 3.

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

<table>
<thead>
<tr>
<th>Season</th>
<th>Amount of DNA after extraction from <em>Cassia alata</em> L. leaves (μg/g of leaves) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>36.5 ± 1.0</td>
</tr>
<tr>
<td>Winter</td>
<td>37.2 ± 1.1</td>
</tr>
<tr>
<td>Summer</td>
<td>39.2 ± 1.2</td>
</tr>
<tr>
<td>Rainy</td>
<td>37.8 ± 2.0</td>
</tr>
</tbody>
</table>

*Results are mean of five experiments.*
Result showed that amount of DNA recovered from *C. alata* L. leaves was highest in summer (39.2 ± 1.2 μg/g of leaves), followed by rainy season (37.8 ± 2.0 μg/g of leaves) and then winter (37.2 ± 1.1 μg/g of leaves). In autumn amount of DNA recovered from *C. alata* L. leaves was found lowest (36.5 ± 1.0 μg/g of leaves). The results, however, were not statistically significant.

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

<table>
<thead>
<tr>
<th>Season</th>
<th>OD values 260 nm(A)</th>
<th>OD values 280 nm(A)</th>
<th>Ratio (A 260/ A 280)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Autumn</td>
<td>0.89</td>
<td>0.44</td>
<td>1.93</td>
</tr>
<tr>
<td>Winter</td>
<td>0.87</td>
<td>0.46</td>
<td>1.89</td>
</tr>
<tr>
<td>Summer</td>
<td>0.87</td>
<td>0.48</td>
<td>1.81</td>
</tr>
<tr>
<td>Rainy</td>
<td>0.85</td>
<td>0.43</td>
<td>1.97</td>
</tr>
</tbody>
</table>

*Results are mean of five experiments.*

It was found out that extracted DNA sample from *C. alata* L. leaves was more pure in summer in comparison to other seasons. Ratio of A 260 and A 280 came 1.81 for extracted DNA samples of summer season. But the same ratios were 1.89, 1.93 and 1.97 for extracted DNA samples of winter, autumn and rainy seasons respectively.

**4. DISCUSSION**

That season has influence on synthesis of chemicals in plants is known in literature.[15,16] Feeny in 1970 showed that amount of oak leaf tannins and nutrients changes with season.[20] In 1977 Gupta observed that amount of active principle constituents of *Eclipta prostrata* L. varies under different seasonal conditions and was maximum during summer.[21] Zhang et al also noted that season, environment stress and refrigerated storage had a big effect on genomic DNA isolation of tung tree.[22] Goldinger et al. showed that season has effect on plant gene expression.[23]

In the present work we have extracted the genomic DNA from *C. alata* L. leaves of different seasons and found that the amount of material obtained in course of extraction of DNA from *C. alata* L. leaves was maximum in autumn followed by rainy season, winter and summer (Fig – 2).
Extracted materials from *C. alata* L. leaves was estimated for quantitation of DNA. Amount of DNA was found maximum in summer followed by rainy season, winter and autumn but the results were not statistically significant (Fig – 3). Seasonal variations in the nucleic acid content and RNA: DNA ratio of the gonad of the scallop *Pecten maximus* was noted by Robbins et al.\(^{24}\) 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.\(^{25}\)
Concentration of DNA was in μg/g of leaves. Result was mean of 5 experiments.

Effect of radiation on higher plants was studied by Jansena et al. who showed that UV-B, a minor component of sunlight and intensity of which varies with season, has a disproportionately damaging effect on higher plants specially on DNA, proteins and membranes.\cite{26} In the present study we have investigated purity of the extracted DNA samples from *C. alata* L. leaves by noting the ratio of A 260 / A 280. Our study showed that isolated DNA sample from the leaves of *C.alata* L. of summer was comparatively pure (1.81) than those DNA samples isolated from *C. alata* L. leaves during winter, autumn and rainy seasons (Fig. - 4). This is in accordance with the findings of Barta et al. who demonstrated that in situ dark adaptation increases the yield and quality of genomic DNA obtained from mature oak leaves.\cite{27}

![Figure 4: Effect of season on purity of extracted DNA from Cassia alata L. leaves.](image)

*Purity was assessed by the ratio of A 260 nm / A 280 nm. DNA is pure if the ratio is 1.8.*

5. CONCLUSION

In the present work we have noted that pure DNA is isolated in high amount from *C. alata* L. leaves during summer (March – May). It is therefore concluded that *C. alata* L. leaves of summer season 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.

REFERENCES


