Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 6 (1): 65-73, 2012
ISSN 1819-3455 / DOI: 10.3923/rjmp.2012.65.73

Modified CTAB Technique for Isolation of DNA from some Medicinal Plants

K.L. Tiwari, S.K. Jadhav and S. Gupta
School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur (C.G)-492010, India

Corresponding Author: S.K. Jadhav, School of Studies in Biotechnology, Pt. Ravishankar Shukla University, Raipur (C.G)-492010, India

ABSTRACT

Medicinal plants utilization and conservation has attracted global attention. Optimization of DNA isolation protocol for genetic characterization of any medicinal plants is the necessary and primary step. So, the standardization of DNA isolation is basic requirement for any further research to be carried out. We report here modified CTAB technique for isolation of genomic DNA from five selected medicinal plants namely *Catharanthus roseus, Tridax procumbens, Tinospora cordifolia, Aloe barbadensis* and *Cissus quadrangularis*. The comparison of genomic DNA yield from all the five medicinal plants and their different organs (leaves, nodal stem and meristematic region) was performed. We got good yield of DNA from leaves, nodal stem and meristematic region of these medicinal plants. Genomic DNA isolated by modified CTAB method was pure, the highest level of purity was obtained from *Aloe barbadensis* was 1.93 from leaves and concentration from *Tinospora cordifolia* was 833.00 μg mL^{-1} from nodal stem. Other plants also gave good yield of genomic DNA with purity range between 1.26 to 1.93 and concentration range from 179.00 to 833.00 μg mL^{-1}. All the five selected medicinal plants for this research gave good yield of DNA from the established modified CTAB protocol. From all the organs of these medicinal plants DNA obtained was pure and quantity was also good in all five plants.

Key words: Blue print, extraction, giloy, eppendorf, ethanol, phenol

INTRODUCTION

Each and every living organism has its specific surrounding medium or environment to which it continuously interacts and remains fully adapted. There is an intact interaction of organisms with each other and also with the present in their habitats (Santra, 2010). It seems to be an axiom of nature that where there is diversity, there is also similarity indeed, nature's variety is boundless. The universe of the cell too is complex and diverse like the world around us, the world of the cell is one of the forms specialized for a particular type of existence (Tyagi, 2009). Cells are the tiny complex bodies, it is difficult to see their structure, more difficult to understand their molecular composition and still difficult to find out the functions of their various components. Each of the cells in our bodies carries our unique genetic code based on our DNA which is chemical inside the nucleus of all cells that carries the genetic instructions for formation of living organisms (Verma and Agarwal, 2007). DNA is our genetic code or the "Blue Print" that we inherit from our parents when we are born, our genetic code or DNA is virtually pristine. Every cell in our body is formed based on our DNA and each cell becomes the holder of our entire genetic blueprint (Sterart and Norbdy, 2009).

Since time immemorial man has been using plant extracts to protect himself against several diseases and also to improve his health and life-style. According to World Health Organization 20,000 plant species are used for medicinal and aromatic purpose (Vural, 2009). The new uses for medicinal plants have been discovered and popularized. So, the Application of molecular technology would increase and facilitate production of these useful substances in medicinal plants by studying their genetic profile (Verma and Agarwal, 2007).

Optimization of DNA isolation protocol for genetic characterization is the primary and necessary step in field of molecular biology (Tan and Yiap, 2009). Studies performed at genetic level are essential for chemical profiling of various medicinal plants like *tridax procumbens* was performed by Jude *et al.* (2009) and for that DNA isolation is the first and foremost step in molecular biotechnology. In the past, the process of extraction and purification of nucleic acids used to be complicated, time-consuming, labor-intensive, and limited in terms of overall through put. Currently, many specialized methods based on use of acid, alkali, detergents, phenol and suitable buffer are developed (Guin, 1966).

The search for a more efficient means of extracting DNA of both higher quality and yield has lead to the development of a variety of protocols, however the fundamentals of DNA extraction remain the same. Firstly DNA must be purified from cellular material in a manner that prevents degradation, for this even crude extraction procedures can still be adopted to prepare a sufficient amount of DNA to allow it for multiple end uses. After that protein digestion and action of detergents during the extraction process destroys the plasma membrane and the nuclear membrane surrounding the DNA. EDTA in extraction buffer is added to prevent DNA from degradation, EDTA chelate the Mg^{2+} needed for enzymes that degrade DNA. For removal of polysaccharides higher concentration of Cetyl Trimethyl Ammonium Bromide (CTAB) is added (Channarayappa, 2007). A range of methods is available to assess the quality of the isolated DNA which include gel electrophoresis, spectrometric analysis, restriction digestion, PCR amplification and chromatographic techniques (Varma *et al.*, 2007).

Agarose gel electrophoresis is a procedure used to separate DNA fragments based on their molecular weight. Quantification of nucleic acids is commonly done in molecular biology to determine the concentrations of DNA or RNA present in a mixture (Channarayappa, 2007). Spectrophotometers are commonly used to determine the concentration of DNA in a solution. It is possible to use UV-spectrophotometer to estimate the purity of a solution of nucleic acids. This method involves measuring the absorbance of the solution at two wavelengths, usually 260 and 280 nm, calculating the ratio of the two absorbance: an characteristic of pure DNA with 1.8 of A260/A280 ratio is considered pure (Nieman and Poulsen, 1963). In this research, we developed a modified CTAB technique for genomic DNA isolation from five selected medicinal plants and their different organs (leaves, nodal stem and meristematic region). The modifications in standard CTAB protocol are made such that good yield of DNA from all the five plants can be obtained and this protocol can become useful for DNA isolation from other medicinal plants.

MATERIALS AND METHODS

Sample collection: The fresh and young tissues of plants namely *Catharanthus roseus* [Sadabahar], *Tridax procumbens* [Ghamra], *Tinospora cordifolia* [Giloy], *Aloe barbadensis* [Ghritakumari] and *Cissus quadrangularis* [Hadjod] was taken from the campus of Pt. Ravishankar Shukla University, Raipur [C.G] in 2010.

Method: In the present study, CTAB protocol given by Doyle and Doyle (1990) was modified and used for the isolation of genomic DNA from five medicinal plants.

The following stock solutions and reagents were prepared by mixing the standard amount of chemicals. Each chemical has its own pH which is very essential to be maintained for the proper functioning of required chemicals. List of these chemicals is Table 1.

For preparation of CTAB buffer (100 mL working solution), 12 mL of Tris HCl was mixed with 30 mL of 5 M NaCl, then 4.5 mL of 0.5M EDTA was added to the solution and mixed thoroughly after that 2.4 g of CTAB powder was added and then 48.9 mL of double distilled water was added in the solution. This was kept in water bath at 60°C for 10-15 min to let the CTAB powder dissolve, after that 600 μL of β-mercaptoethanol was added to CTAB buffer. The collected sample was washed with 90% ethanol and then with double distilled water to remove any contaminant present on that plant. After washing, sample was dried with wattmann filter paper to remove water present on it. That may interfere in isolation protocol.

Taken 1 g of plant parts (leaves, nodal region and meristematic region) each separately from selected plants, they were washed properly and air dried to remove any trace of water present. Then added 2 mL of CTAB extraction buffer to them, after that grinded them properly with the help of mortar and pestle.

Each sample solution was taken in eppendorf tubes and kept them in water bath at 65°C for 45 min after that brought the sample out of water bath and kept at room temperature for 5 min, then centrifuged it at 12,000 rpm for 15 min. Supernatant obtained was taken 1 mL from each tube, transferred them to another tube every time with a wide bore pipette and then added 500 μL of P:C:I to it, shaken for 15 min, after shaking centrifuged at 12,000 rpm for 12 min.

Table 1: List of chemicals needed in CTAB isolation protocol

Name of chemicals	pH	Amount (for 100 mL solution)	Remark
1.0 M Trizma base (Tris-HCL)	8.0	12.11 g	Maintainance of 8.0 pH is essential
0.5 M Ethylene diamine tetra acetic acid (EDTA)	8.0	18.6 g	Maintainance of 8.0 pH is essential and EDTA should be properly dissolved in water
05.0 M sodium chloride (NaCl)	8.0	29.2	NaCl should be properly dissolved in water
Hexadecyltrimethyl ammonium bromide (CTAB)	-	2.4 g	CTAB is added in the extraction buffer and dissolving CTAB requires a temperature of 60°C
2% β-mercaptoethanol	-	600 μL	β-mercaptoethanol should be added after dissolving CTAB powder in extraction buffer
Phenol: Chloroform: Isoamyl alcohol Mixture (P:C:I)	-	25:24:1(mL)	Saturated phenol should be used
Chloroform: Isoamyl alcohol Mixture (C:I)	-	24:1 (mL)	
Iso-Propanol	-	10 mL	Iso propanol should be stored at 4°C
70% ethanol	-	70 mL	Storage at 4°C
Ethidium bromide (EtBr)	-	18.6 g	EtBr should be dissolved properly in gel

Again supernatant was taken in another eppendorf tube and added equal volume of P:C:I, shaken properly, after shaking centrifuged at 12,000 rpm for 10 min. The obtained supernatant after above centrifuge was transferred the in another eppendorf tube, added equal volume of C:I, shaken properly, after shaking centrifuged at 10,000 rpm for 10 min.

After above centrifuge supernatant consisting of DNA was taken in new eppendorf tube and added pre-chilled isopropanol (65% of the total supernatant) kept at -20°C for 2 h, then centrifuged at 8000 rpm for 8 min. Supernatant was discarded from tubes then 300 µL of 70% ethanol was added in to the pellet and centrifuged at 8000 rpm for 10 min, discarded the ethanol after centrifuge and dried the pellets obtained by incubating them at 37°C in dry bath, dissolved these pellets in 100 µL of TAE buffer and stored at -20°C in deep freezer.

Quantification of isolated genomic DNA: With the aid of spectroscopy, the quantitative analysis of nucleic acids and proteins has established itself as a routine method in many laboratories. It includes absorption measurements in the UV range of spectrum, proteins are measured directly at 280 nm, nucleic acid at 260 nm. DNA, RNA, oligonucleotides and even monosaccharides can be measured directly in aqueous solutions in diluted or undiluted form. Aqueous buffer with low ion concentration, TAE Buffers was taken in this research. Purity determination of DNA interference by contaminants can be recognized by the calculation of ratio A260/A280 is used to estimate the purity of nucleic acid (Nieman and Poulsen, 1963).

RESULTS AND DISCUSSION

With five different varieties of medicinal plants in this research we successfully isolated good yield of DNA from the different parts of these plants that are leaves, nodal stem and meristematic region. In present work CTAB protocol was given by Doyle and Doyle (1990) was modified so that a good DNA yield from five diverse plant species can be obtained. We modified the CTAB protocol in such a way that all the five medicinal plants gave good DNA yield.

From all the five medicinal plants comparing the purity and concentration of DNA obtained, good purity range of DNA was seen in *Aloe barbadensis* that was 1.93 from nodal stem and 1.83 from meristematic region *Cissus quadrangularis* showed purity range of 1.76 from leaves. Although poor yield of purity was seen in *Cissus quadrangularis* that was 1.26 from nodal stem and also in *Tridax procumbens* poor purity range of 1.34 from nodal stem (Fig. 1). Similarly, comparing the concentration of all five medicinal plants the good yield of DNA concentration was given by *Tinospora cordifolia* that was 833.00 µg mL^{-1} from nodal stem and 603.37 µg mL^{-1} from meristematic region, after that Aloe *barbadensis* gave DNA concentration of 590.67 µg mL^{-1} from meristematic region. While poor yield of concentration was seen in *Tridax procumbens* which was 179.00 µg mL^{-1} from leaves and *Aloe barbadensis* gave DNA concentration of 179.00 µg mL^{-1} from nodal stem (Fig. 2).

The other plants gave different DNA yield as described here *Tridax procumbens* gave 1.38 (leaves), 1.34 (nodal stem) and 1.43 (meristemetic region) range of purity (Fig. 1) and concentration of 179.00 µg mL^{-1} (leaves), 586.34 µg mL^{-1} (nodal stem) and 461.34 µg mL^{-1} (meristemetic region) (Fig. 2). Also *Cissus quadrangularis* gave 1.76 (leaves), 1.36 (nodal stem) and 1.5 (meristemetic region) range of purity and concentration of 368.34 µg mL^{-1} (leaves), 348.34 µg mL^{-1} (nodal stem) and 299.00 µg mL^{-1} (meristemetic region). Similarly *Catharanthus roseus* gave purity of 1.56 (leaves), 1.46 (nodal stem) and 1.56 (meristemetic region) and concentration of 526.00 µg mL^{-1} (leaves), 447.00 µg mL^{-1} (nodal stem) and 325.00 µg mL^{-1}

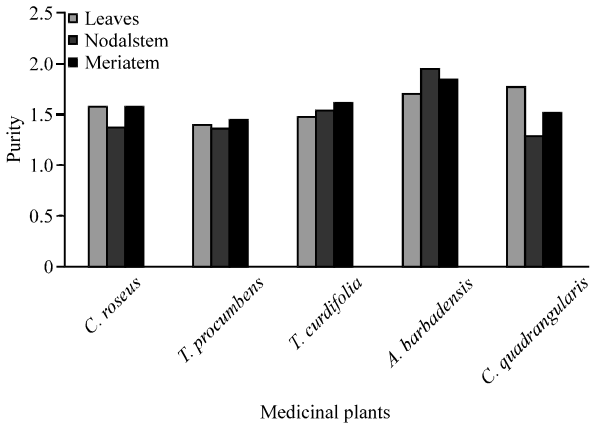

Fig. 1: The DNA purity in different organs of medicinal plants

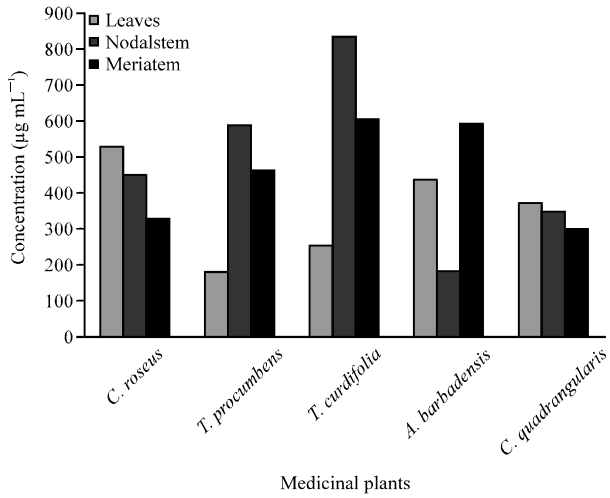

Fig. 2: The DNA concentration in different organs of medicinal plants

(meristemetic region). These were the results of purity and concentration of DNA obtained in research work of modified CTAB protocol (Fig. 1, 2).

Comparison of genomic DNA obtained from all the five medicinal plant showed that *Aloe barbadensis, Cissus quadrangularis* and *Tinospora cordifolia* gave good and pure yield of DNA whereas genomic DNA yield obtained from *Catharanthus roseus* was average and *Tridax procumbens* showed poor yield of genomic DNA.

Again if we compare the DNA yield amongst the three different organs of plants taken that are leaves, nodal stem and meristematic region. Meristematic region gave good purity of 1.43 to 1.83 and concentration of 299.00 to 603.37µg mL^{-1} compared with other parts of medicinal plants taken in study (Fig. 3, 4). The reason behind this is the property of having the most dividing and young cells found in the meristematic region and the production of secondary metabolites at this stage of development is very low almost not produced. Although poor yield of purity ranging from 1.38 to 1.76 and concentration 179.00 to 526.00 µg mL^{-1} was seen in leaves among three organs of plants (Fig. 3, 4). The reason for not getting good yield of genomic DNA from leaves is that the secondary metabolites, pigments and reserved food are mainly stored in leaves which hinder in proper isolation of genomic DNA.

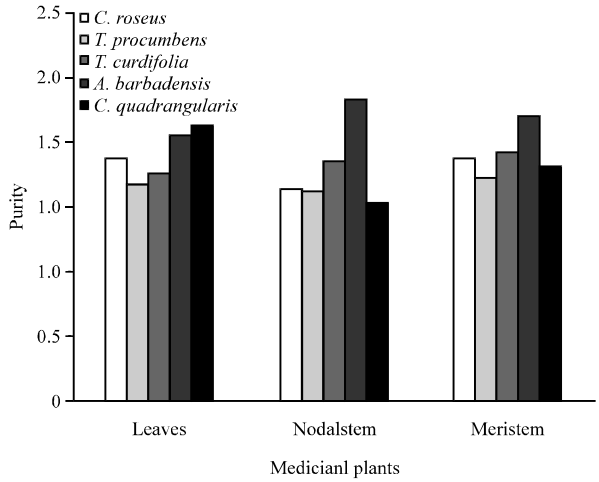

Fig. 3: The comparison of dna purity between different medicinal plants

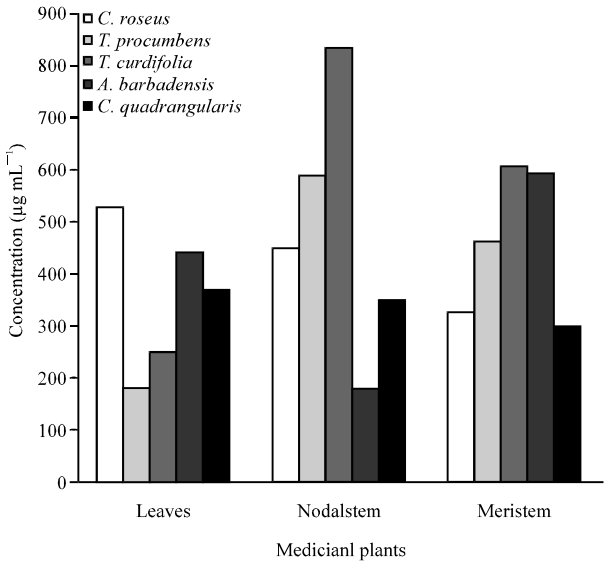

Fig. 4: The comparison of DNA concentration between different medicinal plants

Doyle and Doyle (1990) in their CTAB protocol used 1.4 M NaCl, 20 Mm EDTA, 0.2% **β-** mercaptoethanol in extraction buffer, they heated the sample in water bath only for 30 min at 60°C and extraction only was performed once with Chloroform: Isoamyl alcohol, but in present protocol 5 M NaCl, 0.5 M EDTA and 0.6% **β-**mercaptoethanol was used in extraction buffer, we increased the incubation time to 45 min and temperature to 65°C in water bath and twice P:C:I extraction step was included in this protocol and cold Isopropanol precipitation time was increased to 2 h. These were the modification made in present protocol in comparison to CTAB protocol given by Doyle and Doyle (1990). The increase in NaCl and EDTA concentration in buffer made the extraction of DNA easier with less degradation of DNA due to presence of EDTA with increase in **β-**mercaptoethanol concentration which is needed for denaturation of proteins. Incubation time and

temperature of sample in water bath was increased to make extraction more effective. Applying these modifications in the DNA isolation protocol good yield of DNA from different parts of five medicinal plants was obtained.

Likewise, Khanuja *et al.* (1999) isolated DNA from dry and fresh samples of ten plant species, in the form of leaves, stem, flowers namely *Allium sativum, Artemisia annua, Bacopa monnieri, Catharanthus roseus, Cymbopogon winterianus, Pelargonium graveolens, Mentha arvensis, Ocimum kilmandscharium, Taxus wallichiana* and *Vetiveria zinzaniodes*. They also modified the CTAB technique for DNA isolation by adding 1%PVP to remove polyphenols and absence of P:C:I extraction step was seen in their protocol. DNA yield obtained was 15.00 to 54.00 μg g^{-1} from fresh parts and 20.00 to 85.00 μg g^{-1} from dry plant parts, amongst dry and fresh samples good results were given by dry plant parts. One of the plants taken by them as sample was *Catharanthus roseus* gave DNA yield of 35.00 μg g^{-1} of fresh tissue and 50.00 μg g^{-1} from dry tissues. The modified CTAB protocol used in this research, without the use of PVP the DNA yield from *Catharanthus roseus* was 526.00 μg mL^{-1} from fresh leaves, 447.00 μg mL^{-1} from nodal stem and 325.00 μg mL^{-1} from meristematic region.

Another work on modifying the CTAB protocol for DNA isolation was performed by Khan *et al.* (2007) a simple and efficient protocol for isolating DNA from medicinal plants namely *Asparagus adscendens, Withania somniferia, Asparagus racemosus* and *Chlorophytum borivilianum*. There modification involved use of 3% CTAB, 2 M NaCl, 4% β-mercaptoethanol and 5% PVP in extraction buffer. They got DNA yield range from 48.00 to 68.00 μg g^{-1} from dry tissue and 33.00 to 42.00 μg g^{-1} from fresh tissue of plants. This high amount of chemical and reagents used by them can hinder in extraction procedure and may also cause degradation of DNA. In present modified CTAB isolation without the use of this high amount of chemicals good DNA yield both in terms of purity of 1.26 to 1.93 and concentration of 179.00 to 833.00 μg mL^{-1} taking only fresh parts of them was obtained from all the five plants.

Sahasrabudhe and Deodhar (2010) also modified CTAB protocol to obtain good yield of DNA from *Garcinia indica*. Hence, they made modification like addition of PVP and concentration of NaCl was changed from 2 to 1.4 M in extraction buffer. Also the 0.2% of β-mercaptoethanol was taken in place of 2% and incubation time of 1hr at 60°C in water bath was maintained, these were modifications made by them in CTAB protocol to get good yield of DNA concentration. Srivastava *et al.* (2010) used CTAB isolation protocol given by Doyle and Doyle (1987) for extraction of DNA from different tissues of *Aconitum heterophyllum* medicinal species. They also used 2 other methods for DNA isolation out of which method -1 given by them gave best results of 957.33±1.52 ng μL^{-1} compared to method of Doyle and Doyle.

Dehestani and Kazemi Tabar (2007) isolated DNA of plants with high levels of secondary metabolites. They used three standard protocols for this method out of which one was CTAB protocol which was modified by adding more amount of PVP and high concentration of EDTA with β-mercaptoethanol in extraction buffer, DNA yield of 100-250 μg g^{-1} was obtained from fresh tissues of plant. Shankar *et al.* (2011) isolated DNA from four *in vitro* banana cultivars by using CTAB protocol (Doyle and Doyle, 1987) which gave very low yield of DNA ranging from 341 to 440 μg g^{-1} across all four banana cultivars. This yield was comparatively low and then they used modified CTAB protocol where DNA yield ranged from 712 to 808 μg g^{-1} was obtained which was good compared to Doyle and Doyle CTAB protocol.

CONCLUSION

Medicinal plants are future of herbal biotechnology various tools of biotechnology are essentially needed for every sort of research and new invention in every field of biological studies. No research work is possible without help of basic techniques and tools of biotechnology, one of such basic technique needed for any genetic level of studies is isolation of DNA. DNA isolated by modified CTAB method gave a good yield of concentration and purity. The yield of DNA from all the parts of medicinal plants was average but meristematic region gave best results compared to nodal stem and leaves. Amongst different medicinal plants best results of DNA yield were seen in *Tinospora cordifolia* (Giloy), *Cissus quadrangularis* (Hadjod), *Aloe barbadensis* (Ghritakumari). From this study we conclude that DNA isolated by modification made in CTAB method gave good yield of DNA which is very useful for any necessary researches in the field of molecular biology for the study of medicinal plants.

REFERENCES

Channarayappa, 2007. Molecular Biotechnology, Principles and Practices. 1st Edn., University Press, London, ISBN-13: 9781420051575.

Dehestani, A. and S.K. Kazemi Tabar, 2007. A rapid efficient method for DNA isolation from plants with high levels of secondary metabolites. Asian J. Plant Sci., 6: 977-981.

Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Guin, G., 1966. Extraction of nucleic acids from lyophilized plant material. Plant Physiol., 41: 689-695.

Jude, C.I., C.I. Catherine and M.I. Ngozi, 2009. Chemical Profile of *Tridax procumbens* Linn. Pak. J. Nutr., 8: 548-550.

Khan, S., M.I. Qureshi, T.A. Kamaluddin and M.Z. Abdin, 2007. Protocol for isolation of genomic DNA from dry and fresh roots of medicinal plants suitable for RAPD and restriction digestion. African J. Biotechnol., 6: 175-178.

Khanuja, S.P.S., A.K. Shasany, M.P. Darokar and S. Kumar, 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. Plant Mol. Biol., Rep., 17: 1-7.

Nieman, R.H. and L.L. Poulsen, 1963. Spectrophotometric estimation of nucleic acid of plant leaves. Plant Physiol., 38: 31-35.

Sahasrabudhe, A. and M. Deodhar, 2010. Standardization of DNA extraction and optimization of RAPD-PCR conditions in *cinia indica*. Int. J. Bot., 6: 293-298.

Santra, S.C., 2010. Environmental Sciences. 2nd Edn., New Central Book Agency (P) Ltd., Kolkata, India.

Shankar, K., L. Chavan, S. Shinde and B. Patil, 2011. An improved DNA extraction protocol from four, *in vitro* banana cultivars. Asian J. Biotechnol., 3: 84-90.

Srivastava, N., V. Sharma, B. Kamal, A.K. Dobriyal and V.S. Jadon, 2010. Polyphenolics free DNA isolation from different types of tissues of *Aconitum heterophyllum* wall-endangered medicinal species. J. Plant Sci., 5: 414-419.

Sterart, H.J. and J.J. Norbdy, 2009. Forensic Science: An Introduction to Scientific and Investigative Techniques. 3rd Edn., Taylor and Francis, USA., pp: 757.

Tan, S.C. and B.C. Yiap, 2009. DNA, RNA and protein extraction : The past and the present. J. Biomed. Biotechnol., 2009: 10-10.

Tyagi, A., 2009. Environmental Science. New Edn., Trumann's Specific Series, USA.,.

Varma, A., H. Padh and N. Shrivastava, 2007. Plant genomic DNA isolation: An art or a science. Biotechnol. J., 2: 386-392.

Verma, P.S. and V.K. Agarwal, 2007. Evolution and Ecology. Sehand Publications, Reprint.

Vural, H.C., 2009. Genomic DNA isolation from aromatic and medicinal plants growing in Turkey. Sci. Res. Essays, 4: 59-64.