Genomic DNA Extraction Methods from Wormwood Capillary (*Artemisia capillaris*) for PCR-RAPD Studies

Sayed M. Zain Hasan, Mohammad Shafie B. Shafie And Ramisah M. Shah

Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu,
21030 Mengabang Telipot, Kuala Terengganu, Malaysia.
Corresponding author e-mail: msshafie@yahoo.com.my

ABSTRACT: The protocol evaluated included the Sarkosyl Method, Kit Wizard™ Genomic DNA Purification (Promega), CTAB method, Sodium Dodecyl Sulfate (SDS Method) and Phenol-Chloroform Method. All five protocols evaluated effectively to isolate the DNA from each of the six samples and produced consistently positive results. The quality and quantity of the DNA extracted was compared using UV-spectrophotometer. It was found that the use of Sarkosyl method had good quality among others method (mean 1.4211). However, the CTAB method resulted in the most consistently positive results at the lowest concentrations (mean 795.83 ng). DNA was successfully isolated and RAPD was effectively used to study genetic similarity among *Artemisia capillaris*. [Researcher. 2009;1(1):6-15]. (ISSN: 1553-9865).

Key words: *Artemisia capillaris*, genomic DNA isolation, SDS, CTAB, Sarkosyl, RAPD.

INTRODUCTION

Artemisia capillaris is a species from class Magnoliopsida and family Asteraceae. *Artemisia capillaris* also know as wormwood or wormwood capillary in European (ShamanShop.net, 2002), Yin Chen Hao in China (ShamanShop.net, 2002; Dharmananda, 2002) and in common names are Pokok Ru Nyamuk and Pokok Daun Ru. *Artemisia capillaris* is a member of the parsley family, is a strong-smelling, fennel-like, annual plant reaching a height of about 4 feet or more. *Artemisia capillaris* leaves are alternate, basal large and petiolate, upper often subsessile to sessile, undivided or toothed-shallowly to deeply incised or lobed, palmati or pinnatipartite or pinnatisect. The Flowers is yellowish and tubular. Ray-florets: pistillate and fertile; corolla narrowly tubular, generally tapering upwards, toothed, oblique, eligulate; style exserted, 2-cleft, branches recurved, linear-filiform and terete-oblong, flattened (eFloras.org, 2006). *Artemisia capillaris* was introduced to this country from Asia (Duane and Martha, 2006; Plant for Future, 2006), America and Europe (Duane and Martha, 2006). It is in flower from August to October, and the seeds ripen from September to October for country that had seasonal (Plant for Future, 2002). It is cultivated in China, Japan, Taiwan (ShamShop.net, 2002) and some extent in this country. Small acreages of *Artemisia capillaris* have been grown successfully as a commercial crop.

About 2,000 years before *Artemisia capillaris* has been used in Chinese herbal medicine. This *Artemisia capillaris* considered to be a bitter and cooling herb, clearing "damp heat" from the liver and gall ducts and relieving fevers (Chevallier, 1996). *Artemisia capillaris* widely used in Asia to prevent and treat neonatal jaundice, also effective remedy for liver problems, works on stomach and spleen (Chevallier, 1996; Huang *et al.,* 2003; Abestmall, 2006). Modern research has confirmed that the plant has a tonic and strengthening effect upon the liver, gallbladder and digestive system (Chevallier, 1996). The studied from Hong *et al*., 2004 suggest that *Artemisia capillaries* can be a useful therapeutic agent for endotoxin-induced inflammation and injuries of the liver.

DNA extraction is a routine procedure to collect DNA for subsequent molecular or forensic analysis. Extracted genomic DNA contains nuclear and mitochondrial DNA, if DNA is extracted from plant material it will also contain chloroplast DNA. Each of these types of DNA has forensic, diagnostic and phylogenetic uses, and makes use of the polymerase chain reaction (PCR) to obtain specific information from the DNA. Purified DNA may also be used for studying DNA structure and chemistry, examining DNA-protein interactions, carrying out DNA hybridizations, and for cloning and sequencing (Jimmy and Larry, 2005). The problem of DNA extraction is still an important issue in the field of plant molecular biology. Various plants contain high levels of polysaccharides and many types of secondary metabolites affecting DNA purification (Zidani et al., 2005). According to Henry, (2001) yield and quality of DNA often varies among plant tissue types. Besides, purification of genomic DNA in plant is difficult due to co-extraction of high quantities of tannins, polyphenols and polysaccharides (Shepherd *et al*., 2002). Isolation of plant nucleic acids for use in Southen blot analysis, polymerase chain reaction (PCR) amplifications, restriction fragment length polymorphisms (RFLPs), arbitrary primed DNA amplifications

(RAPD, SSR-PCR), and genomic library construction is one of the most important and time-consuming steps (Zidani et al., 2005).

MATERIAL AND METHODS

Sample Collections.

The samples of *Artemisa capillaris* were collected from the area in Kuala Terengganu, Terengganu. 6 samples were collected randomly around this area.

Kit Wizard™ Genomic DNA Purification (Promega)

DNA from *Artemisia capillaris* leave was extracted from the samples by using Kit Wizard™ Genomic DNA Purification (Promega). About 70 mg of *Artemisia capillaris* leave were used for the extraction of the DNA. 600 µl of nuclei lysis were added to the *Artemisia capillaris* leave into 1.5 ml micro centrifuge tube. The mixture then were homogenized to get the lysat. Then the samples were incubated in the water bath at 65°C for about 15 to 20 minutes. After that, it were treated with 3.0 µl of RNase. The samples then were incubated again in water bath at 37°C for 15 to 20 minutes. Next step, the samples were left at room temperature for 5 minutes.

About 200 µl Protein Precipitation (protenase) were added in the samples and then the samples were vortex at highest maximum speed for about 20 seconds. Then the samples were centrifuged at 14,000 rpm at room temperature for 3 minutes. The supernatant that contain DNA were removed to put into a new micro centrifuge which contains 600 µl of isopropanol. The samples were centrifuged once again at 14,000 rpm at room temperature for 2 minutes. Next step is 600 µl of ethanol (70%) were added to the pellet to wash the DNA. Once again the samples were centrifuged at 14,000 rpm at room temperature for 1 minute. Then the DNA were dried at room temperature for 10 to 15 minutes. Then the DNA were resuspended with 100 µl of "DNA rehydration" for 1 hour. The DNA extraction samples were keep at -20°C to avoid DNA from degradation.

Phenol-Chloroform Method

DNA was extracted based on the Phenol-chloroform method described by Brown *et al.* (1991) with some modifications. Digestion buffer at volume of 500 µl containing (1% (w/v) Sodium Dodecyl Sulphate 0.8%, Triton X-100, 0.5 M NaCl, 0.1 M Tris- Hcl at pH 9, 0.01 M EDTA) were added into 1.5 ml microcentrifuge tube which containing 70 mg all snail body tissue and then the 40 µl of 10 % (w/v) SDS and Proteinase K (20 mg/ml solution) were added. The tube was shaken gently and was incubated at 55^0C for 1 to 2 hours. The sample was treated with 25 µl of RNase. Then, the mixture was left at room temperature for 15 to 30 minutes. The sample were treated with 500µl of phenol:chloroform:isoamyl alcohol (25:24:1) and gently the tube were vortexed to homogenize.

The sample was left at room temperature for 10 minutes before doing centrifugation at 13,000 rpm for 5 minutes. The top later is aqueous and were remove and dispersed into the new microcentrifuge tube. The step of adding phenol:chloroform:isoamyl alcohol were repeated twice. The samples were treated with 500 µl of chloroform:isoamyl alcohol (24:1) and were centrifuged at 13,000 rpm for 5 minutes. The upper aqueous layer was mixed with 1 ml of ice-cold absolute ethanol by rapid inversion of the tubes several times. Then, centrifuge at 6,000 rpm for 30 minutes and after that the precipitated DNA were collected at the bottom tubes as a white pellet. The pellet was washed with 500 µl of 70 % of ethanol and was centrifuge at 6,000 rpm for 15 minutes. The DNA was allowed to dry at room temperature. Then resuspended with 100 µl TE buffer (10 mM Tris and 1 mM EDTA, pH 8) for at least 24 hours at room temperature to fully dissolved before proceeding to the next step. This DNA extraction samples will be kept in – 20^0C to avoid DNA degradation.

CTAB Method

The genomic DNA plantlets were extracted with modified CTAB method (Doyle and Doyle, 1987). Appropriately 50 mg of *Artemisia capillaris* leave were grounded with pre-chilled mortar and pestle in liquid nitrogen. The samples were suspended in 800µl of CTAB buffer [2% (cetryltrimethylammonium bromide) CTAB, 100mM Tris pH8, 20mM EDTA, 1.4M NaCl, 2% PVP 40] and 20µl β-mercaptoethanol will be added per 10ml CTAB solution]. Subsequently, an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) were incubated at 60^oC for one hour. The mixtures were subsequently inverted 6 to 10

times to allow mixing and centrifuge at 12000rpm for 10 minutes. The aqueous phase were transferred into new tube, then 500µl of chloroform:isoamyl alcohol (24:1) were added and centrifuge again at 12000 rpm for 10 minutes. Then the supernatant were transferred into new tube and 750 µl cold isopropanol were added to precipitate the DNA. The tube were gently inverted several times until precipitation occurred, otherwise incubated at -20^{o}C for one hour or over night, then spin at 12000rpm for 12 minutes. The supernatant discarded and the pallets were washed in 500 µl 70% cold ethanol. The pallets were vacuum dry for 1 to 4 minutes and then were dissolves in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The contamination of RNA will be removed by digestion with RNAase (10 µg ml^{-1}) for 30 minutes at 37^{o}C.

Sodium Dodecyl Sulphate Method

DNA were extracted based on the Sodium Dodecyl Sulphate method described by Dellaporta *et al.* (1983) with some modifications. About 70 mg leaves sample were used to extraction of DNA. About 500 µl of extraction buffer were added into 1.5 ml micro centrifuge tubes that contain samples. The mixtures were grinded with grinder until the tissue break into small pieces. Then the samples were incubated in the water bath at 65^{0}C about 1 hour. After that the mixtures were tapping the micro tube gently from time to time. The samples were treated with 2 µl of RNase A. Then, the mixtures were incubated at 37 ^{0}C about 30 minute. The samples were added with 170 µl of 5 M potassium acetate and were mixed gently. Next step is the samples were incubated on ice for 20 minutes.

About 600 µl chloroform:isoamyl alcohol (24:1) were added in the samples and then the samples were mixed gently. Then, the samples were centrifuged at 13, 000 rpm for 10 minute. The top layer is aqueous and were removed (600 - 700 µl) into the new 1.5 micro centrifuge tube. The samples were added with 600 µl isopropanol and were mixed gently by inverting. Then, were centrifuge at 13, 000 rpm for 15 minutes and after that the precipitated DNA were collected at the bottom tubes as pellet. The pellet were washed with 500 µl ethanol 70% and then were centrifuged at 13, 000 rpm for 2 minute. After that the DNA were air dry or leave overnight for get only the DNA. The DNA were dissolved in 100 µl dionise water or depends on the pellet size.

Sarkosyl Nitrogen Method

Fresh and healthy leafs were using and were placed them in a mortar. Then, were freeze in liquid nitrogen and the material were crush to a fine powder with a pestle. The powders were added to 3ml of DNA extraction buffer in a fresh mortar and were homogenized. Then, 1 ml phenol were added and were homogenized again. The mixed were transferred to a test tube (with cap), 2 ml phenol were added again and were centrifuged for 5 minute to separate phase. The upper aqueous phase were transferred into new tube. Then, two volume of ice-cool 95% ethanol were added to the aqueous phase for ethanol precipitated DNA and were centrifuged for 5 minutes, 12, 000 rpm. Then, the ethanol were pour from tube. Precipitated DNA were washed with ice-cool 70% ethanol. DNA were dissolve in 0.5 ml of TE and 2 µg RNAase were added. Then, were incubated at 37 ^{0}C for 15 to 30 minute. 0.25 ml phenol and 0.25 ml chloroform were added and were shaking well. Then, were centrifuged and the upper aqueous phase were transferred into new tube. Then, two volume of ice-cool 95% ethanol were added to the aqueous phase for ethanol precipitated DNA and were centrifuged for 5 minutes, 12, 000rpm. Then, the ethanol were pour from tube. Precipitated DNA were washed with ice-cool 70% ethanol. Finally, will be dissolve in 0.2 to 0.5 ml of TE.

Agarose Gel Electrophoresis

An aliquot of 10 µl of genomic DNA from each sample was mixed with 2.5 µl of loading dye. Then the mixture was separated by agarose gel electrophoresis through 1.0 % of agarose gel in 1.0 X TBE (10 mM Tris, 1mM EDTA pH 8.0). After that the electrophoresis gel were started at 55 volts for 1 to 2 hours. Next the gel was stained with ehidium bromide (EtBr) for 20 to 30 minutes and then was washed with distilled water for 5 to 10 minutes. Then the gel were done photographing with Image Master VDS.

Measurement of DNA Purity and Quality

The genomic DNA extracted was measured using a UV- spectrophotometer at 260 nm and 280 nm. The purity of DNA was determined by calculating the ratio of absorbance at 260 nm to that of 280 nm.

The average value of pure preparations of DNA and RNA is between 1.8 and 2.0 respectively (Sambrook *et al.,* 1989). The DNA concentration was determined by the formula:

DNA concentration = OD_{260} × 50 µg/ml × dilution factor (Linacero *et al.,* 1998).

Screening of PCR

Operon 10 mers Kit A were used in this study. 20 RAPD primers from Kit A (with 60% - 70% G-C) content were screened from a single individual. Primers that have the basic of sharpness, clarity of the profile and the existence of polymorphism were chosen for further study (D'Amato and Corach, 1997). The amplification were programmed at 45 cycles for 30 seconds of denaturation at 94^{o}C, 30 seconds of annealing temperature at 36^{o}C, 1 minutes of primers extension at 72^{o}C and final extension of 2 minutes at 72^{o}C.

RESULTS

Extraction of DNA

The Genomic DNA was successfully extracted and observed to have impurity. Sarkosyl method had a good result, the purity of DNA (gel) is high and clear banding pattern was obtained in this study. The DNA banding patterns were shown in Figure 1.

Purity and Quantity of DNA

The DNA purity of *Artemisia capillaris* with Sarkosyl Method was ranged from 1.3114 to 1.4655, Kit Wizard™ Genomic DNA Purification (Promega) 1.1429 to 1.1593, CTAB Method 1.3097 to 1.5652, SDS Method 1.3828 to 1.7388 and Phenol-Chloroform Method 0.9823 to 1.0382. The range was estimated quantitatively from the ratio between the reading of absorbancy at 260nm and 280nm ($OD_{260}/_{280}$) in UV-Spectophotometer. Quantity of DNA with Sarkosyl Method was calculated ranged from 106.25 to 1567.5µg/mL, Kit Wizard™ Genomic DNA Purification (Promega) ranged from 1182.5 to 2420µg/mL, CTAB Method ranged from 360 to 1268.75µg/mL, SDS Method ranged from 550 to 1350µg/mL and Phenol-Chloroform Method ranged from 2687.5 to 4798.75µg/mL. The values of the DNA purity and quantity are shown in Table 1.

Screening of RAPD Primers

Twenty primers from the Operon 10 mers (Operon Kit A) (OPA 01 to OPA 20) with 60% – 70% GC content were used during the screening of the RAPD primers. The banding patterns which were clear and reproducible bands selected. Photograph showing the RAPD electrophoresis profiles are presented in Figures 2. Out of these, only thirteen of the primers that showed polymorphisms. Number of bands generated by each primer varies, ranging from 0 to 8. Primer OPA 18 formed the highest band number (8). There were 56 fragments generated by these primers (OPA 01 – OPA 20). The sizes of bands were ranged from 250 bp to 1750 bp.

Table 1: Observed density (OD) of DNA purity and quantity of DNA for Genomic DNA extracted (Sarkosyl Method (S1- S6), Kit Wizard™ Genomic DNA Purification (Promega) (K1-K6), CTAB Method (C1-C6), SDS Method (D1-D6) and Phenol-Chloroform Method (P1-P6)).

Sample	OD 260			OD 280			Qualification ratio (Quality)	DNA C (Quantity)(ng)
	1	2	Mean	1	2	Mean		
S1	0.043	0.042	0.0425	0.029	0.029	0.0290	1.4655	106.25
S2	0.627	0.627	0.6270	0.443	0.444	0.4435	1.4138	1567.50
S3	0.452	0.452	0.4520	0.309	0.311	0.3100	1.4581	1130.00
S4	0.359	0.390	0.3895	0.268	0.270	0.2690	1.4480	973.75
S5	0.440	0.438	0.4390	0.307	0.307	0.3070	1.4300	1097.50
S6	0.478	0.478	0.4780	0.365	0.364	0.3645	1.3114	1195.00
K1	0.473	0.473	0.4730	0.408	0.408	0.4080	1.1593	1182.50
K2	0.619	0.619	0.6190	0.537	0.537	0.5370	1.1527	1547.50
K3	0.968	0.968	0.9680	0.848	0.847	0.8475	1.1429	2420.00
K4	0.960	0.961	0.9605	0.835	0.836	0.8355	1.1496	2401.25
K5	0.823	0.823	0.8230	0.714	0.714	0.7140	1.1527	2057.50
K6	0.542	0.541	0.5415	0.466	0.466	0.4660	1.1620	1353.75
C1	0.144	0.144	0.1440	0.092	0.092	0.0920	1.5652	360.00
C2	0.276	0.276	0.2760	0.204	0.203	0.2035	1.3563	690.00
C3	0.507	0.508	0.5075	0.387	0.388	0.3875	1.3097	1268.75
C4	0.272	0.273	0.2725	0.194	0.195	0.1945	1.4010	681.25
C5	0.366	0.366	0.3660	0.262	0.262	0.2620	1.3969	915.00
C6	0.344	0.344	0.3440	0.253	0.253	0.2530	1.3597	860.00
D1	0.352	0.353	0.3525	0.211	0.211	0.2110	1.6706	881.25
D2	0.220	0.220	0.2200	0.130	0.130	0.1300	1.6923	550.00
D3	0.233	0.233	0.2330	0.134	0.134	0.1340	1.7388	582.50
D4	0.316	0.317	0.3165	0.195	0.195	0.1950	1.6231	791.25
D5	0.262	0.263	0.2625	0.164	0.165	0.1 645	1.5957	656.25
D6	0.539	0.514	0.5400	0.390	0.391	0.3095	1.3828	1350.00
P1	1.880	1.880	1.8800	1.904	1.904	1.9040	0.9874	4700.00
P2	1.577	1.577	1.5770	1.519	1.519	1.5190	1.0382	3942.50
P3	1.075	1.075	1.0750	1.062	1.062	1.0620	1.0122	2687.50
P4	1.904	1.909	1.9065	1.931	1.931	1.9310	0.9873	4766.25
P5	1.909	1.913	1.9110	1.931	1.936	1.9335	0.9884	4777.50
P6	1.922	1.917	1.9195	1.954	1.954	1.9540	0.9823	4798.75

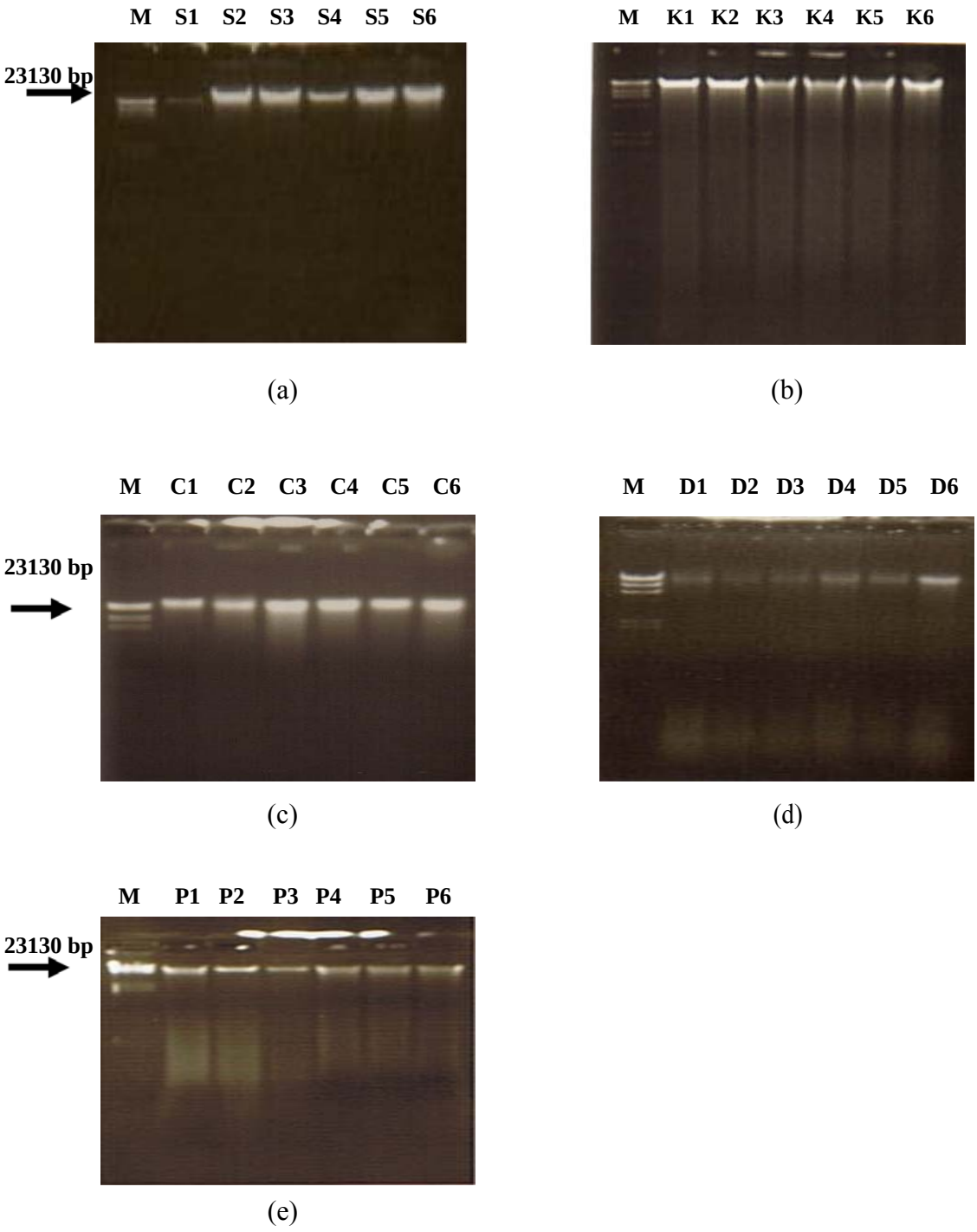
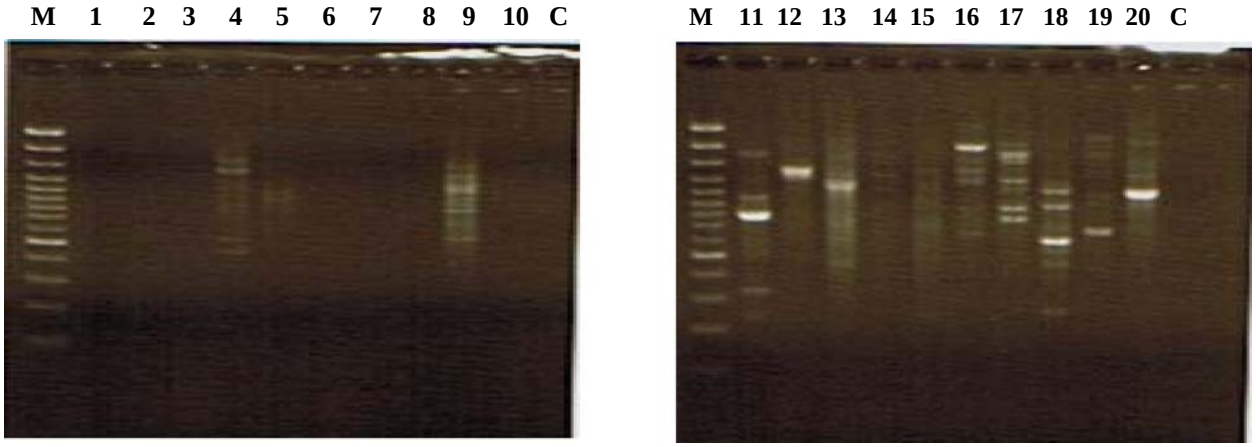


Figure 1: Genomic DNA extracted by Sarkosyl Method (a), Kit Wizard™ Genomic DNA Purification (Promega) (b), CTAB Method (c), SDS Method (d) and Phenol-Chloroform Method (e) on 1.0% agarose gel and stained with 1 µg/mL ethidium bromide (EtBr), λ DNA/Hind III marker (lane M) and samples of *Artemisia capillaris* (lane S1 to S6 (a), lane K1 to K6 (b), lane C1 to C6 (c), lane D1 to D6 (d) and lane P1 to P6 (e)).



(a) (b)

Figure 2: RAPD banding patterns for screening of 1st base primers, (a) OPA 01 to OPA 10 (lane 1 to 10), (b) OPA 11 to OPA 20 (lane 11 to 20), (Lane M is a marker 100bp ladder plus, Lane C is a control).

DISCUSSION

Extraction DNA from samples is the first step for all molecular marker type. DNA can be extract either from fresh, lyophilized, preserved or dried samples but for obtaining good quality DNA fresh material is recommended (Semagn *et al*., 2006). There are difficult to get plant DNA free from contaminating proteins and polysaccharides. Different methods need for different plants that contain divers secondary compounds that interfere with the extraction (Croy *et al*., 1993).

The DNA genomic extracted (Figure 1) from method Kit Wizard™ Genomic DNA Purification (Promega), CTAB Method, Sodium Dodecyl Sulfate (SDS Method) and Phenol-Chloroform Method had high of impurity. Sarkosyl method got clean DNA compare other methods. According to Croy *et al*., 1993, most the plants cells had very tough cell wall and make used vigorous method to breaking the cell. The excessive force make the degradation very high molecular weight molecules thought the shearing.

Agarose gel really use for check whether the DNA is degraded or not. Spectrophotometer measures the intensity of absorbance of DNA solution at 260 nm wavelength, and also indicates the presence of protein contaminants but it does not tell the condition of the DNA which is degraded or not (Semagn *et al*., 2006). The quality of the DNA by Sarkosyl Method was better than others methods. The Sodium Dodecyl Sulfate (SDS Method) had good quality but got poor results for gel electrophoresis and had contamination with RNA. So the best result was Sarkosyl Method that had no contamination by protein or polysaccharides and also good in quality. Kit Wizard™ Genomic DNA Purification (Promega), CTAB Method and Phenol-Chloroform Method got poor quality of DNA. The best quality is between 1.8 – 2.0 (Sambrook *et al.,* 1989). None of the methods gave such high values, the range between 0.9823 – 1.7388. The ratio of 1.3144 – 1.4655 by method Sarkosyl Method was used to RAPD analysis (Table 1).

Plants contain three types of DNA like nuclear, mitochondrial and chloroplast DNA (Rudi et al., 1997). All preparation methods for extraction involve the removal of the cell wall and nuclear membrane around the DNA, cell wall debris, proteins, lipid or RNA. Removal of membranes lipids is facilitated by using detergents such as sodium dodecyl sulphate (SDS), Cetyltrimethylammonium bromide (CTAB), mixed alkyl trimethyl-ammonium bromide (MTAB) (Segman *et al*., 2006) and Sarkosyl (Rudi *et al*., 1997).

DNA should be protected from endogenous nucleases and EDTA complexes magnesium ions were included in the extraction buffer that is a necessary cofactor for most nucleases (Rudi *et al*., 1997; Segman *et al*., 2006). DNA extracts often contain a large amount of RNA, proteins, polysaccharides, tannins and pigments. RNAs are removed using RNA degrading enzyme called RNase A in all method we used. For proteins remover, proteinase-K are using in Phenol-Chloroform Method, Sarkosyl in Sarkosyl Method, Protein Precipitation Solution in Kit Wizard™ Genomic DNA Purification (Promega). Phenol also used as removable for proteins. Polysaccharide more difficult to remove, NaCl, together with CTAB is known to remove polysaccharides (Murray and Thompson, 1980; Rudi *et al*., 1997; Paterson *et al*., 1993). Some protocols replace NaCl by KCl (Thompson and Henry, 1995). According to Fang et al., 1992, polysaccharides remain dissolved in ethanol. Chloroform also used for remove polysaccharides.

The separation by centrifuged when DNA with other compounds such as lipids, proteins, carbohydrates, and/or phenols. The DNA precipitated in salt solution with sodium acetate for Phenol-Chloroform Methods, isopropanol for Kit Wizard™ Genomic DNA Purification (Promega), SDS method and CTAB method, and ethanol for Sarkosyl Method. Plant that had high polyphenolic content, can used phenol that work together with SDS to extract it (Puchooa, 2004). But, SDS-phenol tends to produce low yields of DNA (Rezaian and Krake, 1987).

RAPD is one of the genetic marker studies that had been used in genetic study. RAPD technique had been used in genetic study for wheat (Devos and Gale, 1992) and also had used for filogenetic relations for padi species (Ishii, 1996) . Chosen suitable primers is very important process for PCR-RAPD to get clear and good band. Twenty primers had been used and amplification showed differences banding pattern (Figure 2). From twenty primers, the best primer was primer OPA 18, because produced clear and sharp banding pattern. Differences primers produces differences fragment pattern. These happened because every primer contents G + C and difference base sequences (parenrengi, 2000). Results showed good PCR-RAPD for DNA extraction from Sarkosyl Method (Figure 2). Similarly, quantity and purity of extracted genomic DNA also plays crucial role for analysis of molecular diversity and optimization of different parameters for PCR (Weeden *et al*., 1992; Staub *et al*., 1996).

CONCLUSION

Sarkosyl Method are the best extraction for *Atemisia capillaris* according to concentration of the extracted DNA from gel electrophoresis (DNA yield and purity) and spectrophotometer (DNA quantity) compare the others method. The screening of primers from DNA extraction (Sarkosyl Method) showed OPA 04, OPA 09, OPA 11, OPA 16, OPA 17, OPA 18, OPA 19 and OPA 20 are suitable primers to use in genetic studies for *Artemisia capillaris*. Based on screening results, Sarkosyl Method is suitable to use in PCR-RAPD studies.

Corresponding author:
Mohammad Shafie B. Shafie
Department of Agrotechnology
Faculty of Agrotechnology and Food Science
Universiti Malaysia Terengganu
21030 Mengabang Telipot
Kuala Terengganu
Terengganu, Malaysia
E-mail: msshafie@yahoo.com.my

REFERENCES

Abestmall, (2006). Artemisia Capillaris Herb (Yin Chen Hao) C/S 16 oz: V. [online]. http://www.abestmall.com/best/1862375.html [Accessed on 22sd November 2006]

Brown, T. A. (1991). Essential Molecular Biology, A Practical Approach. *Oxford University Press*. Ney York, pp. 71.

Chevallier, A. (1996) . *The Encyclopedia of Medicinal Plants* Dorling Kindersley. London. ISBN 9-780751-303148. An excellent guide to over 500 of the more well known medicinal herbs from around the world.

Croy, E. J., Ikemura, T., Shirsat, A. and Croy, R. R. D. (1993). Plant Nucleic Acids. Plant Molecular Biology. Bios Scientific Publishers. Blackwell Scientific Publications. UK.

Dellaporta, S. L., Wood, J and Hicks, J. B. (1983). Plant Molecular Biology Rep., 1, 19.

Devos, K. M. and Gale, M. D. (1992). The Random Amplified Polymorphic DNA markers in wheat. Theorical Applied Genetics. 84:567-572.

Dharmananda, S. (2002). CHING-HAO and the Artemisias Used in Chinese Medicine *Ph.D., Director, Institute for Traditional Medicine, Portland, Oregon.*

Doyle, J. J. and Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Focus 12: 13-15.

Duane and Martha. (2006). Instituto de Alternativas para Sobresalientes. Lulu, Inc.
Wormwoods: Botanical and Common Names [online]. http://www.innvista.com/health/herbs/wormwood.htm [Accessed on 25th November 2005]

D'amato, M.E ; Corach, D. (1997) Population genetic structure in the fresh water *anomuran aesla jujuyana* by RAPD analysis.

eFloras.org, (2006). Flora of Pakistan. Asteraceae: Pakistan V. 207 Page 93 [online] http://www.efloras.org/florataxon.aspx?flora_id=5&taxon_id=102682 [Accessed on 2sd December 2006]

Fang, G. S., Hammer, S. and Grumet, R. (1992). A quick and inexpensive method for removing polysaccharides from plant genomic DNA. Biotechniques 13:52–57.

Henry, R. J. (2001). Plant DNA Extraction. In Plant Genotyping - the DNA fingerprinting of plants. (ed. Henry, R.J.), CAB International, United Kingdom. pp. 239 – 249.

Hong, S. H., Seo, S. H., Lee, J. H. and Choi, B. T. (2004). The aqueous extract from *Artemisia capillaris* Thunb. inhibits lipopolysaccharide-induced inflammatory response through preventing NF-κB activation in human hepatoma cell line and rat liver. International Journal of Molecular Medicine 13: 717-720.

Huang, W., Zhang, J. and Moore, D. D. (2003). A traditional herbal medicine enhances bilirubin clearance by activating the nuclear receptor CAR. *J. Clin. Invest.* 113:137-143 (2004). doi:10.1172/JCI200418385.

Ishii, T. (1996). Philogenetic relationship in A-genome species as revealed by RAPD analysis. Genes Genet System 71:195-201.

Jimmy, W. and Larry, S. (2005). Wikipedia. 2006. The free encyclopedia [online]. Mediakiwi. http://en.wikipedia.org/wiki/DNA_extraction [Accessed on 14th August 2005]

Linacero, R., Rueda, J. and Vazquez, A. M. (1998). Quantifying of DNA. Karp, A., P. G. Isaac, and D. S Ingram (Eds.) Molecular Tools for Screening Biodiversity: Plants And Animals. Chapman and Hall. London, Weinheim, New York, Tokyo, Melbourne, Madras. pp 18-21.

Murray, M. G. and Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 8:4321-4325.

Parenrengi, A., Shamsuddin, L., Ismail, P. and Nakisah, M. A. (2000). RAPD Fingerprinting of *Areolated Grouper, Epinephelus arealatus*. On National Fisheries Symposium, 31 October to 2 November 2000, Grand Blue Ware Hotel, Johor Bharu, Malaysia.

Paterson, A. H., Brubaker, C. L. and Wendel, J. F. (1993) . A rapid method for extraction of Cotton (Gossypium spp) genomic DNA suitable for RFLP or PCR analysis. Plant Mol. Biol. Rep. 11:122-127.

Plants For A Future, (2004). Edible, medicinal, and useful plants for a healthier word: *Artemisia capillaris* - Thunb. Yin Chen Hao [online]. http://www.pfaf.org/database/plants.php?Artemisia+capillaris [Accessed on 22sd November 2006]

Puchooa, D. (2004). A simple, rapid and efficient method for the extraction of genomic DNA from lychee *(Litchi chinensis* Sonn.). Afr. J. Biotechnol. 3: 253-255.

Rezaian, M. A. and Krake, L. R. (1987). Nucleic acid extraction and virus detection in grapevine. J. Virol. Methods 17: 277-285.

Rudi, A., Suhaimi, N. and Harikrishna, K. (1997). DNA Technology. A Master Class in New Technologies for the Measurement of Biodiversity. The Universiti Pertanian Malaysia, Serdang, Malaysia.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989). Editors. Analysis and cloning of eukaryotic genomic DNA. In: Molecular cloning: a laboratory manual. 2nd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.

Semagn, K., Bjornstad, A. and Ndjiondjop, M. N. (2006). An overview of molecular marker methods for plants. African Journal of Biotechnology Vol. 5 (25) pp. 2540-2568. ISSN 1684–5315 © 2006 Academic Journals.

ShamShop.net, (2002). Artemisia Capillaris Herb (Yin Chen Hao) Cut & Sifted 500 gm (1.17 lb): V [online]. http://www.shamanshop.net/store/proddetail.cfm/ItemID/160526.0/CategoryID/15500.0/SubCatID/3770.0/file.htm [Accessed on 2sd December 2006]

Shepherd, M., Cross, M., Stokoe, L. R., Scott, L. J. and Jones, M. E. (2002). Highthroughput DNA extraction from forest trees. Plant Mol. Biol. Reptr. 20: 425a-425j.

Staub, J. J., Bacher, J. and Poeter, K. (1996). Sources of potential errors in the application of random amplified polymorphic DNAs in cucumber. Hort.Sci. 31: 262 – 266.

Thompson, D. and Henry, R. J. (1995). Single step protocol for preparation of plant tissue for analysis by PCR. Biotechniques 19:394-397.

Weeden, N. F., Timmerman, M., Hermmat, M., Kneen, B. E. and Lodhi, M. A. (1992). Applications of RAPD Technology to Plant Breeding, Symposium Proceeding, Minneapolis. pp. 12 –17.

Zidani, S., Ferchichi, A. and Chaieb, M. (2005). Genomic DNA extraction method from earl millet (*Pennisetum glaucum*) leaves. African Journal of Biotechnology Vol. 4 (8), pp. 862-866.

Note: This article was primarily published in [New York Science Journal. 2009;2(1):13-21]. (ISSN: 1554-0200).