GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Methodology

Genomic DNA extraction from medicinal plants available in Malaysia using a TriOmic™ improved extraction kit

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Genet. Mol. Res. 10 (4): 2757-2764 (2011)
Received December 6, 2010
Accepted April 1, 2011
Published November 8, 2011
DOI http://dx.doi.org/10.4238/2011.November.8.1

ABSTRACT. DNA extraction was carried out on 32 medicinal plant samples available in Malaysia using the TriOmic™ extraction kit. Amounts of 0.1 g flowers or young leaves were ground with liquid nitrogen, lysed at 65°C in RY1^{plus} buffer and followed by RNAse treatment. Then, RY2 buffer was added to the samples and mixed completely by vortexing before removal of cell debris by centrifugation. Supernatants were transferred to fresh microcentrifuge tubes and 0.1 volume RY3 buffer was added to each of the transferred supernatant. The mixtures were applied to spin columns followed by a centrifugation step to remove buffers and other residues. Washing step was carried

out twice by applying 70% ethanol to the spin columns. Genomic DNA of the samples was recovered by applying 50 µL TE buffer to the membrane of each spin column, followed by a centrifugation step at room temperature. A modification of the TriOmic™ extraction procedure was carried out by adding chloroform:isoamyl alcohol (24:1) steps in the extraction procedure. The genomic DNA extracted from most of the 32 samples showed an increase of total yield when chloroform:isoamyl alcohol (24:1) steps were applied in the TriOmic™ extraction procedure. This preliminary study is very important for molecular studies of medicinal plants available in Malaysia since the DNA extraction can be completed in a shorter period of time (within 1 h) compared to manual extraction, which entails applying phenol, chloroform and ethanol precipitation, and requires 1-2 days to complete.

Key words: Medicinal plant; Molecular study; DNA extraction; TriOmic™

INTRODUCTION

Malaysia's tropical rainforest has been identified as having a large number of medicinal plants. More than 1000 species of plants in the forest have been used in traditional practices by Malaysian ancestors to treat various medical conditions (Yaacob et al., 2009) and also commercialized as dietary supplements in recent years (Chin et al., 2009) in capsules, tonics and tea sachets but not all plants have been scientifically studied (Yaacob et al., 2009).

In recent years, biochemical properties of medicinal plants have been studied for bioactivity against cancer, larvicidal activity and antimicrobial activity, using plant extracts that were extracted by hydro-distillation or solvent extraction methods. Some medicinal plants including *Agaricus subrufescens*, *Andrographis paniculata*, *Piper sarmentosum*, and *Elaeodendron transvaalense* (Lavitschka et al., 2007; Verma and Vinayak, 2008; Zainal Ariffin et al., 2009; Tshikalange and Hussein, 2010) have been identified as having anticancer properties. Bioactivity research carried out on other medicinal plants including *Spilanthes acmella*, *S. calva*, *Aloe barbadensis*, *Saraca indica*, and *Clitoria ternatae* (Pandey et al., 2007; Maurya et al., 2007; Mathew et al., 2009) have shown larvicidal activity against mosquito vector species. While in antimicrobial activity studies, many medicinal plants including *Morus rotunbiloba*, *Spondias pinnata* and *Ornithogalum alpigenum* (Patharakorn et al., 2010; Gupta et al., 2010; Makasci et al., 2010) have been reported to have medicinal properties that can kill microorganisms including bacteria and fungus.

Besides bioactivity studies, medicinal plants have recently become popular because of their antioxidant properties, similar to fruits and vegetables. Antioxidant properties including vitamins, caratenoids, flavonoids, and phenolic acids have been reported to have pharmacological and biological activities such as antioxidative, antiviral and anti-inflammatory effects (Klimczak et al., 2007; Rupasinghe and Clegg, 2007; Pawlowska et al., 2008; Hakiman and Maziah, 2009). In addition, many medicinal plants including *Psidium guava*, *Ficus deltoidea* and *Citrus hystrix* (Qian and Nihorimbere, 2004; Hakiman and Maziah, 2009; Laohavechvanich et al., 2010; Hamid et al., 2010) have been reported to contain antioxidant properties.

In terms of molecular biology, genomics, transcriptomics and proteomics of medicinal

plants are not well established as metabolite studies. Few protocols have been well established for the extraction of genomic DNA from plant samples, with the exception of the CTAB method by Doyle and Doyle (1990), DNA extraction method by Murray and Thompson (1980) and their modifications. The protocols have been used for a wide range of plants. The manual extraction process is time-consuming, taking 1-2 days to complete. Thus, optimization of genomic DNA extraction from several medicinal plants available in Malaysia using the TriOmic™ extraction kit would be very important to future molecular research for medicinal plants since the process can be completed in a shorter period (within 1 h) with a good-quality DNA.

MATERIAL AND METHODS

Sample collection

A number of 32 medicinal plants available in Malaysia were grown either in pots or in the ground. They were collected from areas in Telok Mas, Melaka. Flowers or young leaves were chosen from all the samples prior to DNA extraction (Table 1). All samples were cut into small pieces, weighed to 0.1 g, frozen in liquid nitrogen and stored at -80°C for further use.

Table 1. List of plant samples extracted.

Sample	Scientific name	Local name	Part extracted
H1	*Piper sarmentosum*	Kaduk	Young leaves
H2	*Clitoria ternatea*	Bunga telang	Flowers
H3	*Canna* 'Yellow King Humbert' Burbank	Bunga tasbih	Flowers
H4	*Pandanus odorus*	Pandan wangi	Young leaves
H5	*Pandanus odorus*	Pandan wangi	Shoot meristemic
H6	*Citrus hystrix*	Limau purut	Young leaves
H7	*Vanda* Mimi Palmer	Orkid Vanda	Young leaves
H8	*Zephyranthes rosea*	Rain lily	Leaves
H9	*Andrographis paniculata*	Hempedu Bumi	Young leaves
H10	*Capsicum frutescens*	Cili Padi	Young leaves
H11	*Casuarina* spp	Pokok ru	Young leaves
H12	*Orthosiphon stamineus*	Misai kucing	Young leaves
H13	*Andrographis paniculata*	Hempedu bumi	Young leaves
H14	*Psidium guajava*	Jambu batu	Young leaves
H15	*Manihot esculenta*	Ubi kayu	Young leaves
H16	*Sesbania grandiflora*	Turi	Young leaves
H17	*Ruta graveolens*	Geruda	Young leaves
H18	*Rosa* spp	Ros	Flowers
H19	*Rosa* spp	Ros	Leaves
H20	*Canangium odoratum*	Kenanga	Flowers
H21	*Canangium odoratum*	Kenanga	Leaves
H22	*Euodia ridlevi*	Tenggek burung	Young leaves
H23	*Morinda citrifolia*	Mengkudu	Young leaves
H24	*Gomphrena globosa*	Bunga butang	Flower
H25	*Gomphrena globosa*	Bunga butang	Leaves
H26	*Gynura procumbens*	Sabung nyawa	Young leaves
H27	*Asystasia gangetica*	Daun israel	Young leaves
H28	*Hibiscus esculentus*	Bendi	Young leaves
H29	*Hydrocotyle javanica*	Pegaga gajah	Young leaves
H30	*Coleus scutellarioides*	Daun hati-hati	Young leaves
H31	*Phyllanthus acidus*	Cermai	Young leaves
H32	*Punica granatum*	Delima	Young leaves

DNA extraction using the TriOmic™ kit

Each 0.1 g sample was ground with liquid nitrogen using mortar and pestle until a fine

powder is formed. The samples were transferred to 2-mL microcentrifuge tubes containing 700 µL RY1^{plus} buffer, provided in the TriOmic™ extraction kit (Ecocillus, Malaysia). The mixtures were vortexed for 10 s, incubated at 65°C for 20 min and shaken every 5 min. RNAse treatment was then performed by adding 5 µL RNAse A solution (10 µg/µL) and followed by incubation at 37°C for 15 min. After that, 300 µL RY2 buffer (provided in the kit) was added to all the mixtures and mixed completely by vortexing for 5 s. The mixtures were centrifuged at 4°C at 15,294 *g* (~12,000 rpm) for 2 min. Supernatants were then transferred to fresh 1.5-mL microcentrifuge tubes without disturbing pellets at the bottom of the tubes and the centrifugation step was repeated under the same conditions for 5 min. The supernatants were transferred to fresh 1.5-mL microcentrifuge tubes and 0.1 volume of RY3 buffer (provided in the kit) was added to each of the transferred supernatant and the mixtures were mixed gently 4-5 times. A volume of 750 µL of the mixtures was transferred to separate spin columns (provided in the kit) and centrifuged at 15,294 *g* (~12,000 rpm) at room temperature for 40 s. The pass through was discarded and the steps were repeated for the remaining mixtures. After that, 700 µL 70% ethanol was transferred to each of the spin column and centrifuged at 15,294 *g* (~12,000 rpm) at room temperature for 40 s. The pass through was discarded and another 300 µL 70% ethanol was applied to all the spin columns and centrifuged at the same condition for 2 min to remove excess ethanol. Receiver tubes of the spin columns were then removed and the spin columns were placed onto fresh cap-cut 1.5-mL microcentrifuge tubes. After that, 50 µL TE buffer was pipetted into the membrane of each spin column and allowed to stand for 5 min, followed by centrifugation at 15,294 *g* (~12,000 rpm) at room temperature for 2 min. The genomic DNA recovered was then transferred to fresh 1.5-mL microcentrifuge tubes and stored at -20°C for further use.

DNA extraction using the TriOmic™ kit plus chloroform:isoamyl alcohol (24:1) steps

A modification of the TriOmic™ extraction was carried out by the addition of chloroform:isoamyl alcohol (24:1) steps in the protocol. The samples were ground with liquid nitrogen using mortar and pestle until a fine powder is formed, incubated at 65°C in 700 µL RY1^{plus} buffer (provided in the kit) and treated with RNAse A at 37°C as described above. After that, an equal volume of chloroform:isoamyl alcohol (24:1) was added to all samples and mixed completely by shaking vigorously. The mixtures were then centrifuged at 15,294 *g* (~12,000 rpm) at 4°C for 5 min. The aqueous phase of each sample was transferred to a fresh 2.0-mL microcentrifuge tube. The steps were repeated twice and 0.1 volume RY3 buffer (provided in the kit) was added to the final transferred aqueous phases and mixed gently 4-5 times. The subsequent steps including spin column application, washing steps with 70% ethanol and elution step with TE buffer followed the protocol described in the above subsection.

DNA quantification and agarose gel electrophoresis

The DNA samples isolated were analyzed using a Nanophotometer (Implen, USA) to determine the concentration and purity of the nucleic acids based on the ratios of $A_{260/280}$ and $A_{260/230}$. The yield of DNA extracted from 100 mg samples was calculated by multiplication of the concentration of DNA given by the Nanophotometer and the final volume of DNA recovered. A volume of 5 µL of each sample was loaded on 0.8% (w/v) agarose gels containing 0.003% (v/v) Golden View™ Nucleic Acid Stain (SBS Genetech, China), an alternative to ethidium bromide. The gels were electrophoresed at 100 V for 25 min in 1X TAE buffer using the Mupid®-exU Electrophoresis System

(Takara, Japan) and viewed with the UV Gel Documentation System (Alpha InnoTech, USA).

RESULTS AND DISCUSSION

Genomic DNA extracted from most of the 32 samples showed an increase of total yield when chloroform:isoamyl alcohol (24:1) steps were applied in the TriOmic™ extraction procedure (see Figure 1A-D). The choloroform:isoamyl alcohol (24:1) method is widely used for DNA extraction from plant samples in manual extraction procedures involving DNA precipitation steps using precipitation agents including ethanol or isopropanol (Doyle and Doyle, 1990; Ahmed et al., 2009; Das et al., 2009; Sahasrabudhe and Deodhar, 2010). Unfortunately, manual extraction procedures are time-consuming (1-2 days) since many steps are involved including long centrifugation and incubation period. Extraction of DNA using the TriOmic™ procedure can be completed in a comparatively shorter time than other extraction kits available on the market. A combination of the TriOmic™ extraction procedure with chloroform:isoamyl alcohol (24:1) separation steps with the minimum centrifugation period (5 min) is also not time-consuming since the extraction can be completed within 1 h. Both TriOmic™ procedures with or without the chloroform:isoamyl alcohol (24:1) step resulted in a similar standard of DNA purity for both protein or polysaccharide contamination (see $A_{260/280}$ and $A_{260/230}$ ratios in Table 2).

Table 2. Purity and concentration of DNA extracted.

Sample	Ratio $A_{260/280}$		Ratio $A_{260/230}$		DNA concentration (ng/µL)		Total DNA amount (ng) (in 45 µL)	
	A	B	A	B	A	B	A	B
1	2.000	1.643	1.500	1.917	15.0	11.5	675.0	517.5
2	2.000	1.840	4.000	1.769	10.0	23.0	450.0	1035.0
3	3.000	-	1.500	-	7.5	-	337.5	-
4	2.000	1.600	2.000	2.000	10.0	4.0	450.0	180.0
5	2.500	1.941	1.250	2.750	12.5	16.5	562.5	742.5
6	3.000	2.000	6.000	3.000	17.5	18.0	787.5	810.0
7	-	1.733	-	2.000	15.0	13.0	675.0	585.0
8	2.000	1.800	4.000	2.077	20.0	40.5	900.0	1822.5
9	1.806	1.778	2.800	2.133	28.0	58.0	1260.0	2610.0
10	1.841	1.867	2.455	2.270	40.5	32.0	1822.5	1462.5
11	1.705	1.818	1.339	2.857	37.5	20.0	1687.5	900.0
12	1.917	1.846	2.300	3.429	11.5	12.0	517.5	540.0
13	0.926	2.000	0.510	0.800	12.5	8.0	562.5	360.0
14	1.950	2.000	2.167	3.000	19.5	12.0	877.5	540.0
15	1.600	1.846	2.000	2.400	4.0	12.0	180.0	540.0
16	1.903	1.747	2.458	1.880	29.5	86.5	1327.5	3892.5
17	1.887	1.829	2.489	2.519	58.5	134.0	2632.5	6030.0
18	1.219	1.850	1.300	2.074	19.5	28.0	877.5	1260.0
19	1.429	1.647	1.667	0.795	5.0	17.5	225.0	787.5
20	1.354	1.591	0.607	2.167	44.0	6.5	1980	292.5
21	1.962	1.625	2.550	0.986	25.5	35.5	1147.5	1597.5
22	1.733	1.651	1.857	1.762	13.0	18.5	585.0	832.5
23	2.000	1.762	3.000	2.324	15.0	43.0	675.0	1935.0
24	1.850	1.851	2.176	2.071	18.5	43.5	832.5	1957.5
25	1.885	1.861	1.960	2.310	24.5	33.5	1102.5	1507.5
26	1.829	1.882	1.778	2.370	32.0	32.0	1440.0	1440.0
27	1.600	1.556	1.600	1.077	4.0	14.0	180.0	630.0
28	1.636	1.618	1.000	0.663	9.0	27.5	405.0	1237.5
29	2.120	2.011	2.304	2.261	26.5	82.0	1192.5	3690.0
30	1.682	1.882	0.698	2.462	18.5	16.0	832.5	720.0
31	1.778	1.657	3.200	1.094	8.0	29.0	360.0	1305.0
32	1.868	1.862	1.941	2.077	49.5	54.0	2227.5	2430.0

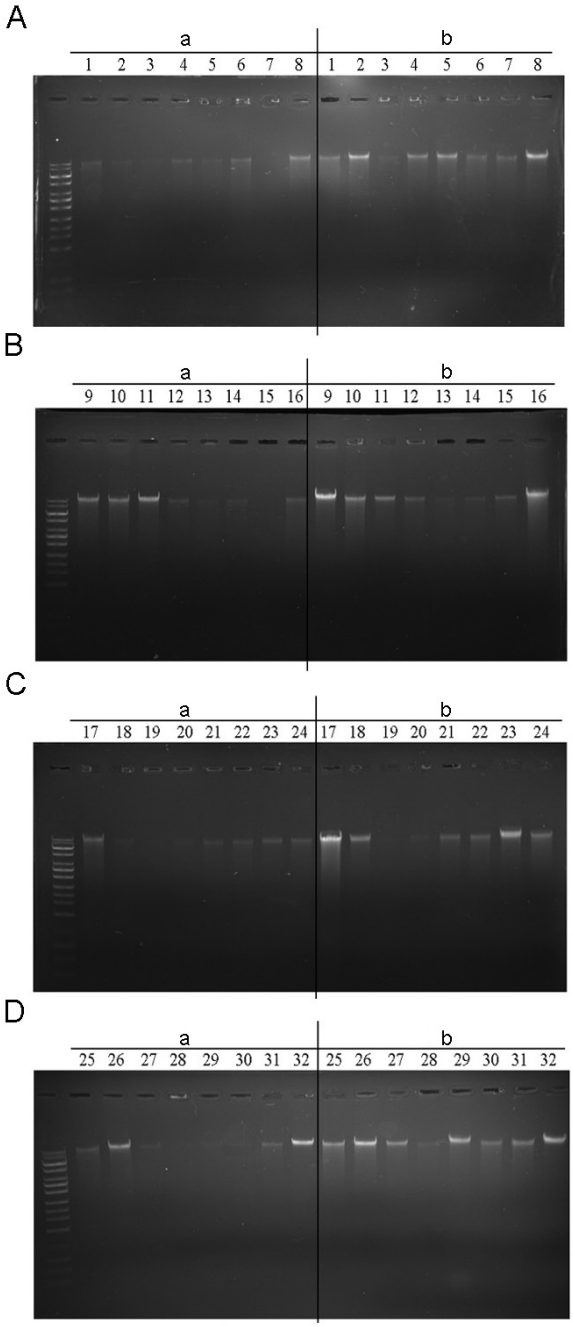

Figure 1. A. Genomic DNA extracted from samples 1-8. **B.** Genomic DNA extracted from samples 9-16. **C.** Genomic DNA extracted from samples 17-24. **D.** Genomic DNA extracted from samples 25-32. The samples were extracted using the TriOmic™ kit (*lanes a*) and the chloroform-isoamyl alcohol extraction step was applied in the TriOmic™ extraction procedure (*lanes b*).

In the original TriOmic™ procedure, RY2 solution (provided in the kit) might play a similar role as chloroform:isoamyl alcohol (24:1) to remove most of the proteins. The excess proteins and other compounds might be eliminated during spin column application in the procedure.

CONCLUSION

The DNA extraction procedure using the TriOmic™ extraction kit can be used with or without chloroform application in molecular studies of medicinal plants that are available in Malaysia. The TriOmic™-improved DNA extraction kit allows researchers to work more productively while facing the peculiar challenges of molecular studies. With shorter periods for DNA extraction, researchers will have more time to focus on the downstream application of the DNA, for example. More importantly, their needs for high yield and purity as well as ease of use are met with the improved kit.

ACKNOWLEDGMENTS

We would like to thank the Malaysia Technology Development Centre (MTDC) and Ministry of Science, Technology and Innovation (MOSTI) for providing research grants to support this project.

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