International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 11: January 2018
UGC Approved Journal No 48951

Original Article | Open Access

Phenol Free and FTA card based Genomic DNA Extraction from Stems and Leaves of *Commiphora wightii* and *C. myrrha*

Sairkar Pramod Kumar[1*], Sharma Anjana [2], and Shukla NP[3]

[1]Centre of Excellence in Biotechnology, M.P. Council of Science & Technology, Bhopal, Madhya Pradesh, India
[2]Bacteriology Laboratory, Department of Post Graduate Studies & Research in Biological Science, Rani Durgawati University, Jabalpur Madhya Pradesh, India
[3] Madhya Pradesh Pollution Control Board, Bhopal, Madhya Pradesh, India.
* Corresponding author, Email: pksairkar@mpcost.nic.in; sairkar_pramod04@yahoo.com

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Sairkar Pramod Kumar, Sharma Anjana, and Shukla NP (2018) Phenol Free and FTA card based Genomic DNA Extraction from Stems and Leaves of *Commiphora wightii* and *C. myrrha*, *Int. J. of. Life Sciences*, Special Issue, A11: 25-30.

ABSTRACT

"Taxonomically difficult" family Burseraceae are drought-tolerant and leafless for most of the year, contains higher amount of polysaccharides, polyphenols and other secondary metabolites in stems and leaves, which make difficulty in the isolation of genomic DNA. Out of three phenol free CTAB based methods (M-1, M-2 and M-3), the isolated DNA from leaves and stem using method M-2 and M-3 respectively, showed a prominent DNA band and these DNA were also performed good amplification with RAPD primers. Whereas, out of 5 sample application methods on Whatman FTA PlantSaver Card, the sample application of first and finally precipitated DNA of CTAB based M-1 method on FTA cards, showed prominent amplification. Good quality and quantity DNA was obtained as higher concentrations of CTAB, PVP and β-mercaptoethenol treatment was given. The isolated DNA and modified sample application method on the FTA PlantSaver card showed good PCR amplification.

Key worlds: Commiphora, *C. wightii, C. myrrha*, Whatman FTA PlantSaver Card, Genomic DNA, CTAB, PVP, β-mercaptoethenol

INTRODUCTION

In the recent years, herb based medicinal systems like Ayurveda, Unani, Homeopathy and folk medicine systems are widely preferred because allopathic medicines have various side effects (Bakhru 1998). Plant drugs constitute as much as 25% of the total drugs in the USA, while in India, the plant drug contribute 80% of the total drug (Mouli and Rao 2009). Every medicinal plant has their own specific medicinal property due to the presence of higher concentrations of polysaccharides, polyphenols, proteins, and other secondary metabolites of plant leaves which may pose hindrance in the isolation of DNA and creates a problem in genomic

downstream studies (Pandey *et al,* 1996; Haque *et al,* 2009; Sahu *et al,* 2012). One of the major factors affecting the success of genomic studies is the purity of DNA as extraction of quality DNA is a major challenge for molecular biologists dealing with higher plants (Anuradha *et al,* 2013). Various chemical treatments are used for eliminating these compounds (Dabo *et al,* 1993; Haque *et al,* 2009; 2010). Mostly young leaves are used for the isolation of genomic DNA, but other parts of the plant such as stem were also used. Whatman FTA (Flinders Technology for Analysis; Guthrie and Susi 1963) card is widely used in the extraction, long term storage and amplification of DNA from animal tissues, including blood in room temperature, while for the extraction of plant genomic DNA a special FTA Plant Saver card was used (Sairkar *et al,* 2013a; Tsukaya *et al,* 2005).

Commiphora spp. of the family *Burseraceae* is being used as a medicinal plant since ancient times and now rated as an endangered plant species (IUCN 2012). At the time of collection of samples in the year 2010, this species was rated as Data Deficiency in India. They are found in the arid to semiarid regions of the world, including the deserts of India, Pakistan, Africa and Saudi Arabia, while in India, it is found in Rajasthan, Madhya Pradesh, Gujarat, Tamilnadu, Orissa and Karnataka. About 185 species of *Commiphora* were found worldwide, out of them especially *C. wightii* (synonym *C. mukul*) and *C. myrrha* found in India (Kirtikar and Basu 1935; Hocking 1993). *C. wightii* contain a bitter gum known as Guggul (Myrrh) in stems and leaves, which is used in the treatment of hypercholesterolemia and cardiovascular diseases (Deng 2007) and it is also shown to have anti-cancerous activity (Xiao and Singh 2008).

The classification and nomenclature of *Burseraceae*, have been called "taxonomically difficult". The drought-tolerant plants are leafless for most of the year and are thus difficult to identify (Gachathi 1997). *Commiphora* species contains higher amount of polysaccharides, polyphenols and other secondary metabolites in stems and leaves, which make difficulty in the isolation of genomic DNA (Haque *et al,* 2009). With keeping of these problems, DNA of *C. wightii* and *C. myrrha* was isolated from young leaves and young stems through different CTAB based methods. Intended for fast and easy removal of polysaccharides, polyphenols and other secondary metabolites and long term storage of genomic DNA at room temperature, the FTA PlantSaver card was also tried with several sample application methods.

MATERIALS AND METHODS

The samples of two different species of *Commiphora* were collected from Bhopal, Prof. TS Murthy Science Station, Obaidullaganj (Madhya Pradesh), Dr. Panjabrao Deshmukh Agricultural University, Akola (Maharashtra), Anand Agricultural University, Anand (Gujarat), and conserved at MPCST Human Herbal Health Care Garden, Bhopal. All the permission related to this work was taken from MP Council of Science and Technology, Bhopal, Madhya Pradesh and Research Degree Committee, Rani Durgavati University, Jabalpur, Madhya Pradesh.

The genomic DNA was isolated using the young leaves as well as young stem of *Commiphora* spp. through three various CTAB based methods i.e. M-1 method (CTAB method of Vijay *et al,* 2009), M-2 method (CTAB method of Haque *et al,* 2008) and M-3 method (modified method of M-2 method). Methods M-1 and M-2 were used for the isolation of genomic DNA from young leaves of *Commiphora* spp. (*C. wightii* and *C. myrrha*), while genomic DNA from young stem was isolated using the M-2 method and its modified version (M-3). In the method M-3, the entire step till drying the DNA pellet was followed according to standard method of M-2. After drying, the pellet was dissolved in 500 µl high salt TE buffer and an equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 13000 RPM for 10 minutes at room temperature. After centrifugation the aqueous phase was transferred to a fresh tube and 500 µl pre-chilled ethanol was added. The mixture was incubated for 30 minutes at -20ºC and centrifuged at 13000 RPM for 15 minutes at 4ºC. Then the supernatant was decanted carefully and the pellet was washed with 70% ethanol twice, after drying at room temperature pellet was dissolved in 50 µl TE buffer.

Samples were applied on to the Whatman FTA PlantSaver Card from 5 various sample application methods and the FTA card disc was purified for further uses (Whatman 2009; Sairkar *et al,* 2013a). In the first three sample application method, namely, directly press method, homogenized with PBS and homogenized with DNAse, RNAse free water was applied as per the instructions of the manufacturer (Whatman 2009). In the last two methods, the first precipitated DNA and finally precipitated DNA of the M-1 method was dissolved in DNAse, RNAse free water and applied directly on the FTA PlantSaver card. The card was allowed to air dry and stored at room temperature. 2.0

mm disc was washed as per the instructions provided by the manufacturer (Whatman 2009).

The yield of DNA was measured using a NanoDrop UV-Spectrophotometer (ND-1000) at 260 nm using TE buffer as blank. Good quality and quantity obtained DNA was rechecked through 1% agarose gel and amplification by RAPD primers RPI-05 (AM 773770) and RPI-10 (AM 750045) (Sairkar *et al,* 2012; Sairkar *et al,* 2013b).

RESULTS

The obtained DNA isolated from young leaves of *C. wightii* and *C. myrrha* using phenol free CTAB based method M-1 described by Vijay *et al,* (2009) was highly contaminated as well as very low in quality (0.84 to 1.02 ratio of A_{260}/A_{280}) and quantity (99.83 to 132.28 ng/µl) (Table 1). The presence of polysaccharides, polyphenols and other secondary metabolites in the leaves may be co-precipitated with DNA and might be the possible reason for the contamination of the isolated DNA. These unwanted compounds from the *C. wightii* DNA could be eliminated by using higher concentrations of CTAB, PVP and β-mercaptoethenol (Haque *et al,* 2008; Samantaray *et al,* 2009). CTAB binds with polysaccharides, while PVP forms insoluble complexes with lactones and phenolic compounds, whereas β-mercaptoethenol helps in the oxidation of phenolic compound (Kim *et al,* 1997). In the M-2 method, good quality (1.79 to 1.91 ratio of A_{260}/A_{280}) and quantity of DNA (1240.73 to 1670.34 ng/µl) was obtained because it had higher concentrations of CTAB, PVP and β-mercaptoethenol (Table 1). The isolated DNA showed good PCR amplification, therefore it can be further used in for molecular downstream applications (Figure 1).

Commiphora species are drought-tolerant plants and does not carry leaf in most seasons of the year (Gachathi 1997). In case of perennial plants, it is important to isolate the DNA at anytime rather than waiting for leaf emergence. Therefore an attempt has been made to isolate the DNA from young stem. In this regard, when the DNA was isolated through M-2 method (which produced suitable DNA from leaves) poor DNA quality (0.81 to 0.96 ratio of A_{260}/A_{280}), but in good quantity (1034.38 to 1452.75 ng/µl) from the young stem was obtained (Table 1).

Table 1: Quantitative and qualitative estimation of genomic DNA isolated from the leaves and youg stem using method M-1, M-2 and M-3.

SL	Sample ID	Method M-1		Methods M-2		Methods M-3	
		Quantity of DNA (ng/µl)	Ratio of A_{260}/A_{280}	Quantity of DNA (ng/µl)	Ratio of A_{260}/A_{280}	Quantity of DNA (ng/µl)	Ratio of A_{260}/A_{280}
1	CW1 (L)	118.21	0.92	1487.12	1.87	--	--
2	CW2 (L)	112.57	0.89	1240.73	1.79	--	--
3	CW3 (L)	99.83	1.02	1402.98	1.80	--	--
	Mean ± SD	**110.20±9.42**	**0.94±0.07**	**1376.94±125.24**	**1.82±0.04**	--	--
4	CM1 (L)	132.28	0.99	1670.34	1.84	--	--
5	CM2 (L)	127.71	0.84	1485.95	1.88	--	--
6	CM3 (L)	112.49	1.01	1491.74	1.91	--	--
	Mean ± SD	**124.16± 10.36**	**0.95±0.09**	**1549 ± 104.83**	**1.88 ± 0.04**	--	--
7	CW1 (YS)	--	--	1228.74	0.87	803.31	1.81
8	CW2 (YS)	--	--	1109.37	0.92	691.74	1.86
9	CW2 (YS)	--	--	1452.75	0.81	1135.92	1.84
	Mean ± SD	--	--	**1263.62 ± 174.33**	**0.87 ± 0.06**	**876.99 ± 231.08**	**1.84 ±0.03**
10	CM1 (YS)	--	--	1356.14	0.96	1098.57	1.89
11	CM2 (YS)	--	--	1270.78	0.84	890.12	1.88
12	CM3 (YS)	--	--	1034.38	0.93	743.69	1.82
	Mean ± SD	--	--	**1220.43 ± 166.68**	**0.91±0.06**	**994.345±147.40**	**1.86±0.04**

C. wightii leave = CW1 (1) to CW3 (L); *C. wightii* Young Stem CW1 (YS) to CW3 (YS);
C. myrrah. Leaves = CM1 (L) to CM3 (L); *C. myrrah.* Young Stem = CW1 (YS) to CW3 (YS)

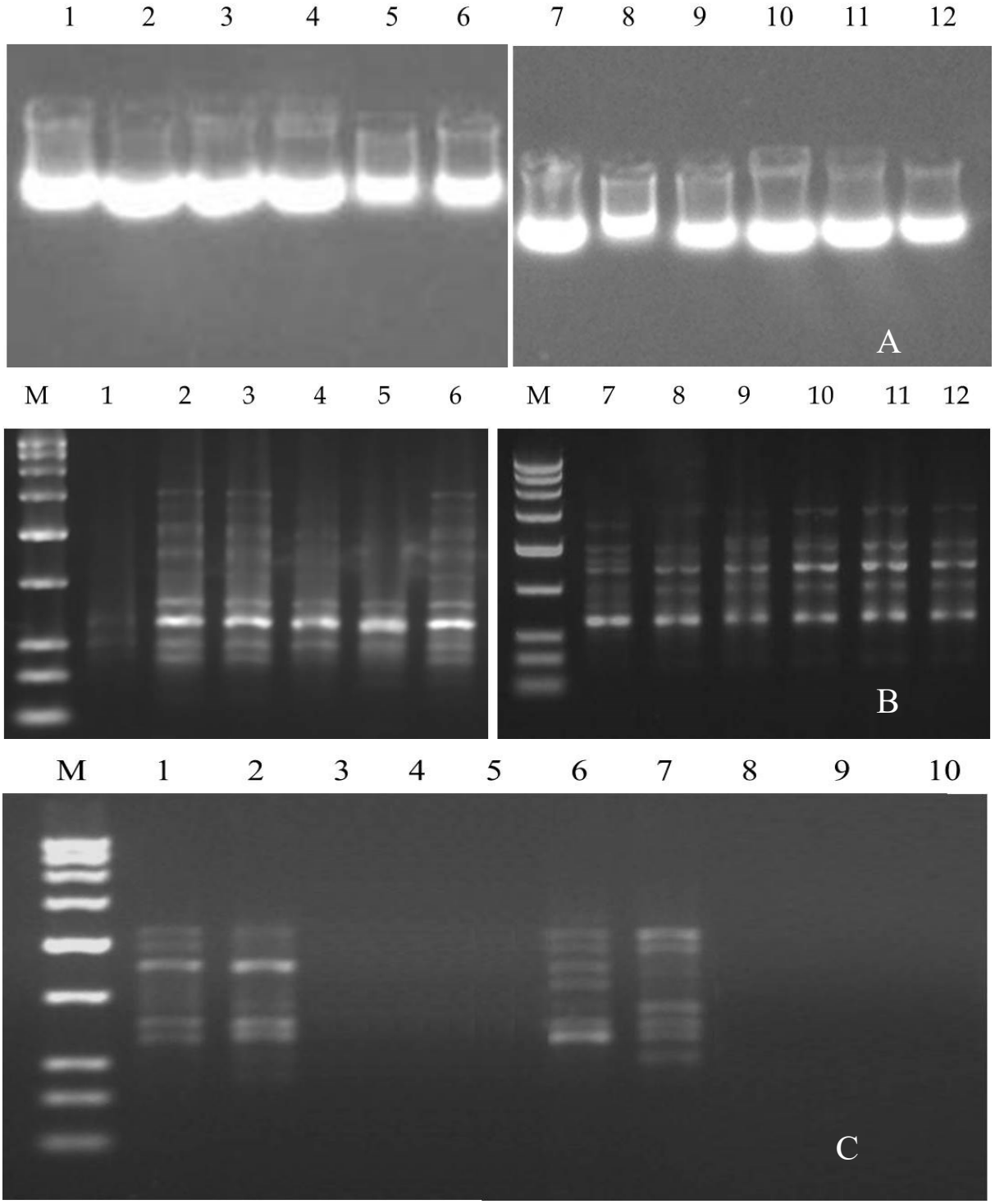

Figure 1: Isolation and Amplification of Genomic DNA of *C. wightti* and *C. myrrah.*

A. Genomic DNA isolation; Lane 1 to 3: DNA isolated from *C. wightti* leaves., **lane 4 to 6**: DNA isolated from *C. wightti* stem**, Lane 7 to 9**: DNA isolated from *C. myrrah* leaves, **lane 10 to 12**: DNA isolated from *C. myrrah* stem.

B. Isolated DNA from leaves and the stem amplified by RPI-05; Lane 1 to 3: Isolated DNA from *C. wightti* leaves, **lane 4 to 6:** Isolated DNA from *C. wightti* stem, **Lane 7 to 9:** Isolated DNA from *C. myrrah* leaves, **lane 10 to 12:** Isolated DNA from *C. myrrah* stem.

C. FTA card trapped genomic DNA amplification; Lane 1-5: amplified by RAPD primer RPI-5, **Lane 6-10:** amplified by RAPD primer RPI-10. **Lane 1 & 6**: laves was directly pressed on FTA card; **lane 2 & 7**: homogenized with PBS; **lane 3 & 8**: homogenized with DNase RNase free water; **lane 4 & 9**: finally recovered DNA of M-1 method was directly applied and **lane 5 & 10**: first precipitate of M-1 method was directly applied.

The poor quality DNA thus obtained might be due to the fact that the young stem had higher amount of polysaccharides and secondary metabolites than the leaves (Sahu *et al,* 2012) which can be overwhelmed through the treatment of higher concentration of NaCl. NaCl facilitates the removal of polysaccharides by increasing their solubility in ethanol so that they do not co-precipitate with the DNA (Fang *et al,* 1992; Choudhary *et al,* 2008). Thus to improve the DNA quality, certain modifications were created in the M-2 method. Due to this modification, the recovered DNA was good in quality (1.81 to 1.89 ratio of A_{260}/A_{280}) as well as in quantity (691.74 to 1135.92 ng/µl) (Table 1). Agarose gel electrophoresis of isolated DNA from leaves and stem using method M-2 and M-3 respectively, showed a prominent DNA band without any smearing and similarly and importantly these DNA served as best templates for good amplification with RAPD primers i.e. RPI-05 and RPI-10 (Figure 1).

The PCR amplification using DNA preserved in FTA PlantSaver card revealed that the following three sample application method i.e. direct press method where the leaves were homogenized with PBS and the leaves were homogenized with DNAse and RNAse free water, showed no amplification with RAPD primers (RPI-05 and RPI-10). This might be due to improper disruption of cell wall of plant or improper washing of the disc, because of these polysaccharides, polyphenols and other secondary metabolites retained by disc (Sairkar *et al,* 2013a). Many researchers suggested that the high concentrations of polysaccharides, polyphenols, proteins, and other secondary metabolites of plant leaves poses problem in polymerase chain reaction amplification (Koonjul *et al,* 1999; Haque *et al,* 2009; Sahu *et al,* 2012; Sairkar *et al,* 2013a).

When polysaccharide and secondary metabolites contaminated DNA precipitate from M-1 method was applied as sample on FTA PlantSaver card, a prominent PCR amplification was observed, which reveals that the major contaminants like polysaccharides, polyphenols and other secondary metabolites were washed off in M-1 method and remaining were removed during the application on FTA PlantSaver card (Figure 1).

CONCLUSION

DNA isolation of *Commiphora* species is a very tedious job for the researcher due to the presence of many secondary metabolites and unavailability of leaves all-round the year. In the present study, a suitable DNA isolation protocol was developed, in which good quality and quantity of DNA was successfully isolated from the young stem of the plant. We suggest that this DNA isolation method may also be used for other latex rich plant species. In addition to this conventional DNA isolation method, FTA PlantSaver card based DNA preservation and amplification method was developed.

Acknowledgment

The authors wish to thank Prof. P. Verma, Director General, MPCST and Dr. RK Singh, Resource Scientist and Group Head, Advance Research Instrumentation Facility, MPCST for the providing laboratory facilities.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

Anuradha HJ, Vijayan K, Nair CV, Manjula A (2013) A novel and efficient protocol for the isolation of genomic DNA from mulberry (*Morus* L.) Emir J Food Agric 25(2):124-131.

Bakhru HK (1998) Herbs that Heal Natural Remedies for Good Health. Orient Paperbacks. Division of Vision Book Pvt. Ltd. New Delhi.

Choudhary K, Mathur N, Choudhary OP, Pillai U (2008) Protocol for Isolation of Genomic DNA from Dry and Fresh Leaves of *Vigna* Species Suitable for RAPD and Restriction Digestion. Advan Biol Res 2(5-6):83-89.

Dabo SM, Mitchell ED, Melcher U (1993) A method for the isolation of nuclear DNA from cotton (*Gossypium*) leaves. Anal Biochem 210:34-38.

Deng R, Yang D, Radke A, Yang J, Yan B (2007) The hypolipidemic agent guggulsterone regulates the expression of human bile salte xport pump: Dominance of trans activation over farsenoid X receptor-mediated antagonism. J Pharmacol Exp Ther 320:1153-1162.

Fang G, Hammar S, Rebecca R (1992) A quick and inexpensive method for removing polysaccharides from plant genomic DNA. BioTechniques 13:52-56.

Gachathi FN (1997) Recent Advances on classification and status of the main gum-resin producing species in the family *Burseraceae*. In: Presented at the Regional Conference for Africa on Conservation, Management, and Utilization of Plant Gums and Resins, October 6 to 10.

Guthrie R, Susi A (1963) A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Am Acad Pediatrics 32:338–343.

Haque I, Bandopadhyay R, Mukhopadhyay K (2008) An optimized protocol for genomic DNA isolation from high secondary metabolites and gum containing plants. Asian J Plant Sci 7:304-308.

Haque I, Bandopadhyay R, Mukhopadhyay K (2009) Intraspecific Variation in *Commiphora wightii* Populations Based on Internal Transcribed Spacer (ITS1-5.8S-ITS2) Sequences of rDNA. Diversity 1:89-101.

Haque I, Bandopadhyay R, Mukhopadhyay K (2010) Population genetic structure of the endangered and endemic medicinal plant *Commiphora wightii*. Mol Biol Rep 37(2):847-854.

Hocking D (1993) Trees for Drylands. Oxford and IBH Publishing Co, New Delhi.

IUCN (2012) "IUCN Red List of Threatened species" Version 2012.2. http://www.iucnredlist.org. Gland, Switzerland.

Kim CS, Lee CH, Shin JS, Chung YS, Hyung JE (1997) A simple and rapid method of isolation of high quality genomic DNA from fruit trees and conifers using PVP. Nucleic Acid Res 27:66-78.

Kirtikar KR, Basu BD (1935) Indian Medicinal Plants Vol.1 Bishen Singh Mahendra Pal Singh, Dehra Dun.

Koonjul PK, Brandt WF, Farrant JM, Lindsey GG (1999) Inclusion of polyvinylpyrrolidone in the polymerase chain reaction reverses the inhibitory effects of polyphenolic contamination of RNA. Nucleic Acids Res 27(3):915–916.

Mouli KC, Rao SDV (2009) Phytoresources as potential therapeutic agents for cancer treatment and prevention. J Glob Pharm Tech 1:4-18.

Pandey RN, Adams RP, Flournoy LE (1996) Inhibition of random amplified polymorphic DNAs (RAPDs) by plant polysaccharides. Plant Mol Biol Rep 14:17-22.

Sahu SK, Thangaraj M, Kathiresan K (2012) DNA Extraction Protocol for Plants with High Levels of Secondary Metabolites and Polysaccharides without Using Liquid Nitrogen and Phenol. ISRN Mol Biol Article ID 205049:1-6.

Sairkar P, Chouhan S, Batav N, Sharma R (2013a) Optimization of DNA isolation process and enhancement of RAPD PCR for low quality genomic DNA of *Terminalia arjuna*. Journal of Genetic Engineering and Biotechnology 11(1):17–24.

Sairkar P, Vijay N, Batav N, Silawat N, Garg RK, Chouhan S, Sharma R, Mehrotra NN (2013b) Genetic Variability in Two Economically Important Medicinal Plants of Madhya Pradesh, India and *Withania Somnifera Rauwolfia Serpentina* Using RAPD Markers. Asian J Exp Biol Sci 4(1):36-43.

Sairkar P, Vijay N, Silawat N, Garg RK, Chouhan S, Batav N, Sharma R, Mehrotra NN (2012) Inter-species Association of *Ocimum* Genus as Revealed through Random Amplified Polymorphic DNA Fingerprinting. Sci Secure J Biotech 1(1):1-8.

Samantaray S, Hidayath KP, Maiti S (2009) An isolation protocol of genomic DNA from *Commiphora wightii* (Arnott.) Bhandari: An endangered medicinal plant. Int J Integ Biol 6(3):127.

Tsukaya H, Iokawa Y, Kondo M, Ohba H (2005) Large-scale general collection of wild-plant DNA in Mustang. Nepal J Plant Res 118:57–60.

Vijay N, Sairkar P, Silawat N, Garg RK, Mehrotra NN (2009) Genetic variability in *Asparagus racemosus* (Willd.) from Madhya Pradsh, India by random amplified polymorphic DNA. Afr J Biotech 8(14):3135-3140.

Whatman INC (2009) FTA™ nucleic acid collection, storage, and purification. http://www.whatman.com/ FTANucleicAcid CollectionStorageandPurification.

Xiao D, Singh SV (2008) Z-Guggulsterone, a constituent of Ayurvedic medicinal Plant *Commiphora mukul*, inhibits angiogenesis in vitro and *in vivo*. Mol Cancer Ther 7(1):171-180.