DNA isolation of clove and lemongrass using modification of the Doyle and Doyle methods and their relation with antioxidant activity

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Abstract. Clove (Syzigium aromaticum) and lemongrass (Cymbopogon winterianus) are essential oil-producing plants that are widely available in Indonesia and have high economic value for medicinal and industrial needs because of their antioxidant activity. However, the information about the relationship between antioxidant activity and the DNA content in these plants was very limited. Especially because DNA isolation of aromatic plants is also not easy to do with methods that are widely used. Therefore, this study aims to isolate DNA from clove and lemongrass using a modified Doyle and Doyle method and to find the relationship between the quality and quantity of DNA with their antioxidant activity. The method used was the use of PVP, isopropanol and sodium acetate for the modification of the Doyle and Doyle method in DNA isolation followed by antioxidant analysis using DPPH. The results showed that the modification to the Doyle and Doyle method had obtained high concentrations of DNA. Besides that, the clove DNA content is higher than lemongrass which is supported by the antioxidant activity of both plants.

1. Introduction

Herbal and aromatic plants like clove and lemongrass have attracted a lot of attention because of their important role in human health. Their chemical compound showed potencies as antioxidant, antimicrobial, antiinflammatory, antieczematic, antipsoriatic, antifungal and antiviral, beside other pharmacological activities[1–4]. Clove (*Syzygium aromaticum*, synonym: *Eugenia cariophylata*) is a type of native Indonesian medicinal plant containing eugenol, eugenyl acetate, caryophyllene, and α-humulene [5,6]. The clove tree has a size of 8-12 m and is a member of the Mirtaceae family originating from the islands of East Maluku. Indonesia is one of the highest clove producers in the world with production reaching 137758 tons in 2020 with an upward trend of 3% per year [7,8]. Other largest clove

producing countries are India, Brazil, Malaysia, Sri Lanka, Madagascar and Tanzania especially the island of Zanzibar. The part of the clove plant that has high economic value is flowers, followed by stems and leaves. The clove flower buds can be harvested after 4 years of planting [2–4].

Lemongrass is a grass plant with a height between 50-100 cm. Chemical compound in citronella oil were Citronellal, Citronellol, geraniol, Isopulegol, Tricyclo[5.2.1.0(1,5)]decane, linalool L, Butanoic acid, Citronellyl acetate, Geranyl acetate, and Elemol as major subtanties[9–11]. The quality of citronella essential oil according to the Indonesian National Standard SNI 06-3953-1995 is that it contains a minimum of 35% citronellal and 85% minimum geraniol [8]. Production of lemongrass in Indonesia was 310 tons in 2014[7–8]. The production centers of lemongrass in Indonesia include the provinces of Aceh, West Java, Central Java, Banten and East Java. Batang Regency is one of the main producers of essential oils in Indonesia, derived from patchouli, cloves, lemongrass, nutmeg and cardamom [12].

Herbal and medicinal plants contain high amounts of many different substances. A number of methods have been reported for efficient DNA isolation of herbal and medicinal plants. However, many of these methods often depend on species. Some of the methods that have been used for DNA isolation from medicinal herbs and aromatic plants are less able to produce good quality DNA for all types of herbal or medicinal plants. This is because each herbal or medicinal plant contains a different type of metabolite which is often in very high amounts that interfere with DNA isolation. There are several things that need to be considered in the DNA isolation process, including producing good quality and quantities of DNA which the method must be effective and applicable to all herbal and medicinal plants. The Doyle and Doyle method using a CTAB was commonly used to obtain DNA in clove and lemon grass plants instead the fact that this method also simple, fast and acurate. The Doyle and Doyle method is widely used for the isolation of plant DNA containing polysaccharides and polyphenol compounds of plants [13–15]

Antioxidants are substances that are able to withstand or slow down the oxidation process of free radicals and protect cells from their harmful effects. This substance is able to inhibit the oxidation of substances that are easily oxidized even in low concentrations. Antioxidant activity shows relationship with plant ability to protect their DNA form oxidative damage[16]. Antioxidants will protect plant DNA from oxidative stress that is mediated by free radicals. Its antioxidant and DNA damage inhibition properties are indicated by its high phenolic compound content. The objective of the study is searching the relationship of DNA from essential oil producing plant with their antioxidant activity. The high antioxidant and DNA damage inhibitor potential of essential oil-producing plants can be used to develop antioxidant compounds for therapeutic applications [17–18].

2. Material and methods

2.1. Material

The materials used in the study is clove and lemongrass leaf from Batang regency

2.2. DNA isolation

DNA isolation was conducted according to Doyle and Doyle method [13] with modification. DNA isolation using the Doyle & Doyle method using buffer CTAB (Cethyl Trimethyl Ammonium Bromide) 2%, 70% ethanol, CIA (Chloroform-Isoamyl-Alcohol), TE buffer and 400 µg of clove and lemongrass leaf samples. The modification was performed with the use of polyvinylpyrrolidone (PVP) 0.01 gr; Isopropanol 2 µl; Sodium acetate (Na-Acetate) 3M 80 ml. It was eliminate the use of merchaptoethanol, ammonium acetate, EDTA, RNase and Sodium chloride. Both ethanol and isopropanol are widely used for recovering DNA from aqueus solution by precipitation with alcohol in the presence of monocovalent like sodium or ammonium. High concentration of EDTA was avoid since it may coprecipitate with the DNA.

2.3. Analiysis of quality and quantity of DNA

The quality and quantity of DNA were analysed using Nanodrop using wavelength 260 nm, 280 nm.

2.4. DPPH Antioxidant Analysis

Determination of DPPH free radical scavenging activity was performaed by added 0.5 mL of extract into 1.5 mL of a 93 µM 1,1-diphenyl-picrylhydrazyl (DPPH) solution in ethanol and vortexed for 2 minutes. The degree of colour change of the solution from purple to yellow indicates the efficiency of free radical scavenger followed by incubation the solution for 30 minutes. The absorbance was measured at a wavelength of 517 nm [14,15]. Free radical scavenging activity is calculated as the percentage reduction in DPPH color using the equation:

$$\text{"Free Radical Counter Activity"} = \left[1 - \frac{\text{Absorbance of Sample}}{\text{Absorbance of Control}}\right] \times 100\%$$

Free radical scavenger activity = 1 - absorbance of control / sample absorbance × 100%

3. Results and discussion

3.1. DNA Isolation of Clove and Lemongrass

The DNA extraction protocol which referred to as Doyle and Doyle method was principally used CTAB, a strong ionic detergent to facilitate the separation of proteins from nucleic acids in extractions of biological materials. This method was commonly used for DNA isolation from plants. According to several researcher this material was not to suitable enough in application to aromatic and medicinal plants to produce high quality and quantity of DNA due to a lot of amount of polysaccharides and polyphenols. Removal of these secondary metabolites requires appropriate reagents for DNA isolation of lemongrass and clove plants. Various methods of DNA extraction were available and being developed experimented for herbal and aromatic plant [13,16–17]. This research were modified from Doyle and Doyle method in the use of polyvinylpyrrolidone (PVP) which removes polyphenolic contamination by binding it through hydrogen bond. The result of DNA isolation from clove and lemongrass was exibited in Table 1 and Table 2.

Table 1. DNA quality and quantity of lemongrass

Sample	Nucleic Acid Concentration	λ_{260}	λ_{280}	$\lambda_{260}/\lambda_{280}$	Sample Type
1	219.1	4.381	2.099	2.09	DNA
2	81.6	1.633	0.71	2.30	DNA
3	96.8	1.935	0.853	2.27	DNA
4	116.6	2.331	1.105	2.11	DNA
5	102.7	2.054	0.921	2.23	DNA
6	124.3	2.486	1.208	2.06	DNA
7	295.9	5.918	2.989	1.98	DNA

The result of extraction using clove and lemongrass leaves using the modification method of Doyle and Doyle with modification produced different results in DNA quality and quantity. DNA concentration and purify of clove leaves were higher than lemongrass. Other researcher gained lower concentration with *Cymbopogon* species about 76 to 90 µg/mL^{-1} of fresh tissues from leaves, while other gaining 179–250 µg/mL^{-1} from *Tinospora cordifolia* and *T. procumbens*. DNA concentration from *Aloe barbadensis, Cissus quadrangulais, Catharanthus roseus* were higher about 390–526 µg/mL^{-1}[18,19]. These concentration were lower comparing with other part of plant due to accumulation of metabolites, pigments and reserved food in leaves of aromatic plants [20,21].

Table 2. DNA quality and quantity of Clove

Sample	Nucleic Acid Concentration	λ_{260}	λ_{280}	$\lambda_{260}/\lambda_{280}$	Sample Type
1	313.4	6.267	4.396	1.43	DNA
2	310.5	6.209	4.448	1.4	DNA
3	445.2	8.904	6.767	1.32	DNA
4	397.1	7.942	6.088	1.3	DNA
5	85.1	1.702	1.25	1.36	DNA
6	248.8	4.975	3.253	1.53	DNA
7	235.8	4.717	3.168	1.49	DNA

The result showed that modification of Doyle and Doyle method conducted in this research was gaining appropriate amount of DNA to be used in further application. Based on the quality of the DNA from leaves of lemongrass and clove, it showed interesting result since both plants showed difference in contamination of DNA based on their purification value. The result of Nanodrop test contained contaminants like RNA and protein. The purity or quality of DNA from plant will have approximately 1.8 to 2.0 at ratio of optical density 260/280 nm that quantified by spectrophotometer [22]. DNA purity below the number 1.8 means that the DNA is contaminated by protein groups and phenolic compounds, whereas if DNA purity is above the number 2.0 it means that DNA is contaminated by RNA groups. A DNA purity of clove less than 2 showed the existence of residual protein or organic solvents in the sample. These contaminant belong to polysaccharides and polyphenols. It was reported that the total phenolic and flavonoid contents of clove were at 474±2.2 mg of GAE/g and 668±1.4 mg of QUE/g. Lemongrass contained phenolic compounds, 125.3 g GAE/g and flavonoids 12,71 mg kg–1 QUE/g. The result also supported by other study in clove plant showed that composition of phytochemical compounds consist of phenolic acids (20.80%); flavonoids (26.81%); tannins (4.90%), saponins (2.60%), alkaloids (1.60%, followed by total protein (17.83%), total carbohydrate (2.23%) and total oil (0.90%)[23,24]. High level of phenolic compound in clove can denature their high proteins and react with cell membrane phospholipid to change cell permeability [25]. Polyphenol is a major component in medicinal plants and present in the vacuole. Polyphenols bind DNA and make downstream processing difficult because it will co-precipitated with DNA [2,26]. Although PVP was use in this research to remove phenolic compounds contaminants from plant DNA extracts by binding it through hydogen bond, it seems that the concentration of protein and phenolic compound need more treatment to be remove efficiently.

Furthermore, DNA purity value of lemongrass was more than 2 which indicated the presence of RNA contaminant. It proves that the modification still need addition of RNase enzymes like other studies, but both of them had lower quantity of DNA. The DNA purification need treatment of genomic DNA with Ribonuclease A (RNase A) to remove the contamination of RNA. This enzyme is an endoribonuclease that catalyzes two-step reaction of hydrolysis of the 3′,5′-phosphodiester linkage of RNA at the 5′-ester bond. The first step will start with transphosphorylation to produce an oligonucleotide terminating in a pyrimidine 2′,3′-cyclic phosphate. The following reaction is the hydrolysis reaction of the cyclic phosphate to give a terminal 3′-phosphate [27,28].

The research result showed that the modification of Doyle and Doyle method using buffer components such as PVP, Isopropanol and Sodium acetate instead CTAB significantly improved the DNA quantity of clove and lemongrass. This modification also reduce using the toxic material like merchaptoethanol which was fatal in contact with skin and also dangerous if swallowed or inhaled.

3.2. Antioxidant Analysis of clove and lemongrass

The results of the DPPH antioxidant analysis of the clove sample were 25.66% inhibition and the lemongrass sample obtained 68.64% inhibition. The value of IC_{50} of DPPH antioxidant potentially indicated both plants to had a very strong activity which clove gained higher value than lemongrass (data not shown) [14,15]. Antioxidant activity was correlated with total phenolic content in medicinal

plant [29–31]. Some studies supported the this research result which found that the total phenolic contents of clove were at 474±2.2 mg of GAE/g while lemongrass contained phenolic compounds about 125.3 g GAE/g [23,32–34].

Plants have an innate ability to protect their DNA by biosynthesize a wide range of non-enzymatic antioxidants to combat induced oxidative damage and avoid their undesirable effects [18-20]. Clove showed higher antioxidant potential comparing with lemongrass which was proven by DNA acquisition at the time of isolation using modification of Doyle and Doyle mehod. Therefore, this result increase our understanding of plant antioxidants importance for the plant itself or if the plant will be used for medicinal purposes. The applications of antioxidant activity from clove and lemongrass will also needed to highlighted for future research in plant antioxidants.

4. Conclusion

DNA isolation of clove and lemongrass using modification of the Doyle and Doyle methods gained high concentration of DNA but need further treatment for gaining beter quality of DNA. This high DNA acquisition is also due to DNA protection by the antioxidant activity produced by the two plants to avoid the harm effects of oxidative damage. This supports the development of the potential for both aromatic plants to be applied for medicinal and industrial purposes.

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