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Developing a rapid and economical DNA extraction protocol for an important medicinal plant *Artemisia judaica* without using liquid nitrogen.

Zahid Khorshid Abbas[1], Mohammad Nasir Khan[1]
[1]Department of Biology, Faculty of Science, University of Tabuk, Kingdom of Saudi Arabia.

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Abstract:

Aims: *Artemisia judaica in Saudi Arabia are exposed to serious threats due to heavy human impacts such as uncontrolled tourism, overgrazing and uncontrolled collection, mining, quarrying and natural drought. These activities warrant careful conservation strategies that would allow inclusion of these plants into development plans of the Saudi Arabia economy in order to ensure their sustainable use. The evaluation of the genetic diversity by using ISSR and RAPD PCR requires a high quality of intact DNA. Therefore the aim of this study was to establish a rapid DNA isolation protocol for Artemisia species without using Liquid Nitrogen.*

Results: *In case of Artemisia species, the DNA protocol was optimized by using solution based method without using Liquid Nitrogen .The quality of DNA was checked on 0.7% agarose gel containing ethidium bromide at a final concentration of 0.5 mg/ml . This provides a very rapid and sensitive means of estimating the nucleic acid concentration. Similarly, the quantity and purity of DNA extracted from Artemisia* species *analyzed by using NanoDrop1000. The λ260/λ280 determined for* Artemisia DNA samples were in a *range of 1.83-2.0 and concentration (ug/ml) (502.7 to 1288.5 ng/µl) indicating a good quality DNA from our method. The protocol was simple, reliable and operative in normal laboratory condition in two hours.*

Conclusion: *The developed protocol proved to be simple, reliable and operative in normal laboratory condition yielding intact DNA from Artemisia species having good quantity (170 to 1200 ng/µl) and quality ($A_{260/280}$ ratio - 1.64 to 1.95) completed in two 2 to 3 hours .*

Key Words: *Artemisia judaica* , Liquid Nitrogen, DNA

Corresponding author: **Dr Zahid Khorshid Abbas,**
Department of Biology, Faculty of Science, University of Tabuk, Kingdom of Saudi Arabia. Email: zahidkhorshid@gmail.com

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INTRODUCTION:

Artemisia judaica L. (Compositae) is a medicinal and aromatic plant growing in the valley bottoms of desert areas and are shrubby herbs growing wildly and distributed in the desert regions of Saudi Arabia including Tabuk region . *Artemisia* species *(family Asteraceae)* is a source of valuable phytochemicals ,essential oils that are used in the treatment of different diseases. (1-2) *Artemisia* species *(family Asteraceae)* are used as a folk medicines in Arabic region for the treatment of inflammatory-related diseases, for instance fungal infections, diabetes, atherosclerosis, cancer and arthritis .Different *Artemisia* species are used for different diseases . *Artemisia annua* is used for the production of antimalarial and possible antibacterial agents as well as serving as a natural pesticide. Artemisinin extracted from the leaves and flowers act as an antimalarial agent against *Plasmodium falciparum* and *Plasmodium vivax including* the multidrug resistant strains. (3-4) *Artemisia tourenefortiana* produces aromatic oil that is used against intestinal worms. Leaves and flowers extract of *Artemisia gmelinii* are used as medicine for headache, cold, cough and abdominal upsets. As this extract contain flavonoids, scopoletin, monoterpenes and sesquiterpene, they are also used in the treatment of hepatitis. *Artemisia sieversiana leaves* contain secondary metabolites like sieversinin and siersin which have antimicrobial properties.

Artemisia dracunculus is commonly known as tarragon and its leaves and flowers extracts are used against toothache and urinary problems, while the roots are useful in treating pharyngitis and lung diseases. (5-6) *Artemisia judaica* L. is used for the treatment of stomach ache, heart diseases, sexual weakness, diabetes, gastrointestinal disorders ,external wounding. Inflammatory-related diseases, for instance fungal infections, diabetes, atherosclerosis, cancer and arthritis. (7-8)

Artemisia species in Saudi Arabia are exposed to serious threats due to heavy human impacts such as uncontrolled tourism, overgrazing and uncontrolled collection, mining, quarrying and natural drought. These activities warrant careful conservation strategies that would allow inclusion of these plants into development plans of the Saudi Arabia economy in order to ensure their sustainable use. (9-10) The sustainable conservation of the threatened *Artemisia* species requires evaluating the genetic diversity by ISSR and RAPD of different populations in different habitats to elucidate the genetic differences between related species and populations of each species. (11-12) Numerous methodologies have been developed for high-throughput and cost-effective extraction of DNA from plant tissues. These include rapid DNA extraction protocols specifically developed for plants, as well as methods applicable to both plant and animal tissues. (13) Intact high molecular weight plant DNA is essential for molecular studies and genomic DNA library construction. *Therefore, the aim of this study was to optimize a rapid DNA isolation protocol for Artemisia species without using Liquid Nitrogen.*

MATERIAL METHODS:

Sample collection

Samples of mature flowering plants were collected from 5 different locations in Saudi Arabia including Tabuk. *Artemisia judaica* L. family *Asteraceae* is an annual, perennial, or shrub; leaves alternate strongly aromatic, densely grayish, tomentose low shrub, 30–80 cm; stems many branched from the base. *Artemisia judaica* L. specimens were collected from different locations in Saudi Arabia including Tabuk-Jordan road (760 m above sea level) .

Each population of *Artemisia judaica* L. consist of about 2 to 3 plants with different age groups. The interval between samples was 2 to 5 m, the pair wise distance between populations was 0.5 to 20 Km, whereas, the pair wise distance between desert divisions was 10 to 200 km. Morphological feature of each plant sample were also documented. About 5 g of young leaves from each representative plant samples were obtained and placed in a ziplock plastic bag containing silica gel which speeded up the drying. The samples were stored at -80°C until use.

PHYSIOLOGICAL PARAMETERS:

The fresh weight the plants were uprooted and washed to remove surface adhered soil particles and wrapped in blotting papers. Dry weight of plants was recorded after drying the plants at 80 °C for 24 h in hot air oven.

Analysis of minerals: The nitrogen and phosphorus Contents:

Nitrogen is actually considered the most important component for supporting plant growth. These starches and sugars are the plant's food. Photosynthesis means “making things with light”. The primary nutrients are needed for plan growth are nitrogen (N), phosphorus (P), and potassium (K).The nitrogen and phosphorus contents were determined using the method of Lindner (1944) (13) and Fiske & Subba-Row (1925) respectively. (14) Potassium was determined with a flame photometer (AIMIL).

Estimation of carbonic anhydrase activity:

Carbonic anhydrases (CAs) are zinc metalloenzymes that catalyze the interconversion of CO_2 and HCO_3^- and are ubiquitous in nature. All carbonic anhydrase CAs are zinc metalloenzymes that catalyze the interconversion of CO_2 and HCO_3^-. The enzymes are ubiquitous in nature and are an example of convergent evolution, as multiple, structurally and sequentially distinct families of CA have been discovered .Activity of carbonic anhydrase (E.C. 4.2.1.1) was measured using the method as described by Dwivedi and Randhawa (1974). (15)The enzyme was expressed as μM CO_2 kg^{-1} leaf FW s^{-1}.

Chlorophyll contents:

Total chlorophyll contents in leaves were estimated using the method of Lichtenthaler and Buschmann (2001). (16) Leaf relative water content (LRWC) was measured by adopting the method of Yamasaki and Dillenburg (1999) (17) using following formula: LRWC (%) = [(FM - DM)/(TM - DM)] × 100 .Estimation of leaf protein content: Leaf protein content was determined according to Bradford (1976) (18) using BSA as a standard.

DNA extraction from *Artemisia judaica* L.

Many DNA extraction methods which are widely used in plant molecular biology, but most of the protocols are time consuming, comparatively expensive and requires liquid nitrogen for grinding Many researchers suggested using the fresh tissue, but it has some limitations such as the glass house or field required for plantation as well as liquid nitrogen is essential for collection and storage. Continuous liquid nitrogen supply is a problem in many developing countries because purchasing time is unpredictable from overseas. We tried to develop a simple and rapid method to isolate DNA under normal laboratory condition (room temperature) from small amount of tissue for large number.

Reagents and chemicals

These included 10% CTAB (w/v), 1 M Tris-HCl (pH 8.0), 0.5 MEDTA (pH 8.0), 4 M NaCl, 3 M sodium acetate, isopropanol, ethanol (70, 100% AR grade), chloroform-isoamyl alcohol (24:1,v/v), β-mercaptoethanol and ribonuclease A (10 mg/ml). The various solutions used are depicted are depicted in table 1 ,2 and 3. Extraction buffer, CTAB DNA solution, chloroform Iso-amylalcohol Phenol (CIP solution) .

Table 1: EXTRACTION BUFFER 100ML

Tris HCL [pH 8],	1M	20ML
EDTA	0.5M	5ML
NaCl	3.5M	5.7ML
SDS	5%	10ML
ddH2O		59.3ml
EXTRACTION BUFFER		100ml

Table 2: 2 X CTAB DNA solution

		100ML
Tris HCL [pH 8],	1M	10ML
EDTA	0.5M	4ML
NaCl	3.5M	40ML
CTAB	2%	2g
B-mercaptoethanol	2 µL/ml	200ul
ddH2O		46ml
2 X CTAB DNA solution		**100ML**

Table 3: chloroform Iso-amylalcohol Phenol (CIP solution)

	100ML
chloroform	91.2ML
Iso-amylalcohol	3.8ML
Phenol	5ML
100ML	100ML

Protocol for DNA extraction from Artemisia species:

a) Artemisia plant leaves were dried for several hours .One gram of Artemisia leaves were weighed and then grounded by mortar and pestle till fine powder was made. During the grinding process, extraction buffer (600 µL) was added followed by incubation at -80 ^{0}C for 5 minutes.
b) The fine powder was poured into 2ml MCT tube and 600 µL of 2 X CTAB solutions was added .Then 400 µL chloroform: isoamyl alcohol: phenol (24:1:5%) mixture was added. The mixture was mixed well by vortexing. Centrifugation was done at 10,000 RPM at room temperature for 10min .
c) The supernatant was transferred into new tubes and two third volume of isopropanol was added and mixed gently by inverting the MCT tubes. The tubes were incubated at room temperature for 10 to 15 min .Then tubes were centrifuged at 10,000 RPM at room temperature for 5 mints.
d) The supernatant was discarded gently and pellet was seen which contained DNA.
e) This DNA pellet was washed with 70% ethanol, air-dried .
f) Finally the DNA was resuspended into 50 µL TE buffer.
g) The quality and quantity of extracted DNA was measured by NanoDrop ND-1000 spectrophotometer

Quality analysis of plant genomic DNA extracted:
The DNA extracted was dissolved in 50 µL TE buffer and stored at -20°C until use. The DNA quality and yield was assessed using Nanodrop (optical density) and 1% agarose gel electrophoresis.

Statistical analysis
Statistical analysis of data obtained from research was done using five replicates of collected plants for each season. The data were analyzed statistically using SPSS-16 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS:
Several morphological, physiological parameters were studied as depicted in the table 4. The *Artemisia* plants were collected from different locations and were screen for several physiological and biochemical attributes.

Table 4.Morphological, physiological parameters of *Artemisia judaica*

Locations	Fresh weight (g)	Dry weight (g)	Total Chl (mg/g FW)	CA activity (µmol CO_2 kg $^{-1}$ leaf FW s^{-1})	Leaf relative water content (%)	Leaf protein content (mg g^{-1} leaf FW)
Location 1	40.38	10.37	2.87	386.52	44.81	4.39
Location 2	37.25	8.78	2.43	388.83	39.72	3.86
Location 3	35.49	7.65	1.98	369.71	42.54	4.51
Location 4	42.58	12.84	2.61	376.54	47.38	4.79
LSD at 5%	**2.87**	**2.49**	**0.16**	**6.91**	**1.68**	0.52

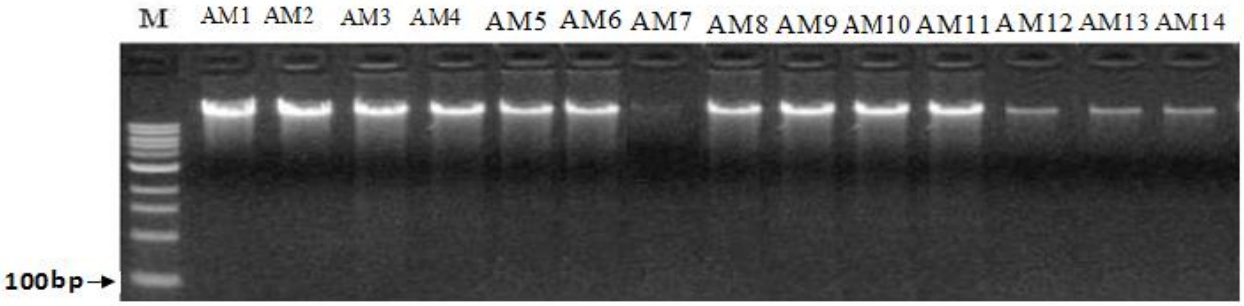
Artemisia judaica L" is a species is widely distributed in Tabuk. We therefore used the *Artemisia judaica* L" as an experimental material in this study to optimize a protocol for rapid DNA isolation. We did not use liquid nitrogen that is used for the storage and grinding of the plant material. In addition, the expensive chemicals have not been used. To trim down the time, the extraction buffer was directly added to the *Artemisia plant during grinding* with extraction buffer.

Quality and quantity analysis of DNA extracted:
The DNA quality, purity and yield were assessed using Nanodrop (optical density) and 1% agarose gel electrophoresis.

Agarose Gel Electrophoresis:
The most commonly used methodologies for quantifying the amount of nucleic acid in a preparation are: Gel Electrophoresis systems comprise a submarine cell including gel tank, gel tray, top lid, casting module and red/black L type electrode. This method of quantification is based on the ethidium bromide fluorescent staining of DNA. Besides quantification, it also provides the advantage of analyzing the quality of the DNA preparation. Native DNA, which migrates as a tight band of high molecular weight (40 kb), presence of RNA, and

degraded/sheared DNA, if any, can be visually identified on the gel. In case of *Artemisia species*, the DNA was electrophoretically separated on a 0.7% agarose gel containing ethidium bromide at a final concentration of 0.5 mg/ml as depicted in figure 1 .The quantity of DNA was estimated by comparing the fluorescent yield of the samples with a series of standards, for instance, 100 bp ladders at varying known concentrations. This provides a very rapid and sensitive means of estimating the nucleic acid concentration.

Figure 1: Genomic DNA extracted from Artemisia plant using solution based method



LEGEND
M-100 bp DNA ladder
-Good quality DNA - AM1- AM13 except
Low quality DNA -AM7

Purity of DNA by Nanodrop analysis

The ratio of absorbance at 260 nm and 280 nm is used to assess the purity of DNA and RNA. A ratio of ~1.8 is generally accepted as "pure" for DNA; a ratio of ~2.0 is generally accepted as "pure" for RNA. We used NanoDrop1000 Spectrophotometer to measure the purity of DNA.

All DNA samples were screened for purity by measuring optical density (OD) at 260nm (OD_{260}) and 280 nm (OD_{280}). The λ260/λ280 ratio ranged from 1.83-2.05 indicating good quality DNA from our method as depicted in table 4.DNA concentration (ug/ml) was calculated based on the OD_{260} reading as depicted in figure 1, 2 and 3.

Table 5: Nanodrop analysis of plant DNA at λ260/λ280 ratio

Sample code	*Code*	*260nm (OD_{260}) / 280 nm(OD_{280}).*	*Concentration*
Artemisia -1	AM-1	1.85	1200 ng/ul
Artemisia -2	AM-2	1.88	366 ng/ul
Artemisia -3	AM-3	1.94	492 ng/ul
Artemisia -4	AM-4	1.80	270 ng/ul
Artemisia -5	AM-5	1.64	172 ng/ul
Artemisia -6	AM-6	2.07	342 ng/ul
Artemisia -7	AM-7	**1.53**	**2486 ng/ul**
Artemisia -8	AM-8	1.82	851 ng/ul
Artemisia -9	AM-9	1.88	366 ng/ul
Artemisia -10	AM-10	1.94	492 ng/ul

Figure 2: Genomic DNA extracted from Artemisia -1 using solution based method

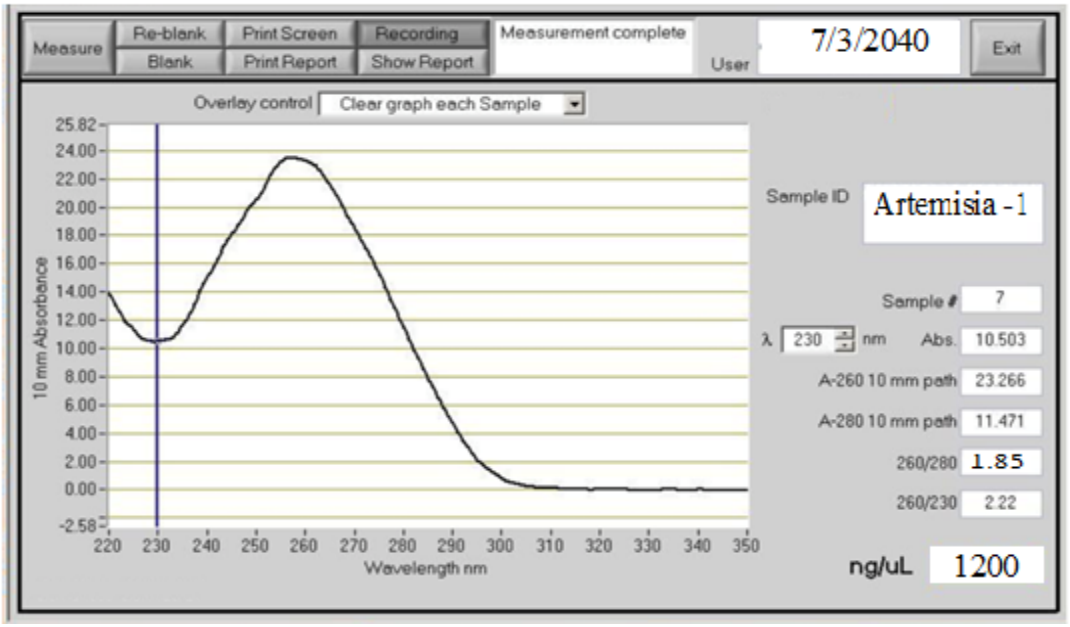


Figure 3: Genomic DNA extracted from Artemisia -7 using solution based method

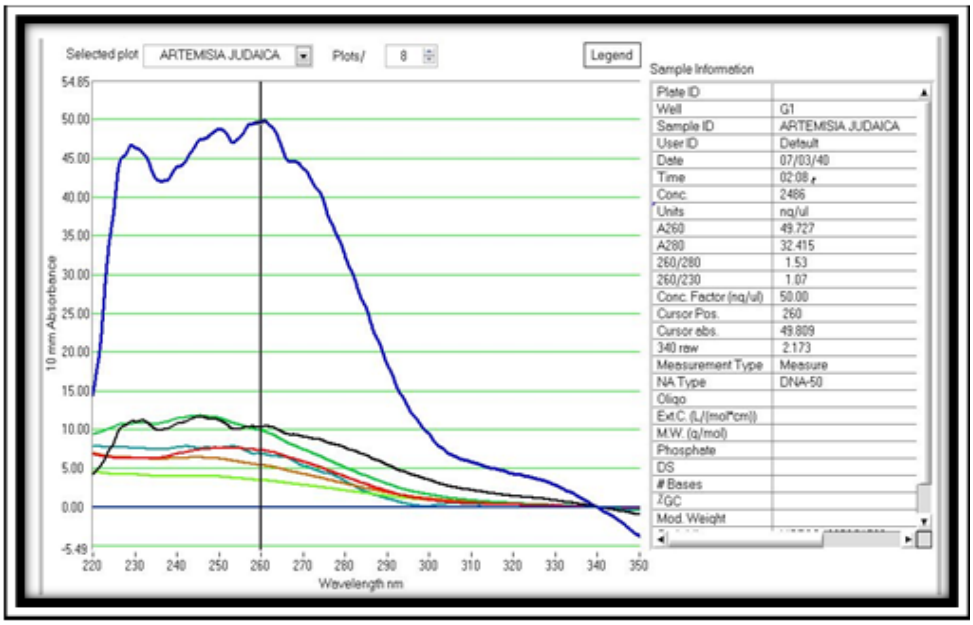
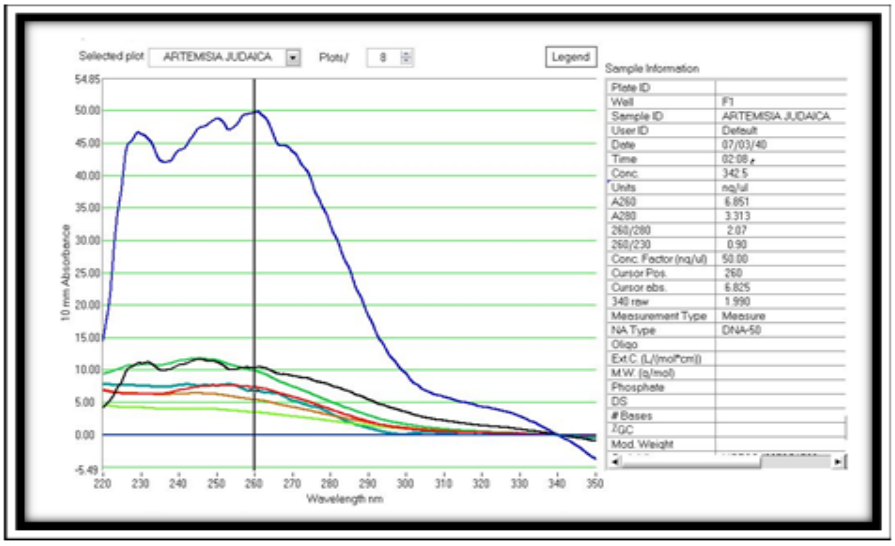


Figure 4: Genomic DNA extracted from Artemisia -6 using solution based method



High quality DNA appeared as a high molecular weight band, without a smear of lower molecular weights that indicate degradation or shearing of the sample. The presence of stable RNAs was detected as a cloud of fluorescence in the low molecular weight region of the gel. Ethidium bromide being mutagen and a carcinogen so it was handled with an extreme care.

DISCUSSION:

Artemisia species in Saudi Arabia are exposed to serious threats due to heavy human impacts such as uncontrolled tourism, overgrazing and uncontrolled collection, mining, quarrying and natural drought. *Artemisia* is an important medicinal plant, used for curing various diseases especially stomach ache, heart diseases, sexual weakness, diabetes, gastrointestinal disorders ,external wounding. Inflammatory-related diseases, for instance fungal infections, diabetes, atherosclerosis, cancer and arthritis. (7) Artemisia species secretes verities of secondary metabolites which hinders in the DNA extraction. Therefore, these contaminants must be eliminated during DNA isolation

Our study describes an efficient DNA extraction protocol for *Artemisia* species based upon the Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method without using hazardous chemicals i.e. liquid nitrogen. In this study, a DNA extraction protocols prepared is cheap and very efficient to recollect DNA from *Artemisia* samples .The commercial kits are generally very fast but too expensive and generate large quantities of contaminant wastes, such as beads, filters, columns .In this study, a method used for the rapid extraction of genomic DNA from small amounts of plant material was modified for the purpose of PCR analysis. The method is applicable to a variety of plant species, and has many advantages, such as dispensing with the use of hazardous chemicals like phenol. Thus, it is fast and complete DNA extraction can be achieved within 2 hours. This method requires small amount of plant tissue to reduce inhibitor agents and could extract optimal amount of DNA.Also, homemade DNA extraction protocols are less efficient in the removal of proteins/carbohydrates and other organic contaminants from samples or extraction solutions used, thus causing enzymatic inhibition in further molecular analysis.

In these cases, the use DNA clean-up steps are required, which can be time and fund consuming. An ideal protocol should optimize DNA yield, minimize degradation, and be efficient in terms of cost, time, labor, and supplies. (13) Although, several DNA extraction protocols have been described for plants containing high concentrations of secondary metabolites, the most of these require a large amount of plant tissue to be ground in liquid nitrogen (14). In addition, liquid nitrogen is unavailable in many regions of the world. In this study, we describe a simple, rapid and efficient method to extract high quantities of quality genomic DNA from little amounts of dry Artemisia species.

CONCLUSION:

The developed protocol proved to be simple, reliable and operative in normal laboratory condition yielding

intact DNA from Artemisia species having good quantity (170 to 1200 ng/µl) and quality ($A_{260/280}$ ratio - 1.64 to 1.95) in two 2 to 3 hours.

Competing interests:
The authors declare that they have no competing interests.

Authors' contributions: -
All authors read and approved the final manuscript. Thanks to all authors for their support and help in this study.

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REFERENCES:

1. Sangwan RS, Sangwan NS, Jain DC, Kumar S, Ranade SA (1999). RAPD profile based genetic characterization of chemotypic variants of *Artemisia annua* L. Biochem. Mol. Biol. Int., 47: 935- 944.
2. Salido S, Valenzuela LR, Altarejos J, Nogueras M, Sanchez A, Cano E (2004). Composition and infraspecific variability of *Artemisia herbaalba* from southern Spain. Biochem. Syst. Ecol., 32: 265-277.
3. Abdin MZ, Israr M, Rehman RU, Jain SK (2003). Artemisinin, a novel antimalarial drug: biochemical and molecular approach for enhanced production. Planta Med. 69: 289-299.
4. Dutcher SK. Power J, Galloway RE, Porter ME (1991). Reappraisal of the genetic map of Chlamydomonas reinhardtii. J. Hered. 82: 295-301.
5. Dellaporta SL, Wood J, Hicks JB (1983). A plant DNA mini-preparation:Version II. Plant Mol. Biol. Reptr.1:19-21.
6. Doyle JJ, Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 1(9): 11-15.
7. Jena RC, Samal KC, Das BK (2010). Opimization of DNA isolation and PCR protocol for RAPD analysis of Mangifera indica L. J. Agric.Technol. 6(3): 559-571.
8. Vallès J, Siljak-yakovlev S (1997). Cytogenetic studies in the genus *Artemisia* L.: fluorochrome banded karyotypes of five taxa, including the Iberian endemic species *Artemisia* barrelieri Besser. Can. J. Bot., 75: 595-606.
9. Barney JN, Ditommase A (2003). The biology of Canadian weeds. 118. *Artemisia vulgaris* L. Can. J. Plant Sci., 83: 205-215.
10. Al-Rawashdeh IM (2011). Genetic variability in a medicinal plant *Artemisia judaica* using random amplified polymorphic DNA (RAPD) Markers. Int. J. Agric. Biol., 13: 279-282.
11. Bremer K, Humphries CJ (1993). Generic monograph of the Asteraceae - Anthemideae. Bull. Nat. Hist. Mus. Lond. (Bot.), 23: 71-177.
12. . Burrows G, Tyrl R (2001). *Toxic Plants of North America*. Ames, Iowa State Univ. Press.
13. .CHEN, H. et al. Evaluation of Five Methods for Total DNA Extraction from Western Corn Rootworm Beetles. PLoS One,v.5, n.8, p.e11963, 2010.
14. Lindner R.C. Rapid analytical methods for inorganic constituents of plant tissues. Plant Physiol. 1944;19:76–89
15. Fiske C.H., Subba-Row Y. The colorimetric determination of phosphorus. J. Biol. Chem. 1925;66:375–400.
16. Dwivedi R.S., Randhawa N.S. Evaluation of rapid test for hidden hunger of zinc in plants. Plant Soil. 1974;40:445–451.
17. Lichtenthaler H.K., Buschmann C. Current Protocols in Food Analytical Chemistry. John Wiley and Sons; New York: 2001. Chlorophylls and carotenoids: measurement and characterization by UV–Vis spectroscopy. pp. F4.3.1–F4.3
18. Yamasaki S., Dillenburg L.R. Measurements of leaf relative water content in araucaria angustifolia. Rev. Bras. Fisiol. Veg. 1999;11(2):69–75.
19. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Annal. Biochem. 1976;72:248–254
20. . Busconi M, Foroni C, Corradi M, Bongiorni C, Cattapan F, Fogher C. DNA extraction from olive oil and its use in the identification of the production cultivar. Food Chem. 83: 127-134 ,2003.