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Full Length Research Paper

Efficient method for the extraction of genomic DNA from wormwood (*Artemisia capillaris*)

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Suitable method for isolation of genomic DNA is really important. The best method can make the best result for genetic study. About five differences methods were used amongst which were Sarkosyl Method, CTAB method, Kit Method, SDS Method and Phenol-Chloroform Method. Isolated genomic DNA showed high purity and high quantity in Sarkosyl method compare the other methods. The Sarkosyl DNA isolation method is suitable for restriction digestion and genetic study for *Artemisia capillaris*.

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

INTRODUCTION

Artemisia capillaris is a species from class Magnoliopsida and family Asteraceae. *A. capillaris* also know as wormwood or wormwood capillary in Europe (ShamanShop.net, 2002), Yin Chen Hao in China (ShamanShop.net, 2002; Dharmananda, 2002) and Pokok Ru Nyamuk and Pokok Daun Ru in Malaysia. *A. capillaris* is a member of the parsley family, a strong-smelling, fennel-like, annual plant reaching a height of about 4 feet or more. About 2,000 years before, *A. 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). *A. capillaris* is widely used in Asia to prevent and treat neonatal jaundice; it is also an effective remedy for liver problems, and 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 report of Hong et al. (2004) suggests that *A. 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). This investigation evaluates various methods for the efficient extraction of genomic DNA from *A. capillaries*.

MATERIALS AND METHODS

Sample collection

The samples of *A. capillaris* were collected from the area in Kuala Terengganu, Terengganu. Six samples were collected randomly

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around this area.

Kit wizard™ genomic DNA purification (Promega)

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

About 200 µl Protein Precipitation (protenase) were added in the samples and then the samples were vortex at highest maximum speed for about 20 s. Then the samples were centrifuged at 14,000 rpm at room temperature for 3 min. The supernatant that contain DNA were removed and placed 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 min. 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 min. Then the DNA were dried at room temperature for 10 to 15 min and resuspended with 100 µl of "DNA rehydration" for 1 h. 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 (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 and 0.01 M EDTA were added into 1.5 ml microcentrifuge tube containing 70 mg of 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°C for 1 to 2 h. The sample was treated with 25 µl of RNase. Then, the mixture was left at room temperature for 15 to 30 min. The sample were treated with 500 µl 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 min before doing centrifugation at 13,000 rpm for 5 min. The top aqueous layer were removed and dispersed into the new microcentrifuge tube. The step of adding phenol: chloroform: isoamyl alcohol was repeated twice. The samples were treated with 500 µl of chloroform: isoamyl alcohol (24:1) and were centrifuged at 13,000 rpm for 5 min. 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 min 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% ethanol and was centrifuge at 6,000 rpm for 15 min. 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 h at room temperature to fully dissolved before proceeding to the next step. This DNA extraction samples will be kept in – 20°C to avoid DNA degradation.

CTAB method

The genomic DNA plantlets were extracted with modified CTAB method (Doyle and Doyle, 1987). Appropriately 50 mg of *A. capillaris* leave were grounded with pre-chilled mortar and pestle in liquid nitrogen. The samples were suspended in 800 µl of CTAB buffer (2% [cetryltrimethylammonium bromide] CTAB, 100 mM Tris pH 8, 20 mM EDTA, 1.4 M NaCl, 2% PVP 40) and 20 µl β-mercaptoethanol were added per 10 ml CTAB solution.

Subsequently, an equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) were incubated at 60°C for 1 h. The mixtures were subsequently inverted 6 to 10 times to allow mixing and centrifuged at 12000 rpm for 10 min. The aqueous phase was transferred into new tube, then 500 µl of chloroform: isoamyl alcohol (24:1) was added and centrifuged again at 12000 rpm for 10 min. 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°C for 1 h or over night, then spin at 12000 rpm for 12 min. The supernatant discarded and the pallets were washed in 500 µl 70% cold ethanol. The pallets were vacuum dry for 1 to 4 min and then were dissolves in 100 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The contamination of RNA was removed by digestion with RNAase (10 µg ml^{-1}) for 30 min at 37°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 extract 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°C about 1 h. After that the mixtures were mixed by 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°C about 30 min. The samples were added to 170 µl of 5 M potassium acetate and were mixed gently. The samples were then incubated on ice for 20 min.

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 min. The top layer is aqueous and were removed (600 - 700 µl) into a new 1.5 micro centrifuge tube. The samples were added with 600 µl isopropanol and were mixed gently by inverting, centrifuged at 13, 000 rpm for 15 min 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 min. After that the DNA were air dry or leave overnight to get only the DNA. The DNA was dissolved in 100 µl dionise water depending on the pellet size.

Sarkosyl nitrogen method

Fresh and healthy leaves were placed in a mortar, freeze in liquid nitrogen and the material were crush to a fine powder with a pestle. The powders were added to 3 ml of DNA extraction buffer in a fresh mortar and were homogenized. Then, 1 ml phenol was added and was homogenized again. The mixed were transferred to a test tube (with cap), 2 ml phenol were added again and were centrifuged for 5 min to separate phase. The upper aqueous phase was 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 min, 12, 000 rpm. Then, the ethanol was pour from tube. Precipitated DNA was washed with ice-cool 70% ethanol. DNA was dissolve in 0.5 ml of TE and 2 µg RNAase were added and incubated at 37°C for 15 to 30 min. 0.25 ml phenol and 0.25 ml chloroform were added, shaked, 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 min at 12, 000 rpm. Then, the ethanol was pour from tube. Precipitated DNA was

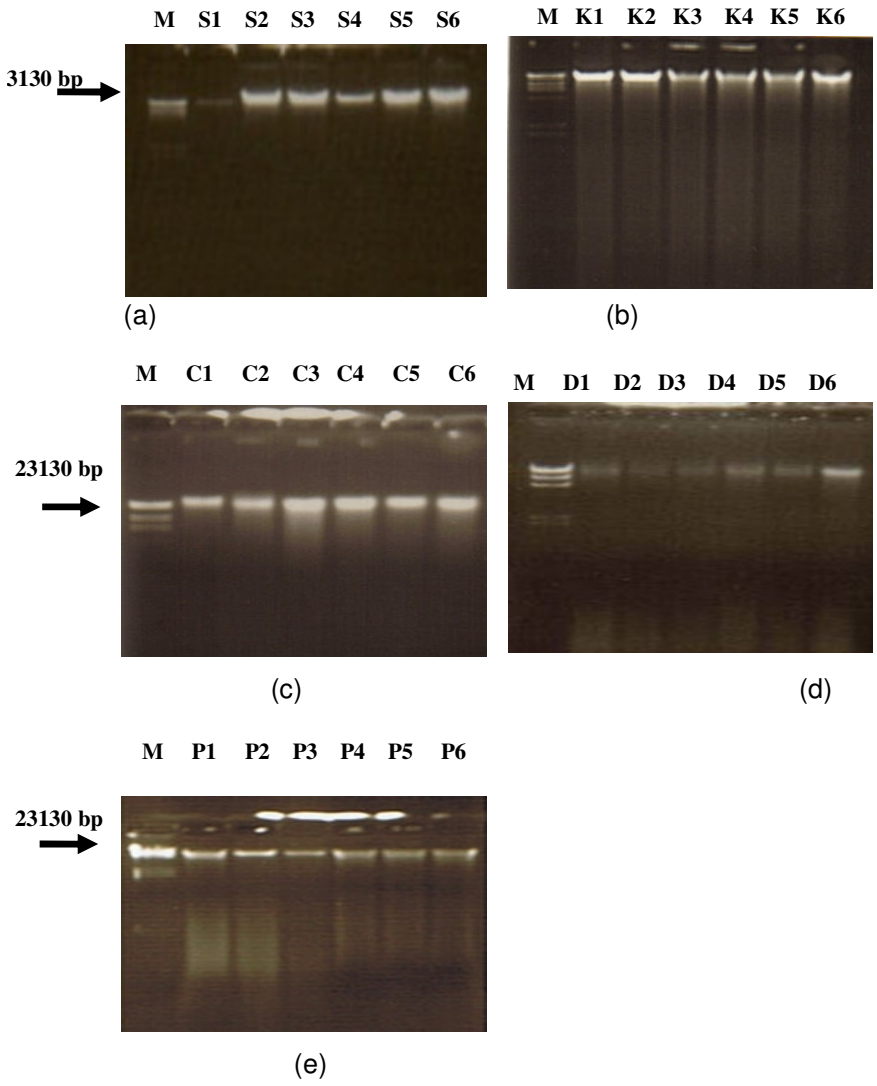

Figure 1. Genomic DNA extracted by Sarkosyl Method (a), Kit Wizard™ Genomic DNA Purification (Promega) (b), CTAB Method (c), SDS Method (d) and Phenol-Chloroform Method (e) on 1.0% agarose gel and stained with 1 µg/mL ethidium bromide (EtBr), λ DNA/Hind III marker (lane M) and samples of *Artemisia capillaris*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

washed with ice-cool 70% ethanol and dissolve in 0.2 to 0.5 ml of TE.

Measurement of DNA purity and quality

The genomic DNA extracted was measured using a UV-spectrophotometer at 260 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).

DNA was electrophoresed on a 1% agarose gel.

RESULTS AND DISCUSSION

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 may need vigorous method to break the cell. The excessive force makes the degradation very high molecular weight molecules thought the shearing.

DNA from Sarkosyl Method showed clear bands that means DNA does not have contamination. While the DNA from other methods showed contamination and

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}			DNA quality (OD_{260}/OD_{280})	DNA conc. (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

smear at gel electrophoreses, which mean DNA is not really pure. The DNA contamination can be from protein and RNA. The amount of RNase and protein removable were suggested to accumulate to get pure DNA. 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).

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 DNA purity of *A. 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 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 range was estimated quantitatively from the ratio between the reading of absorbancy at 260 and 280 nm ($OD_{260}/_{280}$) in UV-Spectophotometer. Quantity of DNA with Sarkosyl Method was calculated ranged from 106.25 to 1567.5 µg/mL, Kit Wizard™ Genomic DNA Purification (Promega) ranged from 1182.5 to 2420 µg/mL, CTAB Method ranged from 360 to 1268.75 µg/mL, SDS Method ranged from 550 to 1350 µg/mL and Phenol-Chloroform Method ranged from 2687.5 to 4798.75 µg/mL (Table 1). 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 contamina-

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)).

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P6	1.922	1.917	1.9195	1.954	1.954	1.9540	0.9823	4798.75

tion by protein or polysaccharides and also good in quality.

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 was 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 used in Phenol-Chloroform Method, Sarkosyl in Sarkosyl Method, Protein Precipitation Solution in Kit Wizard™ Genomic DNA Purification (Promega). Phenol was also used to remove proteins. Polysaccharides are more difficult to remove; NaCl, together with CTAB is known to remove polysaccharides (Murray and Thompson, 1980; Croy et al., 1993; 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 is also used to remove polysaccharides.

The separation was by centrifugation when DNA is contaminated by other compounds such as lipids, proteins, carbohydrates, and/or phenols. The DNA is 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). 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).

According to concentration of the extracted DNA from gel electrophoresis (DNA yield and purity) and spectrophotometer (DNA quantity), we considering Sarkosyl Method the best extraction technique and for DNA isolation in molecular diversity analysis of *A. capillaris*.

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