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EXTRACTION, QUANTITATION & PURITY ESTIMATION OF GENOMIC DNA FROM MURRAYA KOENIGII (LINNAEUS) SPRENG WETTST LEAVES: EFFECT OF SEASON

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ABSTRACT

Medicinal plants play a major role in primary healthcare and are the basis of treatment and cure for various diseases. Therefore, medicinal plants need proper breeding. Study on genetic diversity is also needed for these important plants. For studies on breeding and genetic diversity isolation of pure deoxy-ribonucleic acid (DNA) in good quantity is essential. *Murraya koenigii* Linnaeus Spreng Wettst (*M. koenigii* L.), family Rutaceae, is a medicinal plant possessing several pharmacological activities. Aim of the present work was to isolate genomic DNA from leaves of *M. koenigii* L. and to study purity of the isolated DNA. Seasonal effect on isolation and purity estimation of the DNA from the plant leaves was also done to know the season when optimum amount of pure DNA can be obtained. Leaves of *M. koenigii* L. 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). Genomic DNA was isolated from the collected *M. koenigii* L. leaves of four seasons byconventional method. All DNA samples were quantitated by colorimetric method.Purity estimation of genomic DNA was done by spectrophotometer. Amount of DNA isolated from *M. koenigii* L. leaves of rainy season (June – August), 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 *M. koenigii* L. leaves of rainy season was pure as evidenced by ratio of A260/A280 which was 1.81. But the ratio of A260/A280 of DNA samples isolated from *A. precatorius* L. leaves during winter, summer

and autumn were 2.22, 2.18 and 2.09 respectively. It is concluded that *M. koenigii* L. leaves of rainy season should be used to get maximum amount of pure DNA from the plant leaves.

KEYWORDS: *Murraya koenigii* Linnaeus, DNA extraction, Purity estimation, Effect of season.

1. INTRODUCTION

Since time immemorial plants are being used as an important source of medicine. Even today, WHO (World Health Organization) estimates that about 80 per cent people of the world rely on plants as source of medicine. In India there are about fifteen thousand plants which have medicinal properties. These plants, known as medicinal plants, play a major role in primary healthcare and are the basis of treatment and cure for various diseases in India. From these plants most valuable analgesics, anti - hypertensive, anti cancer drugs have been isolated.[1-3] *Murraya koenigii* Linnaeus Spreng Wettst (*M. koenigii* L.), commonly known as *curry* leaf or *kari patta* in Indian dialects and belonging to family Rutaceae, is one such medicinal plant. In ancient systems of medicine like Ayurveda, Siddha and Unani the plant has therapeutic applications in piles, vomiting, bronchial disorders and in skin diseases.[4] In traditional & folklore medicine the plant is used as anti-helmintic, analgesic and anti-inflammatory agent. It is also used to cure itching, leukoderma, piles, inflammation and blood disorders.[5,6] Researchers found that *M. koenigii* L. leaves have several pharmacological properties such as antioxidant, antidiabetic, antibacterial, antifungal, anti-helmintic, anti-hypertensive etc. The leaves have also cytotoxic activity and can be used in the treatment of bronchial respiratory difficulties.[7] Numerous phytochemicals have been collected from roots, leaves, stems and flowers of *M. koenigii* L. which showed various applications in different fields.[8]

Considering tremendous increase in population and application of *M. koenigii* L., the plant needs proper breeding as 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. Study on genetic diversity, one of the important constituents of biological diversity, is also needed. Both breeding and genetic diversity fall under genetic study and for genetic study of plant, isolation of deoxy- ribonucleic acid (DNA) is essential.[9]

In the present work attempts have been made to extract and quantitate the genomic DNA from *M. koenigii* L. leaves and to estimate purity of the extracted DNA. Effect of season on

extraction, quantitation & purity estimation of genomic DNA from *M. koenigii* L. leaves was also studied.

2. METHODOLOGY

2.1 Collection of plant materials

M. koenigii 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-013/19) 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: Murraya koenigii 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*.[10] with our slide modification.[11] Protocol was as under,

Plant leaves were washed in running tap water followed by distilled water. Leaves were blotted with filter paper to remove water. Leaves were then cut into small pieces. 2gm of the leaves were placed in clean, dry and cold porcelain pestle and mortar and grinded the material

completely. During grinding 8ml of 2 –ME/CTAB extraction solution were added and with the help of spatula the material was transferred to small glass beaker. The mixer was incubated 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. Solution is mixed well by inversion and centrifuged it 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 and the solution was mixed well by inversion. The solution was extracted with equal volume of chloroform / iso amyl alcohol. The solution was then mixed and the top aqueous phase was recovered. 1 volume of CTAB precipitation solution was added. The solution was mixed well by inversion and incubated for 30 min at 65^0 C. The mixer was centrifuged for 5 min at 3000 rpm.

The supernatant was removed and re-suspended the pellet in high salt TE buffer (0.5 to 1 ml per gram of the starting plant material). It was then incubated for 30 min at 65^0C. DNA was precipitated by adding 0.6 volume of isopropanol. The solution was mixed well and centrifuged for 15 min at 10000 rpm. The pallet was washed with 80% ethanol, 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.[12]

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.[13]

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 $p < 0.05$.[14]

3. RESULTS

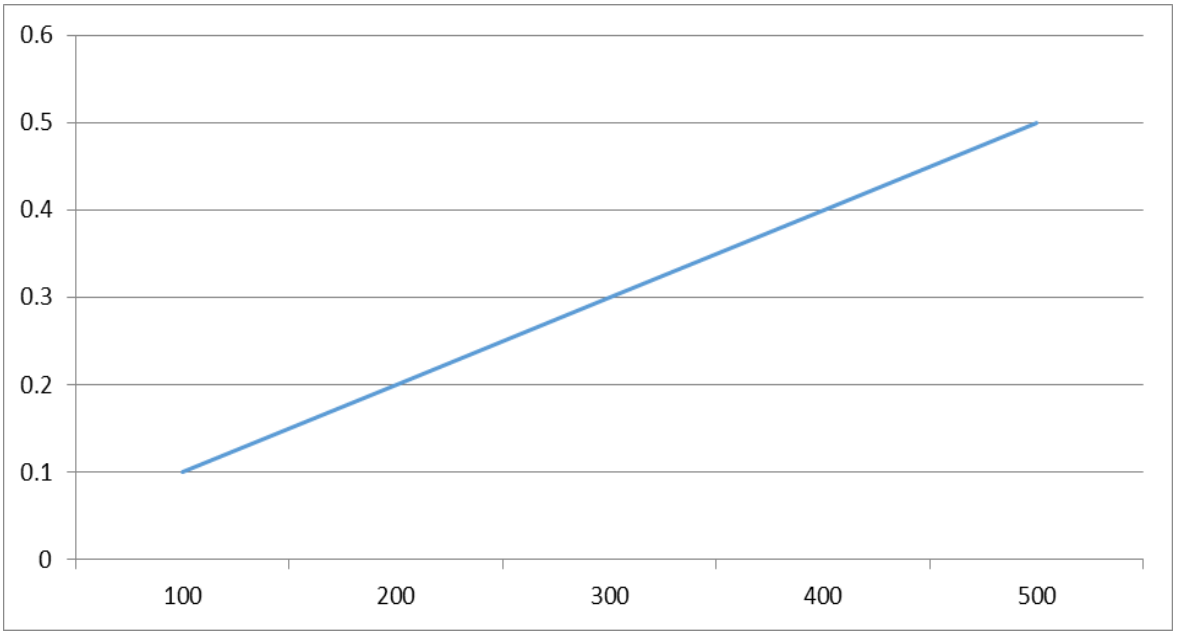
3.1 Effect of seasons on extraction of DNA

As per the protocol of plant DNA extractions discussed in the methodology section extractions of DNA from *M. koenigii* 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 *M. koenigii* L. leaves was given in Table – 1. Result showed that mean amount of the substance recovered after extraction of DNA from *M. koenigii* L. leaves was highest in rainy season (39.30µg/g of leaves) followed by autumn (37.86µg/g of leaves) and then summer (37.28µg/g of leaves). In winter, however, mean amount of the substance recovered after extraction of DNA from *M. koenigii* L. leaves was lowest (36.54µg/g of leaves).

3.2 Quantitation of DNA samples

Standard curve

A standard curve was drawn with different concentrations of standard DNA solutions against optical density taken at 595 nm. The curve is given below.



Vertical Axis: Optical density, Horizontal Axis: Concentration of standard DNA (Microgram).

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

Season	Amount of obtained substance (µg/g of leaves)	Mean amount of the substance (µg/g of leaves)
Autumn	37.9 37.5 38.1 37.8 38.0	37.86
Winter	36.8 36.1 36.6 36.2 37.0	36.54
Summer	38.0 37.1 37.2 36.9 37.2	37.28
Rainy	39.2 38.9 39.1 39.9 39.5	39.30

3.3 Effect of seasons on amount of DNA

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

Result showed that amount of DNA recovered from *M. koenigii* L. leaves was highest in rainy season (33.1± 2.1 µg/g of leaves), followed by autumn (31.5± 1.2 µg/g of leaves) and then in summer (31.0± 2.3 µg/g of leaves). In winter amount of DNA recovered from *M. koenigii* L. leaves was found lowest (30.1± 2.1µg/g of leaves). The results, however, were not statistically significant.

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

Season	Amount of DNA after extraction from *M. koenigii* L. leaves (µg/g of leaves) ± SEM
Autumn	31.5± 1.2
Winter	30.1± 2.1
Summer	31.0± 2.3
Rainy	33.1± 2.1

Results are mean of five experiments.

3.4 Purity estimation of the extracted DNA samples

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

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

Season	OD values 260 nm(A)	OD values 280 nm(A)	Ratio (A 260/ A 280)
Autumn	0.87	0.416	2.09
Winter	0.91	0.409	2.22
Summer	0.89	0.408	2.18
Rainy	0.84	0.464	1.81

Results showed that extracted DNA sample from *M. koenigii* L. leaves was more pure in rainy season in comparison to other seasons. Ratio of A 260 and A 280 came 1.81 for extracted DNA samples of rainy season. But the same ratios were 2.09, 2.22 and 2.18 for extracted DNA samples of autumn, winter and summer respectively.

4. DISCUSSION

As secondary metabolites polyphenolics, tanins, alkaloids and other compounds are present in high amount in most of the medicinal plants. Examples are, tea (*Camellia sinensis*) pokeweed (*Phytolacca dodecandra*), broad bean (*Vicia faba* L.) etc. In presence of high amounts of secondary metabolites it is very difficult to isolate genomic DNA as well as to obtain high quality DNA with good maintenance time from the medicinal plants. With the help of an optimised protocol Haque *et al.* isolated genomic DNA from gum containing plants.[15] Dehestani and Kazemi Tabar used a rapid efficient method and isolated DNA from plants with high levels of secondary metabolites.[16] Kim et al isolated high quality genomic DNA from fruit trees and conifers using PVP by using a simple and rapid method.[17] Friar isolated DNA from plants with large amounts of secondary metabolites.[18] Other workers also isolated DNA from different plants.[19,20] In the present work we have isolated genomic DNA from *M. koenigii*L. leaves.

Many workers studied seasonal variation on bioactive compounds (secondary metabolites) in plants. Fluck and Pharm as early as 1955 showed the influence of climate on the active principles in medicinal plants.[21] Thereafter, series of experiments were conducted in this direction.

Feeny in 1970 showed that amount of oak leaf tannins and nutrients changes with season.[22] Gupta in 1977 observed that amount of active principle constituents of *Eclipta prostrata* L. varies under different seasonal conditions and was maximum during summer.[23] Schultz *et al.*in 1982 showed that leaf quality of two northern hardwoods tree species varies with season.[24] Vasicine contents and their seasonal variation in *Adhatoda vasica* was studied byArambewela *et al.*in1988. They showed *that* vasicine contents in *Adhatoda vasica* were maximum in autumn.[25]

Mauffette and Oechel in 1989 found that leaf chemistry of the coast live oak *Quercus agrifolia* changes with season.[26] Drossopoulos *et al.* 1996 noted that mineral nutrients and carbohydrates in walnut tree leaves were maximum in rainy season.[27] Coli *et al*. in 1997 investigated seasonal and vertical variation in activity of pear thrips (Thysanoptera: Thripidae) within stands of sugar maple and noted maximum activity in autumn [28]. Fernnandez De Sim *et al*. 1999 studied evolution of phenolic compounds of Spanish oak wood during natural seasoning. They found that phenolic compounds were more in rainy season.[29] Ganjewalaand his co-workers, 2000 noted that accumulation of bacoside A in *Bacopa monniera* dependson season and was maximum in summer.[30]

Seasonal variation in anti ulcerogenic effect of *Astilbe rivularis* (saxifragaceae) leaves was studied by Mitra in 2014.[31] Anti ulcerogenic effect of *Astilbe rivularis* was found maximum in July & August. Mitra also studied seasonal variations in antibacterial activity of leaves of titeypati (*Artemisia vulgaris* L.) and found maximum anti bacterial activity during September & October.[32] In other studies also Mitra *et al.* noted that pharmacological activity of medicinal plants varies with season.[33-38]

In the present investigation we have extracted for the first time the genomic DNA from *M. koenigii* L. leaves of different seasons and found that the amount of material obtained after extraction of DNA from *M. koenigii* L. leaves was maximum in rainy season followed by autumn, summer and winter (Fig – 2).

Amount of DNA after extracting *M. koenigii* L. leaves was quantitated. It appeared that amount of DNA was maximum in rainy season followed by autumn, summer and winter but the results were not statistically significant (Fig – 3).

We have also studied purity of the extracted DNA samples from *M. Koenigii* L.leaves by noting the ratio of A 260 / A 280. Our study showed that isolated DNA sample from the leaves of *M. koenigii* L.of rainy season was comparatively pure than those DNA samples isolated from

M. koenigii L. leaves during autumn, summer and winter. (Fig - 4). Zhang *et al* noted that season, environment stress and refrigerated storage had a big effect on genomic DNA isolation of tung tree.[39]

5. CONCLUSION

Our present study has shown that pure DNA in good amount may be extracted from *M. koenigii* L. leaves during rainy season. DNA extraction from *M. koenigii* L. leaves during other seasons like summer, winter and autumn will neither yield good amount of DNA nor produce pure DNA.Therefore, in need of extraction of DNA from *M. koenigii*L.leaves, leaves of *M. koenigii*L. of rainy season may be utilized.

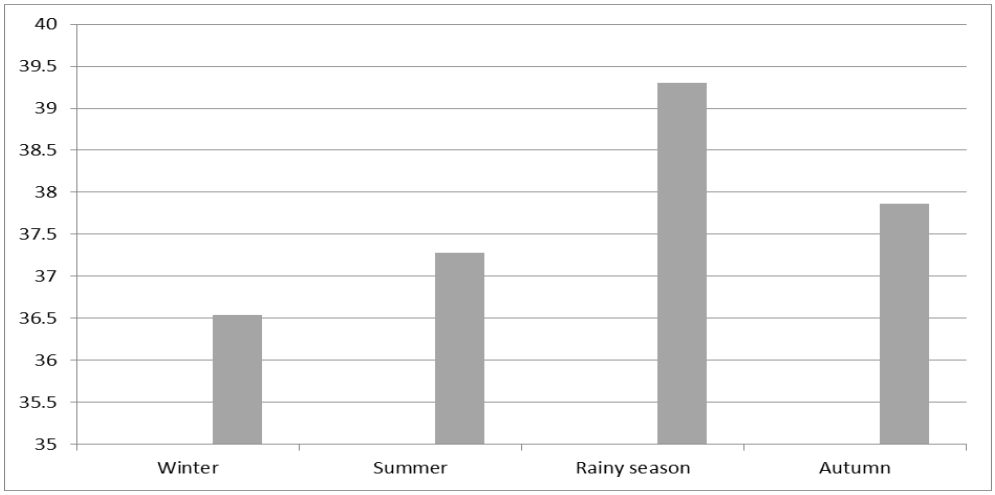


Figure 2: Effect of season on amount of material obtained after extraction of DNA from *M. koenigii* L. leaves.

Amount of the substance was in µg/g of *M. koenigii* L.leaves. Results were mean of 5 experiments.

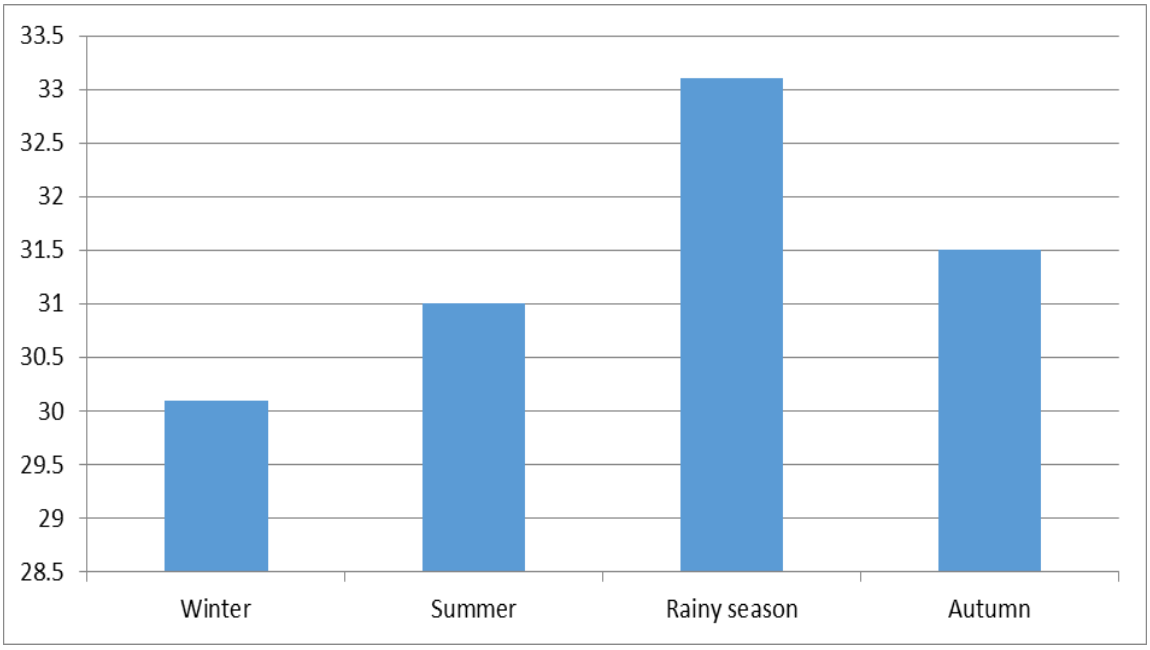


Figure 3: Effect of season on concentration of extracted DNA from *M. koenigii*L. leaves.

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

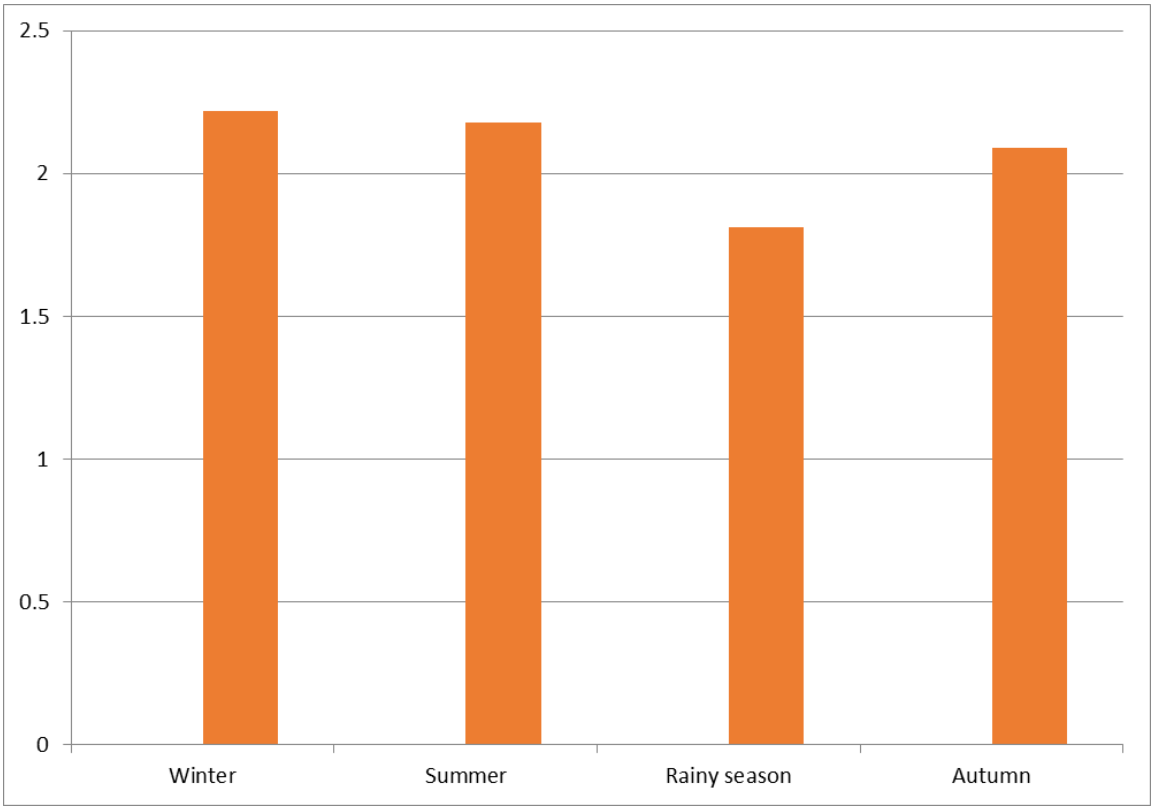


Figure 4: Effect of season on purity of extracted DNA from leaves of *M. koenigii* L.

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

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

REFERENCES

1. Katzung BG. Basic and Clinical Pharmacology, London: Prentice Hall International (UK) Limited, 1995; 6.
2. Pezzuto J. Taxol® production in plant cell culture comes of age. Nature Biotechnol, 1996; 14: 1083.
3. Roberts MF. Medicinal products through plant biotechnology. In: Robins RJ, Rhodes MJC. (eds). Manipulating Secondary Metabolism in Culture. Cambridge: University Press, 1988; 201-16.
4. Kumar VS, Sharma A, Tiwari R, Kumar S. Murraya koenigii (curry leaf): a review. J Med Arom Plant Sci, 1999; 21(4): 1139-1141.
5. Nadkarni KM, Indian Materia Medica, Popular Prakashan, Mumbai, 1976; 3(1): 196.
6. Kirtikar KR, Basu BD. Indian Medicinal Plants, Oriental Enterprises, Uttarchal Pradesh, 1981; 2(1): 473.
7. Khuntia TK, Panda DS. Evaluation of antibacterial, antifungal and anthelmintic activity of Murraya koenigii Spreng. Pharma Sci Monit, 2011; 2(2):105-110.
8. Chakrabarty M, Nath A, Khasnobis S, Chakrabarty M, Konda Y, Harigaya Y, Komiyama K. Carbazole alkaloids from Murraya koenigii. Phytochemistry, 1997; 46(4): 751-755.
9. Hickey JM, Chiurugwi T, Mackay I, Powell W, Eggen A, Kilian A, Jones C, Canales C, Grattapaglia D, Bassi F, et al. Genomic prediction unifies animal and plant breeding programs to form platforms for biological discovery. Nat. Genet, 2017; 49: 1297.
10. Choudhary K, Mathur N, Choudhary OP, Pillai U. Protocol for Isolation of Genomic DNA from Dry and Fresh Leaves of Vigna Species Suitable for Rapd and Restriction Digestion. Advances in Biological Research, 2008; 2 (5-6): 83-89.
11. Ribek Thapa, Baibhav Karki, Sudarshan Chettri,Tanaya Ghosh, Prasenjit Mitra, Prasanta Kumar Mitra. Seasonal effect on extraction, quantitation & purity estimation of genomic DNA from *Cassia alata* l. leaves. World Journal of Pharmacy & Pharmaceutical Sciences, 2018; 7(7): 1419-1430.

12. Gendimenico GJ, Bouquin PL, Tramposch KM. Diphenylamine-colorimetric method for DNA assay: a shortened procedure by incubating samples at 50 degrees C. Anal Biochem., 1988; 15, 173(1): 45-8.
13. Psifidi A, Dovas CI, Banos G. A comparison of six methods for genomic DNA extraction suitable for PCR-based genotyping applications using ovine milk samples. Mol. Cell. Probes, 2010; 24(2): 93-8.
14. Bliss CI. Statistics in biology, Statistical methods for research in the natural sciences, McGraw Hill Book Company, NY, 1967; 558: 1.
15. Haque I, Bandopadhyay R, Mukhopadhyay K. An Optimised Protocol for Fast Genomic DNA Isolation from High Secondary Metabolites and Gum Containing Plants. Asian Journal of Plant Sciences, 2008; 7(3): 304-308.
16. Dehestani A, Kazemi Tabar SK. A Rapid Efficient Method for DNA Isolation from Plants with High Levels of Secondary Metabolites. Asian Journal of Plant Sciences, 2007; 6(6): 977-981.
17. Kim CS, Lee CH, Shin JS, Chung YS, Hyung NI.A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. Nucleic Acids Res., 1997; 25(5): 1085–1086.
18. Friar EA. Isolation of DNA from plants with large amounts of secondary metabolites. Methods Enzymol, 2005; 395: 3–14.
19. Abu Almakarem AS, Heilman KL, Conger HL, Shtarkman YM, Rogers SO. Extraction of DNA from plant and fungus tissues in situ. BMC Res Notes, 2012; 5: 266.
20. Bashalkhanov S, Rajora OP. Protocol: a high-throughput DNA extraction system suitable for conifers. Plant Methods, 2008; 4: 20.
21. Fluck H, Pharm M. The influence of climate on the active principles in medicinal plants. J. Pharm.Pharmacol, 1955; 7: 361-383.
22. Feeny P. Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology, 1970; 51: 565–581.
23. Gupta PL. Variation in morphological characters and active principle constituents of *Eclipta prostrata* Linn. under different seasonal and soil conditions. JRIM, 1977; 12(1): 80-84.
24. Schultz JC, Nothnagle PJ, Baldwin IT. Seasonal and individual variation in leaf quality of two northern hardwoods tree species. American Journal of Botany, 1982; 69: 753–759.
25. 25. Arambewela LSR, Ratnayake CK. Vasicine contents and their seasonal variation in *Adhatoda vasica*. Fitoterapia, 1988; 59(2): 151-153

26. Mauffette Y, Oechel WC. Seasonal variation in leaf chemistry of the coast live oak*Quercus agrifolia* and implications for the California oak moth *Phryganidia californica.* Oecologia, 1989; 79: 439–445.
27. Drossopoulos B, Kouchaji GG, Bouranis DL. Seasonal dynamics of mineral nutrients and carbohydrates by walnut tree leaves. J Plant Nutr, 1996; 19: 493–516.
28. Coli WM, Hollingsworth CS, Hosmer TA. Seasonal and vertical variation in activity of pear thrips (Thysanoptera: Thripidae) within stands of sugar maple. Canadian Journal of Research, 1997; 27: 667-673.
29. Fernandez De, Simon B, Cadahta E, Conde A, Garcta MC. Evolution of phenolic compounds of Spanish oak wood during natural seasoning.First results. J. Agric. Food Chem, 1999; 47: 1687–1694.
30. Ganjewala D, Srivastava AK, Luthara R. Ontogenic and seasonal variation in accumulation of bacoside A in *Bacopa monniera*. Journal of Medicinal and Aromatic Plant Sciences, 2000; 22(4A): 233-237.
31. Mitra Prasanta Kumar. Seasonal variation in anti ulcerogenic effect of *Astilbe rivularis* (saxifragaceae) leaves. Acta Biomedica Scientia, 2014; 1(3): 129 –132.
32. Mitra Prasanta Kumar. Seasonal variation in antibacterial activity of leaves of titeypati (*Artemisia vulgaris* L.). J Pharma Biol, 2014; 4(4): 173-176.
33. Mitra Prasenjit, Ghosh Tanaya, Mitra Prasanta Kumar. Seasonalvariationin hepatoprotective activity of *Cassia alata* linn. leaves on antitubercular drugs induced hepatotoxicity in rats. Int. J Pharmacy Practice & Drug Research, 2016; 6(1): 40-44.
34. 34. Mitra Prasenjit, Ghosh Tanaya, Mitra Prasanta Kumar. Seasonal variation in anti ulcerogenic activity of *ageratum conyzoides* l. leaves. International Journal of Biopharmaceutics, 2016; 7(2): 63 – 68.
35. Mitra Prasanta Kumar, Ghosh Tanaya, Mitra Prasenjit. In vitro anti thiamine effect of *Murrya koenigii* (linn.) spreng wettst leaves: effect of season. International Journal of Biopharmaceutics, 2016; 7(2): 69 – 72.
36. Mitra Prasenjit, Ghosh Tanaya, Mitra Prasanta Kumar. Isolation of Anti Thiamine Compound from the Leaves of *Abrus Precatorius* Linnaeus: Effect of Season on Yield of the Compound. European J Biomedical and Pharmaceutical Sciences, 2018; 5(3): 581-586.
37. Ghosh Tanaya, Mitra Prasenjit, Mitra Prasanta Kumar. Seasonal variation in antibacterial activity of leaves of *Murrya koenigii* (linn.) spreng wettst. European J Biomedical and Pharmaceutical Sciences, 2018; 5(4): 782-786.

38. Mitra Prasenjit, Ghosh Tanaya, Mitra Prasanta Kumar. Effect of season on in vitro anti oxidant activity of *Mentha spicata* L. leaves. World J Pharmacy and Pharmaceutical Sciences, 2018; 7(4): 1737 – 1747.
39. Lingling Zhang, Yue Pan, Jinmin Fu, Junhua Peng. Season, Environment Stress and Refrigerated Storage Affect Genomic DNA Isolation of Tung Tree American Journal of Plant Sciences, 2012; 3: 1562-1567.