



INNSPUB

RESEARCH PAPER

Journal of Biodiversity and Environmental Sciences (JBES)

ISSN: 2220-6663 (Print) 2222-3045 (Online)

Vol. 12, No. 6, p. 67-78, 2018

<http://www.innspub.net>

OPEN ACCESS

## Efficient DNA extraction and purification protocol of medicinal plants which producing large amount of secondary metabolites

Farhad Hariri Akbari<sup>1\*</sup>, Mansoor Omid<sup>2</sup>, Sepide Torabi<sup>3</sup>, Seyed Mostafa Pirseyedi<sup>4</sup>, Sayyed Sajjad Moravveji<sup>5</sup>, Mehdi Shafiee Alavijeh<sup>6</sup>, Baharak Behjat Sasan<sup>7</sup>, Saeed Parvaneh<sup>8</sup>

<sup>1</sup>Department of Agriculture & Plant Breeding, Islamic Azad University, Research & Science Branch, Tehran, Iran

<sup>2</sup>Department of Agronomy and Plant Breeding, University of Tehran, Karaj, Iran

<sup>3</sup>Department of Agriculture & Plant Breeding, Islamic Azad University, Research & Science Branch, Tehran, Iran

<sup>4</sup>Department of Plant Sciences, North Dakota State University, United States of America

<sup>5</sup>Kish International Campus, University of Tehran, Tehran, Iran

<sup>6</sup>Department of Biotechnology, University of Zabol, Zabol, Iran

<sup>7</sup>Department of Horticultural, Islamic Azad University, Research and Science Branch, Tehran, Iran

<sup>8</sup>Department of Gardening, University of Zabol, Zabol, Iran

Article published on June 09, 2018

**Key words:** Medicinal plants, DNA extraction and purification method, Genetic diversity, AFLP markers, Transfer and gene over expression.

### Abstract

Using medicinal plants is very valuable and beneficial. So, it is essential that the genetic and other molecular properties must be determined for the purposes of economy, sanitary and health, as well as maintaining natural diversity and avoiding the disappearance of endangered species in natural habitats. Although, Medicinal plants in various tissues and different stages of growth have varying amounts of organic compounds that make difficulties in the extraction process of genome in some cases or even impossible. Therefore, providing a convenient, fast, practical and low-cost method that works in all laboratories and situations with available materials seems very important. In this research, the genomic DNA has been extracted from various tissues and organs of different medicinal plant families. In addition, this protocol was achieved by studying, performing and using different methods of genomic extraction method on various medicinal plants. Whether the sample has been contaminated with different metabolites, rapid and efficient purification methods are available. The quantity and quality of the extracted DNA were determined by electrophoresis of 1% the Agarose gel and spectrophotometer tool respectively. The results show that the genomic DNA extracted is relatively pure and desirable. The results of this study have been used directly in various genetic molecular researches such as assessment of the genetic diversity with the AFLP molecular marker, transfer and gene over expression; it must be noted that the results of this study in similar resources were not observed and done for the first time.

\*Corresponding Author: Farhad Hariri Akbari ✉ [hariri.a.farhad@gmail.com](mailto:hariri.a.farhad@gmail.com)

## Introduction

Using medicinal plants are very valuable as the major and minor products (Majnoun Hosseini and Davazdah Emami, 2007). In addition, there are a lot of advantages through direct cultivation and sale of the extracts from these plants (Kadkhoda *et al.*, 2011). This type of plant in different tissues and different stages of growth has variable and large amounts of organic compounds or secondary metabolites, which can be referred to as proteins, polysaccharides, colored pigments, alkaloids, tannins and phenolic compounds, terpenes, etc. (Sahu *et al.*, 2012). These compounds have medicinal and medical properties but recently due to the resistance of the pathogens to chemical agents and antibiotics, alternative sources are needed to meet the sanitary and health needs and also reduce the harmfulness of chemicals drugs (Pirttii, 2001; Hariri Akbari *et al.*, 2010; Kazemi and Seyed Hajizadeh, 2012). therefore, clear that the determination of genetic, molecular, biochemical, morphological, physiological and ecological characteristics of this group of plants for the purpose of sustainable economic exploitation, sanitary and health goals, as well as maintaining their diversity in natural areas, preserving and saving of endangered species from their natural habitat seems necessary (Karimi and Farokhi, 1998).

In the meantime, molecular topics are important in various biotechnology studies. With using biotechnology tools such as genomics, genetic engineering and gene library development, it is possible to maintain and protect billions of genotypes in low volume and low cost. It can be said that the extraction of nucleic acids is the first and most important step in molecular genetic studies (Ghareyazi, 1996; Farsi and Zolali, 2003; Kazemi and SeyedHajizadeh,2012). In many cases, the extraction of the genome has also had limitations because of organic compounds, conditions as well as the reagents used for that purpose usually are expensive or cause a high environmental impact (Kazemi and Seyed Hajizadeh, 2012). Although, several successful DNA extraction protocols for plant species that

contain polyphenolics and polysaccharides compound have been developed, none of these are universally applicable to all plants (Varma and Padh, 2007) and the published protocols are also limited (Kazemi and Seyed Hajizadeh, 2012; Sahu *et al.*, 2012). That it is necessary to provide a convenient, fast, practical and low-cost method that can be done in all laboratories with available materials (Shafiee *et al.*, 2009).

The search for efficient protocol for extracting genomic DNA that have both higher quality and yield has led to the development of several protocols for isolating and extracting DNA from plants containing high levels of secondary metabolites (Sahu *et al.*, 2012), in some cases, changing in the physical conditions and the materials of the experiment will be improved the quantity and quality of the extracted DNA (Vojdani, 1996; Naghavi *et al.*, 2005).

In this study, by studying and conducting various experiments on variety of medicinal plants such as *Anethum graveolense*, *Artemisia annua*, *Mentha arvensis*, *Ferula assa-foetida*, *Datura stramonium*, *Ducrosia anethifolia*, *Aloe vera* and Callus of *Taxus baccata* was achieved an easy, convenient, affordable and low cost way to extract the genome from medicinal plants. Most of these plants are native to Iran and parts of Afghanistan. In order to demonstrate the importance and applicability of this method in molecular studies, some genomic DNA that were extracted by following method as well as *Anethum graveolense*, *Ducrosia anethifolia*,

*Artemisia annua* which were used directly to determine the genetic diversity with using the AFLP molecular marker, genome of opium poppy was used for the effect of exacerbating the expression of the TYDC gene for increasing the production of important alkaloids (Koohezadi, 2009) and for Callus of *Taxus baccata* the method Just used for proof testing that this method could extract DNA from different tissues (Fig. 3) (Behjat Sasan *et al.*, 2014) and all of the genome samples which were extracted by this method used in molecular studies successfully.

## Material and methods

### *Plant cultivation and callous production*

Seeds of *Anethum graveolense* have been collected from natural habitats and placed in dish pads on wet paper and then transferred to plastic pots after germination. When the plant length reaches 10 to 15 centimeters and the leaves are well grown, it is ready to perform extraction (Fig. 1).

Seeds of *Ducrosia anethifolia* were grown in Murashige and Skoog medium (Mostafavi and Afzali, 2005; Shafiee *et al.*, 2012) with glucose in 2-2% and agar in 8 g/L, growth regulators including auxin and cytokinin at concentrations of 2 mg / L and 1 culture medium, in dark space and temperatures of 26 and 24 degrees Celsius were cultivated for the production of calluses (Fig. 2) (Ashtari, 2008; Kadkhoda *et al.*, 2011; Omidi *et al.*, 2012).

Taxus was occurred on 1/2 MS medium which had one-fourth nitrogen (KNO<sub>3</sub>, NH<sub>4</sub>NO<sub>3</sub>) supplemented with glutamine, 1mg/l 2,4-D and 1 mg/l Kin from stem (Fig. 3) (Behjat Sasan *et al.*, 2014) and the Callus was use for DNA extraction.

*Artemisia annua* (Lari Yazdi *et al.*, 2002; Baraldi *et al.*, 2008) and other plants were planted in the field and greenhouse for the production of seedlings and fresh leaves (Fig. 4) (Bovard *et al.*, 2016).

### *DNA extraction method*

Plant organs and calluses were put in a mortar which autoclaved and placed in a freezer at -20 ° C and with helping of liquid nitrogen, the specimens are completely ground (Naghavi and Ghareyazi, 2007) and transferred to tubes of 1.5 ml. into each of the tube, add 900 µl of the SDS solution 4% (0.4 g SDS in 10 cc of Tris solution, EDTA, NaCl) to the buffer solution and place it at 65 degrees Celsius for 45 minutes. It should be noted that the inverting procedure should be done alternately. Add 300 µl of potassium acetate to each tube and place it in a container of ice for 25 minutes. The tubes are centrifuged at 3 ° C for 15 minutes and 12000 rpm.

Remove 750 µl of the upper liquid and transfer to a new tube, then add 750 µL of isopropyl alcohol and place it at 25 ° C for 5 minutes. The tubes were again centrifuged at 3 ° C for 15 minutes and 12000 rpm. Discard the upper liquid and place the tubes at 37 ° C in the incubator for 20-30 minutes. Until the pellet (Fig. 5) is completely becoes dry.

### *Purification*

Add 700 ml of autoclaved DDW to each of the tubes, It is kept at 4 ° C for 1 hour. Then we slowly tap into the Pellet formed at the end of the tube to be completely dissolved after that the chloroform solution 24/1 (24: 1) is added to the volume of 700 microliters next, Shake gently for 20 seconds to obtain a uniform emulsion.

Centrifuge with 13000 rpm for 10 minutes, which consists of two phases: The lower phase is chloroplast isoamyl alcohol and the supernatant phase contains DNA. The supernatant phase is transferred to a new 2 ml tube And 0.1 volume of upper liquid, Sodium acetate (3 mol, pH = 8) was added. 2.5 times the upper liquid volume, absolute alcohol (100%) added and then waiting 5 minutes to dewater.

Centrifuge 13000 rmp for 10 minutes, Discard the upper liquid and pellet, 100 µl of alcohol was added to 70% . Removing the alcohol and place in the incubator for 20-30 minutes at 37 ° C to dry completely. Then add 100 µl of TE or distilled water (DDW).

### *Electrophoresis of Agarose gel*

Different methods such as ion exchange chromatography and electrophoresis can be used for separation of nucleic acids. The performance of all these methods is based on the difference in electric charge (Clark and Pazdernik, 2005). In this study, simple and fast electrophoresis method of 1% agarose gel was used.

Mix 5 µl of prepared DNA samples with 5 µl of dye, after that 9.5 µl of DNA and dye mixture were placed

in 0.1% agarose gel wells. Agarose gel for 35 minutes at a constant voltage of 90 V, was electrophoresis. after staining with ethidium bromide (for 30 minutes), DNA is observed and photographed under UV light on the Geldoc device. It is also possible to measure the DNA quantity by spectrophotometer (Fig. 6).

#### *Electrophoresis of polyacrylamide gel*

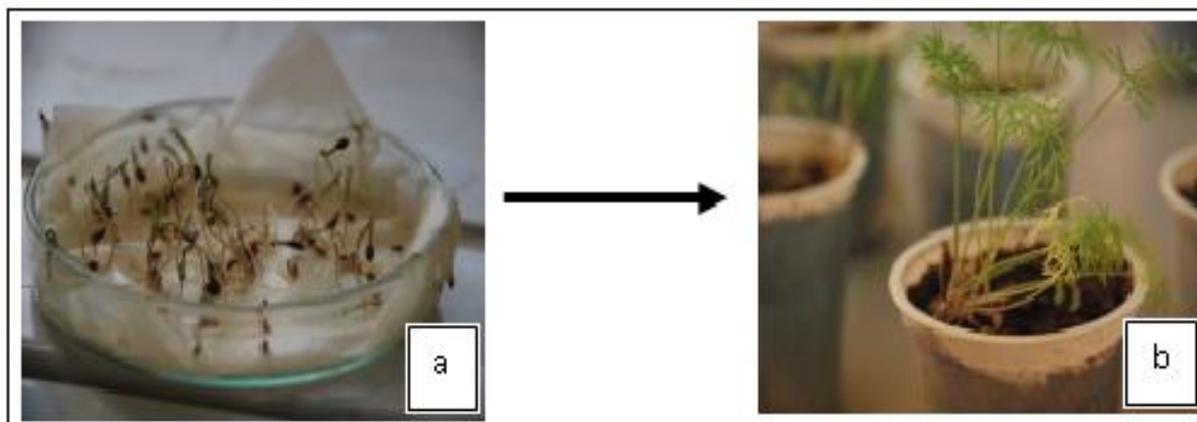
Molecular markers, in particular DNA based markers provide reliable genetic information because of the independence of the confounding effects of environmental factors (Powell *et al.*, 1996; Etminan *et al.*, 2012).

The AFLP procedure was performed with appropriate modifications of the method described by Vos *et al.*, (1995). In order to demonstrate the applicability, high quality and quantity of the genomic DNA which was extracted by this method, assessment of genetic diversity by AFLP molecular markers for *Anethum graveolense*, *Artemisia annua* and *Ducrosia anethifolia* (Fig. 8) has been done.

#### **Results and discussion**

##### *Results of extraction and purification process*

The DNA obtained was free of any contaminating proteins, polysaccharides and coloured pigments.



**Fig. 1.** A view of the cultivated plant samples of *Anethum graveolense*. The seeds were put on Wet paper. (a) and transferred to the plastic pot after germination(b) A view of the cultivated plant samples.

The study of gel electrophoresis shows high-weight and good resolution bands for all specimens, and also these bands prove that DNA does not have fracture and also are desirable quality for molecular experiments (Fig.6).

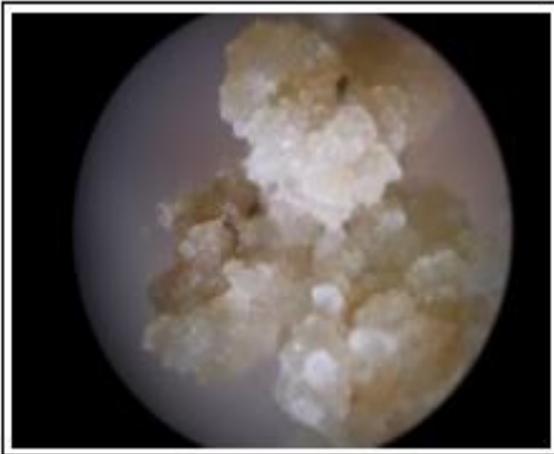
Genome quantitative determination was done by spectrophotometer and the purity Index with radiating two different wavelengths ranging from 260 to 280 nm shows 1.76 to 2.01 and prove that genomic DNA has a good quantity.

Considering that the extraction of DNA from medicinal plants due to the high level of phenolic, alkaloid, tannin, oily compounds, etc. is a problem and requires purification methods. However, using

non-differentiated tissues in this method, such as fresh leaves, young ones, as well as callus, allowed the removal of the purification step. consequently, DNA extraction can be made at a faster rate and cost less.

As shown in Fig. 6 of the electrophoresis gel, without applying RNase, Protease and purifying process with phenol and chloroform isoamyl alcohol, the resulting DNA has a relatively good quality.

In the case of contamination with metallolites, the purification step can be used to extract pure DNA, Of course, this should be noted that the purification steps lead to the fracture and compression of the DNA, therefore, carefulness in the work process is important so that there is no need for repetition.



**Fig. 2.** View of callus of *Ducrosia anethifolia* (Shafiee *et al.*, 2012).

The right side genomic DNA is much purer than the left one. It should be noted that this DNA is so pure and after purification step, it has been used directly in molecular tasks like AFLP marker for assessment of genetic diversity (Fig. 7 and Fig. 8).

*Using the extracted genome in molecular studies*

The isolated genomic DNA was found suitable for restricted enzyme and digestion process. In addition,



**Fig.3.** Callus of *Taxus baccata* was used for extracting genome by the method (Behjat Sasan *et al.*, 2014).

This DNA extraction protocol can also be used in molecular studies such as genomics and genetic engineering, some of which are PCR-based protocols. Genome required for using in molecular markers, determination of polymorphism and genetic

diversity should have high quality and quantity and the results of the polyacrylamide gel and the study of the genetic diversity with the AFLP molecular marker showed that the extracted genome has been suitable for molecular studies (Fig. 8). As well as The Genome of Opium poppy which was extracted by the method for the effect of exacerbating the expression of the TYDC gene for increasing the production of important alkaloids (Fig. 9). (Koozhadi, 2009; Koozhadi *et al.*, 2013).



**Fig. 4.** View of the greenhouse.

**Discussion**

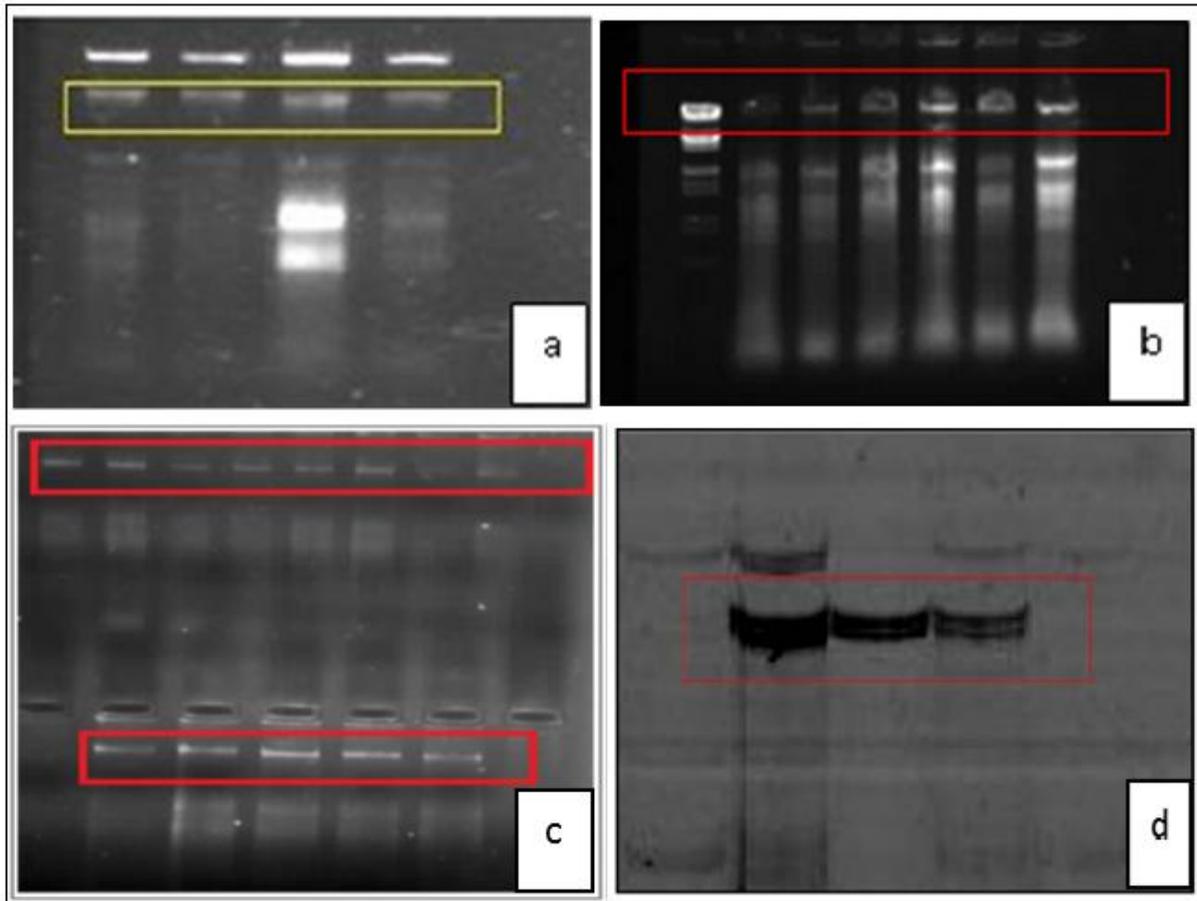
The extraction of the genomic DNA from any plant sample needs the basic requirement that is first to break the cell to release all cellular constituents like DNA, RNA, polysaccharides, carbohydrates, enzymes, etc.



**Fig. 5.** Shows that a very clear pellet and does not indicate any contamination.

The cell membrane can be ruptured by detergents like SDS or CTAB. EDTA is used as a chelator of most metal ions requires for cellular nucleases activity. Most proteins are removed by chloroform extraction while the polysaccharides are removed by salt (NaCl, KCl or NaAc) together with detergent (Murray and Thompson, 1980).

Some changes in the process of extraction and purification are required in terms of temperature, time, and amounts of materials needed, which can provide a suitable method for the extraction of genomic DNA in molecular research (Keb-Llanes, 2002).



**Fig. 6.** 1% agarose gel was used for extracting the genome of medicinal plant sampels: *Anethum graveolense*(a), *Ducrosia anethifolia*(b),*Artemisia annua*(c ) and*Taxus baccata* (d).

(a)Agarose gel electrophoresis containing DNA samples along with a DNA ladder Which represents the concentration of extracted DNA.

(b) Agarose gel electrophoresis of samples containing DNA from callus of *Ducrosia anethifolia*, which has transparent bands in all four replicates.

(c )DNA extracted from leaves of *Artemisia annua*.

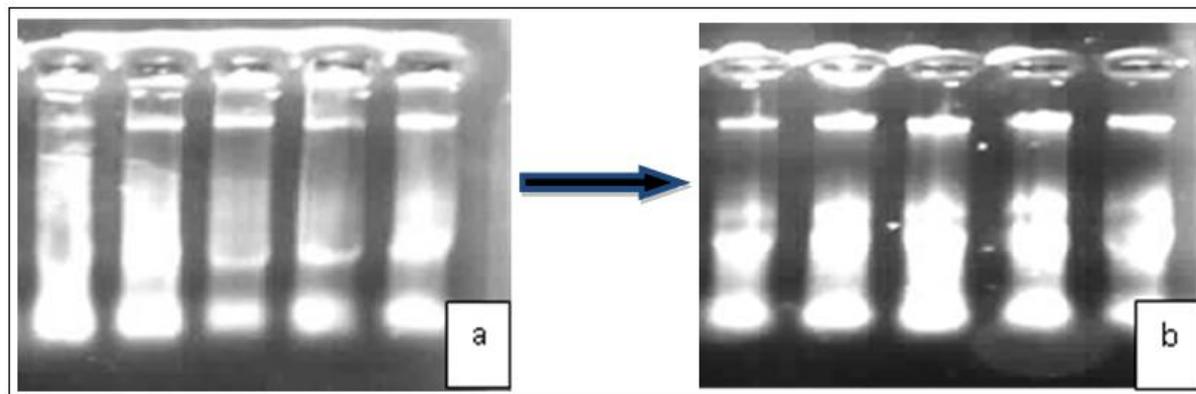
(d ) DNA extracted from callus of *Taxus baccata*.

The isolation of pure, intact, and high-quality DNA is very crucial for any molecular studies. However, DNA isolation from plants is usually compromised by excessive contamination by secondary metabolites. The DNA isolation methods need to be adjusted to

each plant species and even to each plant tissue because of the presence of these unlike metabolites (Lutz *et al.*, 2011). Some changes in the process of extraction and purification are required in terms of temperature, time, and amounts of materials needed,

which can provide a suitable method for the extraction of genomic DNA in molecular research (Keb-Llanes, 2002). The search for a more efficient method of extracting DNA of both higher quality and

yield has led to the development of several protocols for isolating DNA from plants containing high levels of secondary metabolites (Sahu *et al.*, 2012).



**Fig. 7.** Extracting Genomic DNA from the *Anethum graveolense*

(a) Shape left before purification.

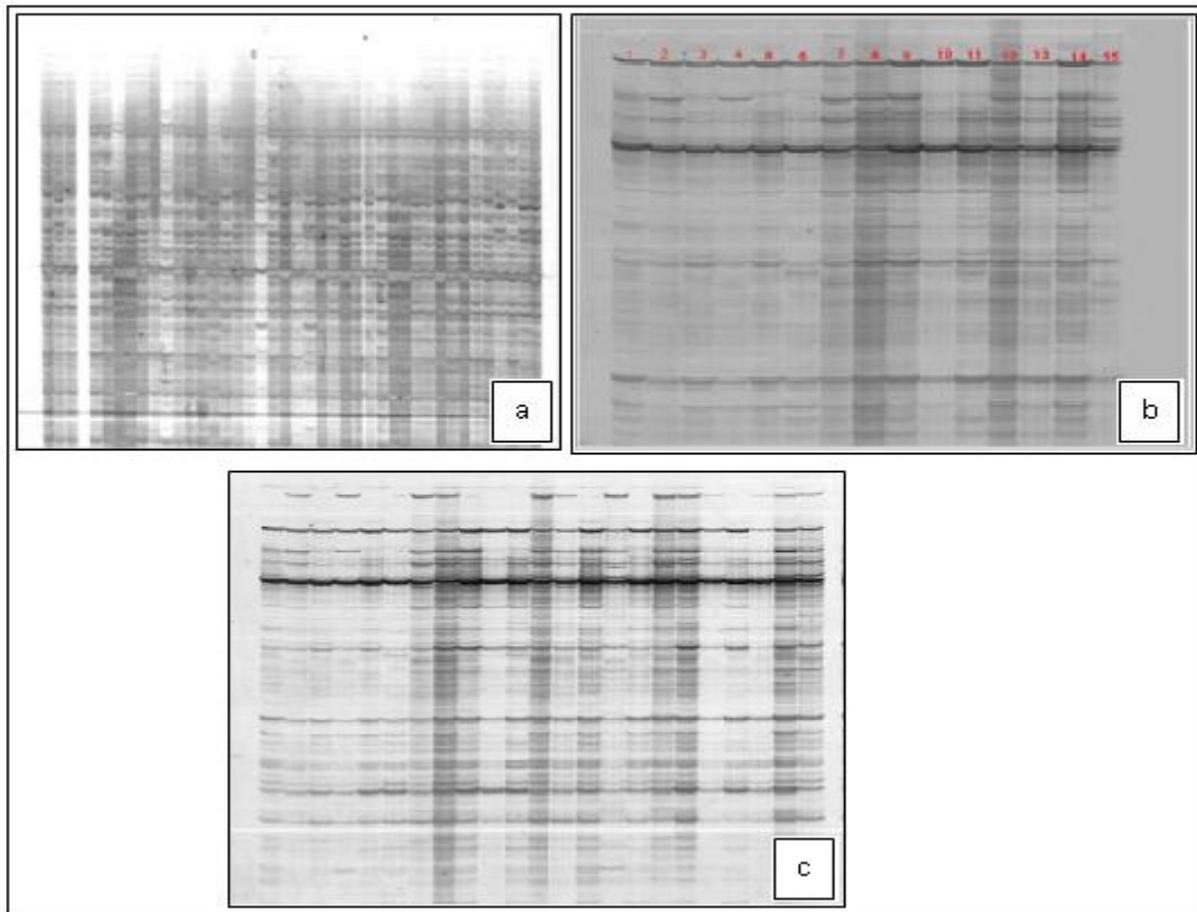
(b) Shape right after purification.

In this research, applying various methods such as CTAB, Dellaporta, Qiagen DNeasy Mini Plant Kit to extract DNA on medicinal plants, it was concluded that the DNA produced by this method has a much better quality and quantity for molecular experiments. The method is very simple, easy, functional and low cost compared with the use of genomic extraction kits that are common in genetic laboratories today, it significantly reduces the cost of the test. On the other hand, this method has fewer steps than CTAB methods, so in a short time it is possible to extract more samples with better quality and quantity (Kazemi and Seyed Hajizadeh, 2012).

By comparing the various methods of extracting DNA, if the extracted specimen from the genomic plant is not rich in metabolites, Using routine laboratory methods are efficient. But if the herb has a high level of metabolism, especially when exposed to environmental stress in this case, the use of common protocols is not efficient or it should take a lot of time to optimize it. But this method is applied in various medicinal plants which are very diverse in terms of treatment conditions or culture media and showed that good and acceptable genomic DNA can be

obtained at little cost and time. In this way, there's no need to use the Triton X-100, the polyvinylpyrrolidone (PVP), which is used in a CTAB buffer-based method.

According to Ribeiro and Lovato (2007), the extraction of the genome by using the Mini Plant Kit from young leaves yields very good DNA but if the plants is somewhat were affected by environmental stress or become elderly, the extracted genome greatly reduced in terms of quality and quantity or will be not efficient. Polymerase chain products (PCR products) prove this, because the polymerase enzyme (Taq DNA Polymerase) does not perform well in the presence of metabolites, metabolites inhibit the activity of the substances which were used in various stages of extraction, also, if the number of samples or the repetition of the test increases, good results are not achieved with plant DNA extraction kit. But after the extraction of the genome, if contamination with the metabolites will be observed, the purification steps that provided in this protocol could be performed. It is better to perform the purification step once; otherwise, the DNA may be squeezed or broken.



**Fig. 8.** Polymorphism investigation of *Anethum graveolense*, (a) (Hariri Akbari *et al.*, 2014), *Artemisia annua* (Bovard *et al.*, 2016 ) (b), and *Ducrosia anethifolia* (c) by using the amplified fragment length polymorphism (AFLP) technique with using the genome which was extracted by mentioned method.

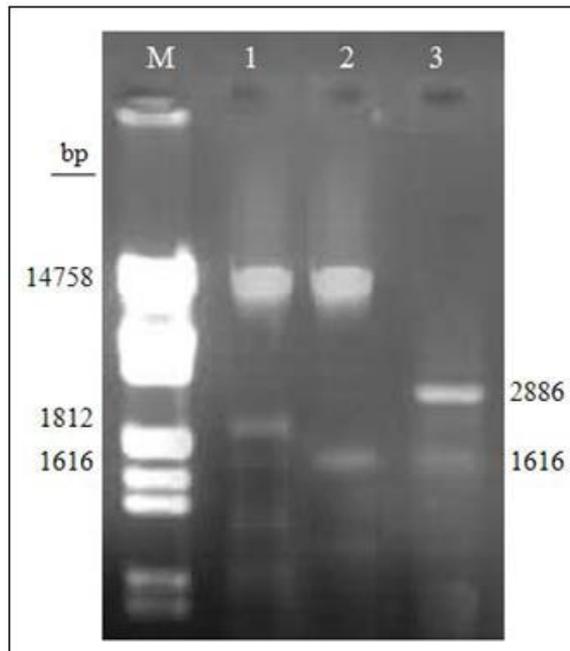
Detergent sodium dodecyl sulfate (SDS), which is used in the first steps, has the potential for uniforming the electrical charge of the proteins and destroying of the membrane of the cell, in contrast of other methods that has used simpler way but SDS could be tangible in laboratories.

According to Al-Saghir (2009), it should be noted that none of these methods are suitable for woody and rough texture such as rough stems and their seeds and to extract the genome from these tissues, there is a need for alternative methods. Because of crushing plant tissues at the sub- cellular levels and the collapse of cellular organelles are not possible with the simple methods available, in other hand not only the genome of fresh organs could be extracted by this method but also Somewhat old and rough organs too. In this genome extraction method, liquid nitrogen

due to reduced cost and the unavailability of advanced tools crushing plant tissue is used, in addition plays the role of brittle plant tissue and it is prevented from the rapid corruption of crushed texture. In this study, different tissues of the plant are used to extract the genome successfully like fresh leaves, green and Grass Shoot or even callus of *Ducrosia anethifolia* and *Taxus baccata*.

Maria *et al.*, (2001), by extracting DNA from medicinal plants with aromatic compounds using CTAB, managed to extract the desired quality genome, but the time and number of steps have diminished DNA quality and their research required the use of PVP and Mercaptoethanol. These materials should be used with special tools and Laminar flow, as well as adding lithium chloride (LiCl) in the CTAB buffer, it prevents DNA from being isolated from the

RNA However, in this method, we have removed the RNA at the last step by adding 2 Macro liters of RNase. In spite of, This method does not require RNAase, making it advantageous over common protocol.



**Fig. 9.** The genome of the *Opium Poppy* was extracted and used for overexpressing to increase its medicinal alkaloids contents.

Various genomic DNA isolation methods like Dellaporta *et al.*, (1983), CTAB (Murray and Thompson, 1980), and methods were tried and Khanuja *et al.*, (1999) method is the well suited to most of the medicinal plants. Further improvement of Khanuja *et al.*, (1999) method was developed by modification of few steps in the genomic DNA isolation protocol to get the good quantity and quality of the genomic DNA which is used for further downstream processes like Polymerase Chain Reaction etc.

The age of the leaf also affects the quality of extracted DNA (Moreira and Oliveira, 2011).

The young leaves yield a good quality and quantity of DNA with fewer impurities (secondary metabolites etc.). It was found that the young, frozen leaves yield good quality of DNA. (Shafiee *et al.*, 2009).

## Conclusion

Using the method that presented in this study, with such a wide variety of medicinal plants and their various tissues including herbs, fresh leaves, old tissues and even calluses can extract the genome and use it in various molecular experiments, has not been observed. The results of this study, has been used in molecular research, including determining genetic diversity with molecular markers AFLP, transfer and gene overexpression. This research, due to the easy method and the use of available materials, can be a good alternative to kits-based methods.

## Acknowledgements

My Appreciation goes to colleagues of the Department of Biotechnology from Iranian Institute of Medicinal Plants (IMP), Mahdi Faghihi (PhD), Masoud Zohrabi (PhD), Mahdi Yousofzadeh (PhD), Parisa Alizadeh (PhD), Ali Sahebifard (MSc), Hosein Rafieian (MSc), Hossein Rofoogar (MSc), Sara Eshmoel (MA), Samane Rastegar (MSc), Neda Sadat (MSc) and Mahdiah Shahedi (MA) to assist in preparation of this article.

## References

- Al-Saghir MG.** 2009. Rapid and efficient method of genomic DNA extraction from aromatic plants. *Plant Molecular Biology Reporter* **19**, 273a–f.  
<http://dx.doi.org/10.3923/rjb.2009.70.73>
- Ashtari R.** 2008. Effect of hormones and explants on tissue culture of *Ducrosia anethifolia* plant. Master Thesis. Faculty of Agriculture, Zabol University.
- Baraldi R, Isacchi B, Predieri S, Marconi G, Vincieri FF, Bilia AR.** 2008. Distribution of artemisinin bioactive flavonoids from *Artemisia annua* L. during plant growth. *Biochemical Systematics and Ecology Journal* **36**, 340–348.
- BehjatSasan B, Omid M, Naghavi MR, Hariri Akbari F, KalateJari S, Shafiee M, Shafiee M.** 2014. Effect of explants, salts concentration medium and hormone treatments on *Taxus baccata* in vitro

culture. *International Journal of Biosciences (IJB)* **5** (6), 1-9.

ISSN: 2220-6655 (Print) 2222-5234(Online)

<http://www.innspub.net>

**Bovard R, Naghavi MR, Omidi M, Torabi S, Rezazadeh SH, Hariri Akbari F, Shafiee M, Behjat Sasan B, Parvaneh S, Safakish Kashani MB.** 2016. The study of genetic diversity in different plant populations of *Artemisia Annua* L. native to northern Iran by molecular marker AFLP. *Journal of Biodiversity and Environmental Sciences (JBES)* **9** (2), 51-59.

ISSN: 2220-6663 (Print) 2222-3045 (Online)

<http://www.innspub.net>

**Clark D, Pazdernik N.** 2005. *Molecular biology*. Elsevier Inc. All rights reserved

**Dellaporta SL, Wood J, Hicks JB.** 1983. A plant DNA mini-preparation: Version II. *Journal of Plant Molecular Biology Reporter* **1**(4), 19-21.

<http://dx.doi.org/10.1007/BF02712670>

**Etminan A, Omidi M, Majidi Hervean E, Naghavi MR, Reza zadeh SH, Pirseyedi M.** 2012. The study of genetic diversity in some Iranian accessions of *Hyoscyamus* sp. using amplified fragment length polymorphism (AFLP) and retrotransposon/ AFLP markers. *African Journal of Biotechnology* **11**(43), 10070-10078.

<http://dx.doi.org/10.5897/AJB11.2106>

**Farsi M, Zolali J.** 2003. *Principles of Plant Biotechnology*. Ferdowsi University Press, Mashhad 495. (translated).

**Ghareyazi B.** 1996. Application of DNA marker in plant breeding. 14th Congress of Agromomy and Plant Breeding in Isfahan. Isfahan, Iran.

**Hariri Akbari F, Omidi M, Oladzad A, Ghaderi A, Safakishkashani MB.** 2010. Biotechnology and secondary metabolism engineering strategies to make

resistance for drought, salt, height in plants. 2<sup>nd</sup> National Congress on Water Crisis in Agriculture and Natural Resources, Islamic Azad University, Shahre-Rey Branch. Iran.

**Hariri Akbari F, Omidi M, Torabi S, Khoshkharam M, Bovard R, Shafiee M, Safakish Kashani MB, Saeed P, Behjat Sasan B.** 2014. Assessment of genetic diversity of Iranian population of *Anethum graveolens* L. (dill) using AFLP molecular markers. *Journal of Biodiversity and Environmental Sciences (JBES)* **4**(6), 141-152. ISSN: 2220-6663 (Print) 2222-3045 (Online)

<http://www.innspub.net>

**Kadkhoda Z, Sedaghat S, RezazadehSh, Naghdibadi H, TaghizadFarid R, Hariri Akbari.** 2011. Determination amount of different alkaloids *Papaver bracteatum* Lindl. collected from different regions of Iran by high-performance liquid chromatography (HPLC) method. *Journal of Medicinal Plants* **2**(1). [www.jhd.iaushk.ac.ir](http://www.jhd.iaushk.ac.ir)

**Karimi M, Farokhi H.** 1998. Investigating Iranian medicinal plants in different areas and determining medicinal priority types.

**Kazemi M, Seyed Hajizadeh H.** 2012. An efficient protocol for genomic DNA extraction from *Alcearosea*. *Journal of Agricultural Technology* **8**(1), 319-326. ISSN 1686-9141

<http://www.ijat-aatsea.com>

**Keb-Llanes M.** 2002. Plant DNA Extraction Protocol. *Plant Molecular Biology Reporter* 20: Pistachio Trees (*Pistacia vera* L.), Academic Journal Inc, ISSN 1816-4919.

**Khanuja SPS, Shasany AK, Darokar MP, Kumar S.** 1999. Rapid isolation of DNA from dry and fresh samples of plants producing large amounts of secondary metabolites and essential oils. *Journal of Plant Molecular Biology Reporter* **17**(1), 1-7.

<http://dx.doi.org/10.1023/A:1007528101452>

- Koohzadi F.** 2009. The effect of exacerbating the expression of TYDC gene to increase the production of Papaver somniferum alkaloids. Master Thesis. Zabol University, Faculty of Agriculture.
- Koohzadi F, Omidi M, Solouki K, Mahdinejad N, Alizadeh H.** 2013. Overexpression of TYDC2 in medicinal plant Opium Poppy to increase its medicinal alkaloids contents. *Modern Genetics Journal (MGJ)*. **4(31)**, 343-352.
- Lari Yazdi H, Khavarinejad R, Roustaian A.** 2002. Quantitative analysis of Artemisinin antimalarial in *Artemisia annua* extract of northern Iran. *Journal of Medicinal Plants* **1**, 41-37.
- Lutz KA, Wang W, Zdepski A, Michael TP.** 2011. Isolation and analysis of high quality nuclear DNA with reduced organellar DNA for plant genome sequencing and resequencing. *BMC Biotechnology Journal* **11(1)**, 54.  
PMID: 21599914  
PMCID: PMC3131251  
<http://dx.doi.org/10.1186/1472-6750-11-54>.
- Majnoun Hosseini N, Davazdah Emami S.** 2007. Agronomy and production of some herbs and spices. Isfahan University of Technology Publication Center.
- Moreira PA, Oliveira DA.** 2011. Leaf age affects the quality of DNA extracted from *Dimorphandra mollis* (Fabaceae), a tropical tree species from the Cerrado region of Brazil. *Genetics and Molecular Research Journal* **10(1)**, 353-358.  
<http://dx.doi.org/10.4238/vol10-1gmr1030>.  
<https://www.ncbi.nlm.nih.gov/pubmed/21365551>
- Mostafavi A, Afzali D.** 2005. Chemical compounds of extract of *Ducrosia anethifolia* (DC.) Boiss. Faculty of Pharmacy, Shahid Bahonar University of Kerman.
- Murray MG, Thompson WF.** 1980. Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Research Journal* **8(19)**, 4321-4325.  
PMCID: PMC324241 PMID: 7433111  
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC324241/>
- Naghavi M, Ghareyazi B, Hosseini Salkadeh GH.** 2005. *Molecular Markers*. University of Tehran Press.
- Naghavi M, Ghareyazi B.** 2007. *Molecular markers*. University of Tehran Press.
- Omidi M, ShafieeAlavijeh M, Solooki M, Oladzaad A.** 2012. Root culture of medicinal plant *Ducrosia anethifolia*. 12th Iranian Genetic Congress 21-23.  
[https://www.civilica.com/Paper-CIGS12-CIGS12\\_1057.html](https://www.civilica.com/Paper-CIGS12-CIGS12_1057.html)
- Pirttilä AM, Hirsikorpi M, Kämäräinen T, Jaakola L, Marja A, Hohtola A.** 2001. DNA Isolation methods for medicinal and aromatic plants. *Journal of Plant Molecular Biology Reporter* **19(3)**, 273-273f.
- Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S, Rafalski A.** 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular Breeding Journal* **2(3)**, 225-238.  
<http://dx.doi.org/10.1007/BF00564200>
- Ribeiro RA, Lovato MB.** 2007. Comparative analysis of different DNA extraction protocols in fresh and herbarium specimens of the genus *Dalbergia*. *Journal of Genetics and Molecular Research* **6(1)**, 173-187.  
PMID: 17469067
- 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. *International*

Scholarly Research Network ISRN Molecular Biology  
**2012** (2012), Article ID 205049, 6 pages

PMID: 27335662

PMCID: [PMC4890884](https://pubmed.ncbi.nlm.nih.gov/PMC4890884/)

<http://dx.doi.org/10.5402/2012/205049>

**ShafieeAlavijeh M, Omid M, Solooki M, Oladza A.** 2012. The embryogenesis of the *Ducrosia anethifolia* plant through cultivation of cell suspension. 12th Iranian Genetic Congress.

**Shafiee M, Omid M, Oladza A, Etminan A, Hariri Akbari F.** 2009. Presentation a quick method for extracting DNA from callus of *Ducrosia anethifolia*. 2009. 6<sup>th</sup> Iranian Horticultural Science Congress. Rasht, Guilan University.

[https://www.civilica.com/Paper-BAGHBANIO6-BAGHBANIO6\\_251.html](https://www.civilica.com/Paper-BAGHBANIO6-BAGHBANIO6_251.html)

**Shafiee M.** 2011. Endangered plant tissue culture and suspension *Ducrosia anethifolia*. Master Thesis, Faculty of Agriculture, Department of Biotechnology, University of Zabol.

**Solouki M, Riginejad N.** 2006. Plant Biotechnology 113-137.

**Varma AH, Padh N.** 2007. Plant genomic DNA isolation: an art or a science. *Biotechnology Journal* **2**, 386-392.

**Vojdani P.** 1996. The importance of conservation methods in natural habitat and its role in conservation and exploitation of plant reserves. Key articles collections of the fourth congress of agronomy and plant breeding in Iran, Isfahan University of Technology. Isfahan. Iran.

**Vos P, Hogers R, Bleeker M, Rijans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M.** 1995. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research Journal* **23(21)**, 4407-4414.

<http://dx.doi.org/10.1093/nar/23.21.4407>

**Yazdi D, Shahnazi S, Seyfi H.** 2004. Planting and harvesting medicinal plants. Jahad University Publications.