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COMPARISON OF DNA EXTRACTION METHODS FROM *GERANIUM* (GERANIACEAE)

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The genus *Geranium* (Geraniaceae); with about 320 species throughout the temperate regions, is chemically characterised by the presence of tannins, flavonoids, anthocyanins and essential oils which interfere with the extraction of pure genomic DNA. It is necessary to optimise the extraction protocols to reduce the effects of the presence of these compounds to the lowest level.

The present study compares the plant genomic DNA extraction Kit (DNP™ Kit), CTAB DNA extraction method by Murray and Thompson and Sahu *et al.*, from the extracting DNA point of view *Geranium* species. The results showed significant differences in DNA contents between the three methods. Quantity and quality of extracted genomic DNAs were compared by employing the spectrophotometer, Nano-Drop, agarose gel electrophoresis, and polymerase chain reaction (PCR) methods and molecular marker such as (ITS and trnL-F) and ISSR. The method of Sahu *et al.*, provided the best results (200 ng/μL) in terms of quantity and quality of DNA, therefore, this method was taken and optimised for DNA extraction. Our results proposed that this method could be effective for plants with same polysaccharides, proteins and polyphenols components. The advantage of this method is that it omits the use of liquid nitrogen and toxic phenols which are expensive. The success of this method in obtaining high-quality genomic DNA has been demonstrated in the *Geranium* species group and the reliability of this method has been discussed.

Key words: DNA extraction, *Geranium*, ISSR, PCR, secondary metabolites

INTRODUCTION

Molecular markers are extensively used in the development of genetic and physical maps of genomes for different purposes as germplasm characterisation, genetic diagnostics, characterisation of transformants, the study of genome organisation, phylogenetic analysis, marker-assisted selection, mapping quantitative trait loci (QTL), etc. (Gupta *et al*. 1999). The thermostable *Taq* DNA polymerase caused the development of several PCR-based markers, such as random amplified polymorphic DNA (RAPD), inter-simple sequence repeats (ISSR), sequence tagged microsatellite site (STMS) and amplified fragment length polymorphism (AFLP), etc.

The genus *Geranium* L. (Geraniaceae) with about 320 species distributed throughout the temperate regions, is chemically characterised by the presence of tannins, flavonoids, anthocyanins and essential oils which affect the extraction of pure genomic DNA (Aedo *et al.* 1998, Esfandani-Bozchaloyi *et al*. 2018*a*, *b*, *c*). There are 25 annual and perennial species of this genus in Iran according to Flora Iranica (Schönbeck-Temesy 1970). Some species of the genus *Geranium* (cranesbill) are utilised as an anti-diabetic, haemostatic, anti-haemorrhoid, and anti-diarrhoea, and as a remedy for tonsillitis, cough, whooping cough, urticaria, dysentery, pain, fevers, and gastrointestinal ailments in some folk medicines (Bate-Smith 1973, Baytop 1999).

DNA extraction is a critical step for genomic analysis especially from the plant materials with high accumulation of interfering substances including polysaccharides, proteins, polyphenols and secondary metabolites, which affect DNA restriction, amplification and another related process (Zamboni *et al.* 2008).

General problems in the isolation and purification of high molecular weight DNA from medicinal and aromatic plant species include: (1) degradation of DNA due to endonucleases, consolation of highly viscous polysaccharides, and (2) inhibitor compounds like polyphenols and other secondary metabolites which directly or indirectly interfere with the enzymatic reactions (Weising *et al.* 1995). The presence of polyphenols, as oxidising agents present in many plant species, can reduce the production of the purified extracted DNA (Loomis 1974, Porebski *et al.* 1997).

Several DNA extraction protocols have been successfully utilised to plant species (Doyle and Doyle 1990, Reichandt and Rogers 1994). Doyle and Doyle's method (1990) is applied to extract DNA in fruit trees (Jenderek *et al.* 1997). The extraction technique of Lodhi *et al.* (1994) has been utilised for the grape, apple, apricot, peach, cherry and snapdragon. Sarkhosh *et al.* (2006) used the Bi *et al.* (1996) method for some Iranian pomegranate (*Punica granatum* L.) genotypes. Murray and Thompson (1980) method were used for DNA extraction in cabbage, olive, rose (Csaikl *et al.* 1998) and sweet cherry (Khadivi-Khub *et al.* 2008).

Saghai-Maroof *et al*. (1984)'s method was used for DNA extraction in mangroves and salt marsh species (Sahu *et al*. 2012). Talebi-Baddaf *et al.* (2003) introduced Murray and Thompson (1980)'s method as the most appropriate method to achieve high-quality DNA extraction from pomegranate leaves. Because plants contain high amounts of many different substances, it is unlikely that just one nucleic acid isolation method suitable for all plants can ever exist (Loomis 1974). Therefore, it is necessary to modify the extraction protocols.

A perfect method is the one that is highly reproducible and represents the highest yield and pure DNA with the lowest content of contaminants that could be amplified in PCR.

Therefore, the present study aims to compare three different DNA extraction methods to isolate high-quality DNA from *Geranium* leaves. In this study, we showed the results of tests from several DNA extraction protocols that were made to overcome the problems that mainly arise from polysaccharide contamination. We evaluate a highly effective method for high-quality DNA isolation from young and mature leave of *Geranium* species.

ISSR amplification was also performed in order to evaluate the suitability of the DNA extraction methods for PCR-based techniques. As far as we know, this is the first report on DNA extraction from *Geranium* species leaves in Iran, and we expect that the suggested protocol can be an incentive to perform further studies in order to investigate the genetic diversity among the plants with same chemical components as *Geranium* species.

MATERIALS AND METHOD

Plant samples for DNA isolation

In present study leaves of 22 species *Geranium* were collected from different habitats in Iran (Table 1). The leaves dried by silica gel powder (Chase and Hills 1991), were used in all experiments. One gram of young and mature leaf were collected and stored at –80 °C until extraction. For molecular studies we used different number of plant specimens, as they were required. For example, in ISSR analysis, we used 22 samples of 22 species, while for cp-DNA 10 species, and for nrDNA ITS 10 species were used (Esfandani-Bozchaloyi *et al.* 2017*a*, *b*, *c*, *d*, 2018*a*, *b*).

Extraction methods

Plant genomic DNA extraction Kit (DNP™ Kit), CTAB DNA extraction method by Murray and Thompson (1980) and a modified method of Saghai-Maroof *et al.* (1984), which was used by Sahu *et al.* (2012), is used and evaluated here for *Geranium* species. Among all the tested protocols, Saghai-Maroof's method, which was used by Sahu *et al.* (2012) produced good quality DNA. Therefore, this method was taken and optimised for DNA extraction by modifications of the concentration of Tris-HCl, NaCl, β-mercaptoethanol, and PVP (polyvinyl pyrrolidone).

Table 1
Geranium species and populations, their localities and voucher numbers

No	Species	Locality	Section	Voucher no.
1	*G. dissectum*	Guilan, Siahkal, Ezbaram	*Dissecta*	HSBU 201658
2	*G. columbinum*	Guilan, Lahijan	*Geranium*	HSBU 201659
		E Azerbaijan, Kaleybar cheshme ali akbar		HSBU 201660
		E Azerbaijan, Kaleybar, Shojabad		HSBU 201661
3	*G. rotundifolium*	Tehran, Tuchal	*Geranium*	HSBU 201662
4	*G. collinum*	Tehran, Damavand	*Geranium*	HSBU 201663
5	*G. platypetalum*	E Azerbaijan, Kaleybar	*Tuberosa*	HSBU 201668
6	*G. sylvaticum*	E Azerbaijan, Kaleybar cheshme ali akbar	*Geranium*	HSBU 201669
7	*G. pratense*	E Azerbaijan, Kaleybar, Shojabad	*Geranium*	HSBU 201670
8	*G. ibericum*	Mazandaran, Tonekabon-jannat rudbar	*Tuberosa*	HSBU 201671
9	*G. gracile*	Mazandaran, Noshahr, Kheyrud kenar Forest	*Tuberosa*	HSBU 201672
10	*G. linearilobum*	Tehran, Firuz kuh	*Tuberosa*	HSBU 201673
11	*G. kotschyi*	Alborz, Karaj- Qazvin	*Tuberosa*	HSBU 201674
12	*G. tuberosum*	E Azerbaijan, Kaleybar cheshme ali akbar	*Tuberosa*	HSBU 201675
13	*G. trilophum*	Tehran, Tuchal	*Trilopha*	HSBU 201676
14	*G. molle*	E Azerbaijan, Kaleybar, Shojabad	*Batrachioidea*	HSBU 201677
		E Azerbaijan, Kaleybar, cheshme ali akbar		HSBU 201678
15	*G. pyrenaicum*	E Azerbaijan, Kaleybar, road side	*Batrachioidea*	HSBU 201679
		E Azerbaijan, Kaleybar cheshme ali akbar		HSBU 201680
		E Azerbaijan, Kaleybar, Shojabad		HSBU 201681
		E Azerbaijan, Babak fort		HSBU 201682
16	*G. pusillum*	E Azerbaijan, Kaleybar, road side	*Batrachioidea*	HSBU 201683
		E Azerbaijan, Kaleybar cheshme ali akbar		HSBU 201684
		E Azerbaijan, Kaleybar, Shojabad		HSBU 201685
17	*G. purpureum*	E Azerbaijan, Kaleybar, cheshme ali akbar	*Ruberta*	HSBU 201686
		Guilan,Gole rodbar		HSBU 201687
		Guilan,Gole rodbar, road side		HSBU 201688

Table 1 (continued)

No	Species	Locality	Section	Voucher no.
18	*G. robertianum*	Guilan,Gole rodbar	*Ruberta*	HSBU 201689
19	*G. albanum*	Guilan, Sangar, road side	*Divaricata*	HSBU 201690
		Guilan, Lahijan		HSBU 201691
		Guilan, Jirandeh		HSBU 201692
		Mazandaran, Siah bisheh to Chalus		HSBU 201693
		Golestan, Ramian		HSBU 201694
20	*G. divaricatum*	E Azerbaijan, Kaleybar	*Divaricata*	HSBU 201695
		Tehran, Darband		HSBU 201696
21	*G. lucidum*	E Azerbaijan, Kaleybar cheshme ali akbar	*Lucida*	HSBU 201697
22	*G. mascatense*	Khuzestan, Shushtar- Masjed solyman	*Trilopha*	HSBU 201698

Standardised extraction method

1. Preheat suspension buffer (pH 8) containing 50 mM EDTA, 120 mM Tris-HCl, 1 M NaCl, 0.5 M sucrose, 2% Triton-X 100, 5% PVP and 0.2% β-mercaptoethanol in water bath at 60 °C for 60 min.

2. Grind 0.03–0.05 g of dried leaves to have a fine powder at room temperature and a mortar and pestle. – Note: To avoid usage of liquid nitrogen, this method is successfully employed.

3. Transfer the content in 2 mL micro centrifuge tubes and add 400–500 µl of suspension buffer.

4. Invert and mix gently and incubate at 60 °C for 40 min.

5. Centrifuge the suspension at 10,000 rpm for 15 min at room temperature.

6. Add 1.5 mL of extraction buffer containing 20 mM EDTA, 100 mM Tris-HCl, 1.5 M NaCl, 2% CTAB, 1% β-mercaptoethanol and incubate at 60 °C for 30 min.

7. Centrifuge at 12,000 rpm for 15 min at room temperature. Carefully transfer the aqueous phase into a new tube. – Note: Use wide-bore tips for transferring the aqueous phase to avoid mechanical damage to DNA.

8. Add double volume of chloroform: isoamyl alcohol (24 : 1), and invert gently 15 to 20 times and centrifuge at 12,000 rpm for 15 min. – Note: If the aqueous layer appears translucent, repeat the step until the solution is transparent.

9. Add double volume of chilled isopropanol and keep at –20 °C for 1–24 hrs to precipitate the DNA. – Note: The longer the chilled incubation, the more the precipitation.

10. Centrifuge at 12,000 rpm for 15 min and discard the supernatant. To the pellet, add 300 μl of 70% chilled ethanol and spool out the pellet carefully and centrifuge again at 12,000 rpm for 15 min.

11. Discard the supernatant and vacuum dry or air dry the pellet at room temperature. – Note: Make sure that there is no residual ethanol, this is very critical especially if the DNA is to be used directly for PCR. Overdrying should also be avoided as it makes the pellet difficult to suspend.

12. Add 100 μL of high salt TE buffer (0.5 M NaCl, 10 mM Tris-HCl, 1 mM EDTA (pH 8) and keep at 37 °C for 30 min.

13. Add 300 μL of chloroform : isoamyl alcohol (24 : 1), and shake slowly for 15 min on a rocker.

14. Centrifuge at 12,000 rpm for 15 min at room temperature.

15. Carefully transfer the aqueous phase into a new tube. Add 300 μl of chilled ethanol precipitation in the presence of 3 M sodium acetate (pH 5.2) and keep at –20 °C for 1–2 hrs to precipitate the DNA.

16. Centrifuge at 12,000 rpm for 15 min and discard the supernatant. To the pellet, add 300 μl of 70% chilled ethanol and spool out the pellet carefully and centrifuge again at 12,000 rpm for 15 min.

17. Discard the supernatant and vacuum dry or air dry the pellet at room temperature.

18. Add 50 μL (depending upon the pellet) of TE 139 buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) to dissolve the precipitate. – Note: Chelator present in TE can affect PCR and restriction digests. DNA in TE should be suitably diluted before use in such reactions.

We have made some modification to the Sahu *et al.* (2012) protocol:

1. Grind 0.03–0.05 gram of dried leaves to have a fine powder at room temperature with a mortar and pestle.

2. Add an equal volume of chilled isopropanol and add 30 to 50 μL of 3 M sodium acetate (pH 5.2) and keep at –20 °C for 1–24 hrs to precipitate the DNA, step 10 Sahu *et al.* (2012).

3. We omitted also step15 Sahu *et al*. (2012); (Add 3 μL RNase 10 mg/mL and keep at 37 °C for 30 min).

High level of β-mercaptoethanol successfully removes the polyphenols (Suman *et al.* 1999). It was evident that high concentration of β-mercaptoethanol resulted in high-quality DNA. Using of NaCl concentrations higher than 0.5 M, along with CTAB, was previously recorded to be efficient in removing polysaccharides during DNA extraction (Moreira and Oliveira 2011, Paterson *et al.* 1993). It was also efficient in the present study with 1.5 M of NaCl concen-

tration. Polysaccharides and secondary metabolites of *Geranium* species were bounded by PVP and it is in concordance with previous studies (Chaudhry *et al.* 1999, Couch and Fritz 1990, Zhang and Stewart 2000). More replications for using chloroform : isoamyl alcohol resulted in better removing of proteins in *Geranium* species. Sahu *et al.* (2012) used sodium acetate and isopropanol only in step 15, but we used one more time of this material in order to have the better precipitation of DNA and removing most of the secondary metabolites and polysaccharides from the DNA. The presence of higher quantities of polyphenols and polysaccharides in mature leaves are proved by Porebski *et al.* (1997), which makes it very difficult to isolate DNA of good quality. So we used fresh and young leaves to overcome this problem.

Concentration, purity and quality of the DNA extracted

The quantity (concentration and extraction efficiency) and quality (purity and intactness) of the DNA obtained in ratio of 1:49 (20 μL of DNA stock solution + 980 μL of double distilled sterile water) were assessed spectrophotometrically at 260 and 280 nm, and the A260/A280 ratio was used to assess contamination with proteins by employing the spectrophotometry (Hitachi U-2001 UV/VIS), Nano-DropTM (Thermo Scientific) described by Brodmann (2008) and Wilmington (2008), and agarose gel electrophoresis, PCR methods and molecular markers, such as ITS and ISSR. This spectrophotometric analysis was performed in triplicate on the samples of extracted DNA using spectrophotometer. In order to verify DNA integrity, 5 μL DNA from 7 sample were subjected to gel electrophoresis on 0.8% (w/v) agarose gel, stained with ethidium bromide, and a constant voltage of 120 V for 90 min. The DNA bands were visualised and images were acquired using Gel Doc XR+ Imaging system (Bio-Rad Laboratories Inc., Germany).

ISSR amplifications

The quality of extracted DNA was examined by running on 0.8% agarose gel. 10 ISSR primers; $(AGC)_5GT$, $(CA)_7GT$, $(AGC)_5GG$, UBC 810, $(CA)_7AT$, $(GA)_9C$, UBC 807, UBC 823, $(GA)_9T$ and $(GT)_7CA$ commercialised by UBC (the University of British Columbia) were used (see Table 2). The final volume of 12 μL was tested in PCR reaction (2.5 μL PCR reaction buffer 10x, 0.875 μL $MgCl_2$ 50 mM, 0.5 μL dNTPs 10 mM, 1.0 μL primer 10 μM, 0.2 μL *Taq* DNA polymerase 5 unit/μL, 2.0 μL template DNA (5 ng/μL). The amplification reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94 °C, followed by 40 cycles of 1 min at 94 °C; 1 min at 52–57 °C and 2 min at 72 °C. The reaction was completed by a final extension step of 7–10 min at 72 °C. The amplification products were

Table 2
Primer sequences used in this study

Region	Primer Sequences (5′-3′)	Tm	Reference
TAB_C	CGAAATCGGTAGACGCTACG	56	Taberlet *et al*. (1991)
TAB_F	ATTTGAACTGGTGACACGAG	56	Taberlet *et al*. (1991)
ITS4	TCCTCCGCTTATTGATATGC	57	White *et al*. (1990)
ITS5	GGA AGT AAA AGTCGT AAC AAG G	57	White *et al*. (1990)
UBS807	AGAGAGAGAGAGAGAGT	54	UBS set no. 9
UBS810	GAGAGAGAGAGAGAGAT	54	UBS set no. 9
UBC 823	TCTCTCTCTCTCTCTCC	56	UBS set no. 9
$(AGC)_5GT$	AGC AGC AGC AGC AGC GT	56	UBS set no. 9
$(CA)_7GT$	CACACACACACACAGT	56	UBS set no. 9
$(AGC)_5GG$	AGC AGC AGC AGC AGC GG	56	UBS set no. 9
$(CA)_7AT$	CACACACACACACAAT	56	UBS set no. 9
$(GA)_9C$	GAGAGAGAGAGAGAGAGAC	56	UBS set no. 9
$(GA)_9T$	GAGAGAGAGAGAGAGAGAT	55	UBS set no. 9
$(GT)_7CA$	GTGTGTGTGTGTGTCA	55	UBS set no. 9

observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

ITS-sequences

The ITS region was amplified using PCR with following primer pairs ITS4 and ITS5 (White *et al*. 1990). The final volume of 12 μL was tested in PCR reaction (2.5 μL PCR reaction buffer 10x, 0.875 μL $MgCl_2$ 50 mM, 0.5 μL dNTPs 10 mM, 1.0 μL primer 10 μM, 0.2 μL *Taq* DNA polymerase 5 unit/μL, 2.0 μL template DNA (5 ng/μL). The amplification, reactions were performed in Techne thermocycler (Germany) with the following program: 5 min initial denaturation step 94 °C, followed by 35 cycles of 1 min at 94 °C; 45 sec, at 57 °C and 2 min at 72 °C. The reaction was completed by a final extension step of 7–10 min at 72 °C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany). The ITS and *trn*L-F regions were amplified using primers reported as universal primers by White *et al*. (1990) and Taberlet *et al.* (1991), respectively, for flowering plants (see Table 2).

cp-DNA- sequences

The sequent of *trn*L-F intergenic spacer was amplified with the forward primer pair of 5′-CGAAATCGGTAGACGCTACG-3′ and reverse primer pair of 5′-ATTTGAACTGGTGACACGAG-3′ (Taberlet *et al.* 1991). The final volume of 12 μL was tested in PCR reaction (2.5 μL PCR reaction buffer 10x, 0.875 μL $MgCl_2$ 50 mM, 0.5 μL dNTPs 10 mM, 1.0 μL primer 10 μM, 0.2 μL *Taq* DNA polymerase 5 unit/μL, 2.0 μL template DNA (5 ng/μL). The PCR amplification program was 5 min at 95 °C, followed by 35 cycles of 1 min at 95 °C, 45 sec at 56 °C, and 2 min at 72 °C, with a final extension step of 7 min at 72 °C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

RESULTS AND DISCUSSION

Comparison of different DNA extraction methods on agarose gel electrophoresis

The quality of 7 extracted DNA sample was verified spectrophotometrically using a NanoDrop instrument and agarose gel electrophoresis. DNA purity and yield were compared between these three extraction methods. Plant genomic DNA extraction Kit (DNP™ Kit) did not give proper results for

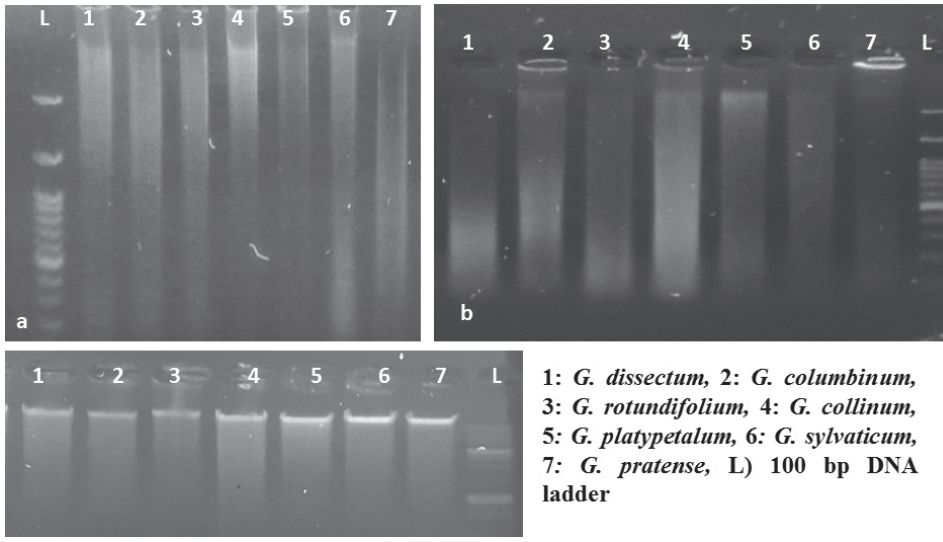

Fig 1. Electrophoretic pattern of DNA extracted by the three different methods from *Geranium* leaves. The electrophoresis was performed in 0.8% (w/v) agarose gel. The extraction methods were: a) Kit (DNP™ Kit); b) Murray and Thompson (1980); c) Sahu *et al.* (2012)

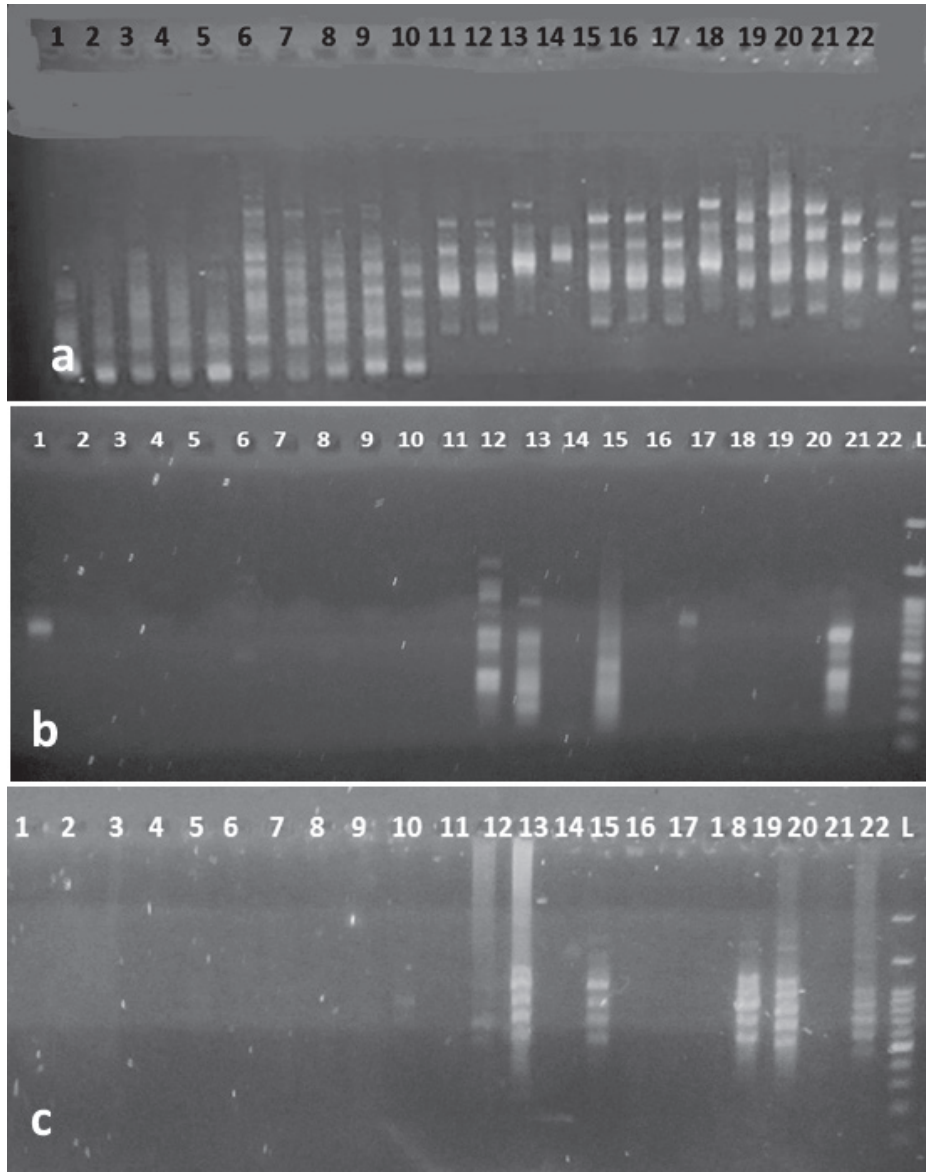

Fig. 2. Amplification of DNA from *Geranium* leaf using three different extraction methods by ISSR amplification and the primer $(AGC)_5GG$. – Fig. 2a): Sahu *et al.* (2012); Fig. 2b): Kit (DNP™ Kit); Fig. 2c): Murray and Thompson (1980) (1 = *G. dissectum*, 2 = *G. columbinum*, 3 = *G. rotundifolium*, 4 = *G. collinum*, 5 = *G. platypetalum*, 6 = *G. sylvaticum*, 7 = *G. pratense*, 8 = *G. ibericum*, 9 = *G. gracile*, 10 = *G. linearilobum*, 11 = *G. kotschyi*, 12 = *G. tuberosum*, 13 = *G. trilophum*, 14 = *G. molle*, 15 = *G. pyrenaicum*; 16 = *G. pusillum*; 17 = *G. purpureum*, 18 = *G. robertianum*, 19 = *G. albanum*, 20 = *G. divaricatum*, 21 = *G. lucidum*, 22 = *G. mascatense*)

Geranium species due to the presence of polysaccharides and proteins in the pellet and show brown or yellow DNA precipitate that presents the gDNA gel image (Fig. 1*a*). In Murray and Thompson (1980)'s method of extraction, we encountered many difficulties from the cell lysis to DNA separation phases in the supernatant and subsequent reactions. The presence of phenolic compounds caused a brownish pellet (Fig. 1*b*).

The results confirmed that extracted DNA by Sahu *et al*. (2012)'s method from leaves possess better quality in comparison with the other extraction methods as well as Kit (DNP™ Kit) and Murray and Thompson (1980)'s methods (Fig. 1c).

Due to the elimination of polysaccharides or protein contaminations DNA has been extracted with high quality. We believe that this method will be efficient for molecular studies of many other aromatic and herbal plants.

Clear banding patterns were observed in the ISSR study by Sahu *et al.* (2012)'s method (Fig. 2*a*) that possess better quality in comparison with the other extraction methods as well as Kit (DNP™ Kit) (Fig. 2*b*) and Murray and Thompson (1980)'s methods (Fig. 2c).

PCR tests outcomes in the ITS (Fig. 3*a–c*) and trnL-F (Fig. 3*d–f*) study showed that the extracted DNA by Sahu *et al.* (2012)'s method (Fig. 3a, d) from leaf samples brings an acceptable quality for PCR, and as the most appropriate method in aspect of quality of DNA extracted from young leaves of *Geranium.* The PCR-amplified DNA fragments of ITS and trnL-F for 10 samples showed a clean single band product when examined on an agarose gel (Fig. 3). The PCR products were of about 800 bp.

UV spectrophotometer and NanoDrop™ 1000 spectrophotometer

In spectrophotometer procedure, absorption of double-stranded DNA in wavelength of 260 nm is 50 μg/μL. In fact, the ratio of absorption amount resulted in 260 nm to 280 nm is range from 1.7 to 2. It shows the most absorption is done by nucleic acids and therefore extracted DNA is well-qualified and its purity is acceptable. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm. The results showed that the DNA yield and DNA purity obtained from one g of the fresh leaf tissue in different methods using UV spectrophotometer was statistically significant ($P \leq 0.01$). A higher DNA yield was obtained with method of Sahu *et al.* (2012) (450±88.1 ng/μL fresh weight), while the lowest was obtained with method of Murray and Thompson (1980) (292±44.4 ng/μL fresh weight) (Table 3). Therefore, the results confirmed that extracted DNA by Sahu *et al.* (2012)'s method from leaves of *Geranium* possess better quality and quantity in comparison with the other

methods. DNA sample was measured with a UV spectrophotometer for the ratio of OD260/OD280 using TE buffer. The ratio of OD260/OD280 was determined in order to assess the purity and concentration of the DNA sample. DNA concentration was calculated according to the equation of Wilmington (2008). DNA concentration (ng/μL) = OD260 × a (dilution factor) × 50.

Absorbance measurements made on a spectrophotometer, including any Thermo Scientific NanoDrop Spectrophotometer, will include the absorbance of all molecules in the sample that absorb at the wavelength of interest.

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. If the ratio is appreciably lower in either case, it may indicate the presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

Some researchers encounter a consistent 260/280 ratio change when switching from a standard cuvette spectrophotometer to a NanoDrop spec-

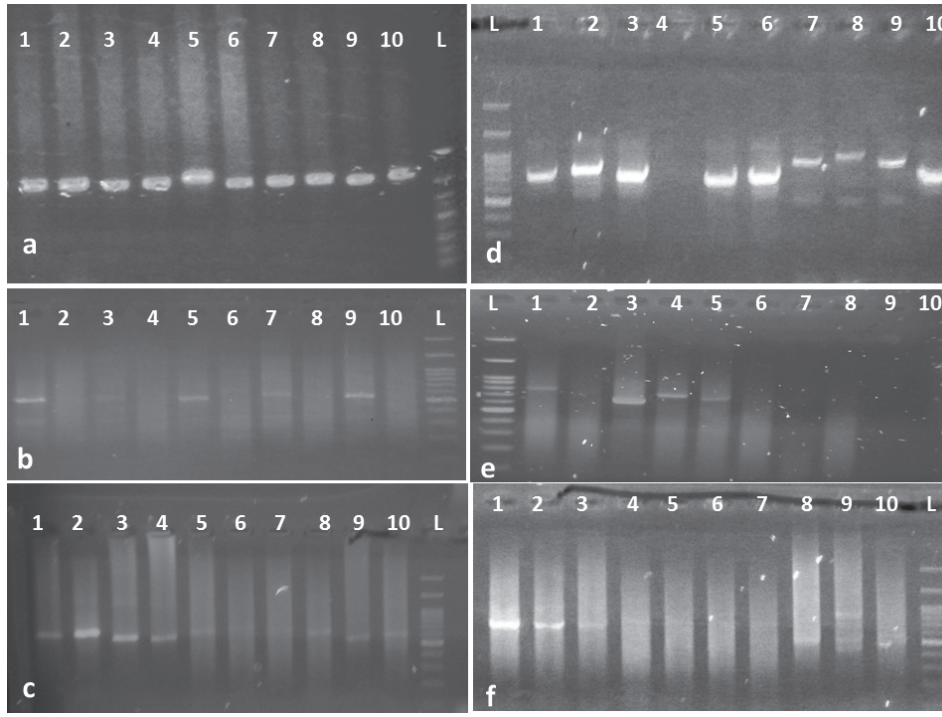

Fig. 3. Agarose gel (1.5%) showing the PCR amplified ITS (Fig. 3a, b, c) and trnL-F (Fig. 3d, e, f) of the plant materials used in the present study. – Fig. 3a, d): Sahu *et al.* (2012); Fig. 3b, e): Kit (DNP™ Kit); Fig. 3c, f): Murray and Thompson (1980) (1 = *G. dissectum*, 2 = *G. columbinum*, 3 = *G. rotundifolium*, 4 = *G. collinum*, 5 = *G. platypetalum*, 6 = *G. sylvaticum*, 7 = *G. pratense*, 8 = *G. ibericum*, 9 = *G. gracile*, 10 = *G. linearilobum*) 100 bp DNA ladder)

Table 3
Comparison of means for efficiency of three different DNA extraction methods in leaf samples of leaves *Geranium* using Duncan's multiple range test ($P \leq 0.01$)

Methods	Spectrophotometer		Nano-Drop	
	DNA yield (ng/μL)	DNA purity (ng/μL)	DNA yield (ng/μL)	DNA purity (ng/μL)
Sahu *et al.* (2012)	450±88.1	2.1±0.15	590.4±86.5	1.84±0.15
Kit (DNP™ Kit)	323±33.8	1.8±0.18	767.5±11.8	1.70±0.09
Murray and Thompson (1980)	292±44.4	1.7±0.19	555±76.4	1.78±0.07

trophotometer. The three main explanations for this observation are listed below: Small changes in the pH of the solution will cause the 260/280 to vary*. Acidic solutions will under-represent the 260/280 ratio by 0.2–0.3, while a basic solution will over-represent the ratio by 0.2–0.3. If comparing results obtained using a NanoDrop spectrophotometer to results obtained using other spectrophotometers, it is important to ensure that the pH of an undiluted sample measured on our instruments is at the same pH and ionic strength as the diluted sample measured on the conventional spectrophotometer.

The NanoDrop absorbance is useful for detection of contaminants such as protein, salts, and polysaccharides, which can inhibit and interfere in DNA sequencing. The NanoDrop 1000 spectrophotometer has the capability to measure highly concentrated samples without dilution. The ratio of 260 and 280 nm absorbance is used to assess the purity of DNA and RNA. This ratio is between 1.7 and 1.8, and this range is generally accepted as "pure" for DNA (Table 3).

CONCLUSIONS

In this study, three DNA extraction methods were compared to isolate high quality DNA that can be efficiently amplified using PCR. Murray and Thompson (1980) and DNP™ Kit resulted in brown or yellow DNA precipitate that could not be reliably amplified by PCR. Therefore, we used the protocol of Sahu *et al*. (2012)'s method that produced good quality DNA from these sources that of the methods also was applied successfully to mangroves and salt marsh plants containing elevated concentrations of polysaccharide and polyphenolic compounds (Sahu *et al.* 2012).

Sahu *et al*. (2012)'s method helped us to provide a pure DNA with high efficiency in *Geranium* species. Advantages of the present method for studying medicinal plants with secondary metabolites are as follows: 1) omission of liquid nitrogen, 2) decrease of toxic effects, hazardous, expensive of some component as phenol in other methods, 3) lower amount of dried or fresh

plant material, without any conservation specific condition. Although this method has many advantages, it is time-consuming. The DNA extracted using this protocol can be used for whole-genome sequencing, advanced sequencing technologies, and bioinformatics tools.

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