

ANALYSIS OF NYAMPLUNG (*Callophylum inophyllum*) SEED EXTRACT AS ANTIOXIDANTS THROUGH VARIOUS EXTRACTION METHODS

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ABSTRACT

Nyamplung (*Callophylum inophyllum*) is a plant that commonly grows in coastal locations. The seeds of nyamplung contain natural antioxidants, making them potential raw materials for medicinal and cosmetic products. This study aimed to examine the potential of nyamplung seed extract as a source of antioxidants. The extraction processes of the nyamplung seeds were carried out using two methods (i.e., maceration and ultrasound-assisted extraction) and two solvents (i.e., ethanol and ethyl acetate). The results showed that the extraction of nyamplung seeds using maceration and ultrasound-assisted extraction with ethanol as solvent produced extract containing phenolic, flavonoids, tannins, and steroids. Meanwhile, the use of ethyl acetate as a solvent only produced phenolic, flavonoids, and steroid compounds. Functional group and component analysis showed that the maceration method resulted in a higher percentage of compound content than the ultrasonic method. The antioxidant value was very high with an IC₅₀ value of <50 g/mL. These findings prove that nyamplung seeds are one of the natural ingredients rich in antioxidants.

Keywords: *Callophylum inophyllum*, Extraction, Antioxidants, Maceration, Ultrasonic.

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INTRODUCTION

Indonesia has the second-largest biodiversity in the world. This biodiversity should be well-preserved and managed to provide plenty of benefits for the community and the world.¹ Utilization of biodiversity is primarily needed to develop a country and meet the people's need for energy, product materials, food, the environment, and the pharmaceutical industry. Plants are one of the biological diversities that play an essential role in the survival of living things. They provide raw materials for clothing, food, medicine, cosmetics, and more.² several plants in Indonesia can be used as the foundation for cosmetics and medication, including nyamplung (*Callophylum inophyllum*). Nyamplung is one of the mangrove plants that typically live in coastal areas. It has different names in different regions. Indonesians call it *Nyamplung*, while in Aceh Province, it is popularly known as *Boeh Beunot*.³ In England, it is called *Tamanu*, whereas, in Hawaii, it is referred to as *Kamani*.⁴ Nyamplung can tolerate sandy soil but grows best when it contains minerals (sand-clay). Nyamplung can also live in places with high humidity, good drainage (pH 4 to 7.4), an altitude of 0 to 800 m above sea level (e.g., mountains, swamps, and forests⁵, and rainfall type A and B with rainfall of 1.000-4.100 mm/year. In Indonesia, nyamplung can be found in Sumatra, Java, Kalimantan, Papua, Bali, and Nusa Tenggara.⁶ The species commonly found in Aceh Province is *Callophylum inophyllum*. Nyamplung has single leaves with alternate leaf arrangements. The leaf blade is oval and elongated, and the leaf base is round with a length of 10 to 21 cm, 6 to 11 cm in width, and a flat leaf edge. The leaves are green and pinnate, and the stem size ranges from 1.5 cm to 2.5 cm. The flowers and fruit of nyamplung grow directly from the dormant buds in the upper leaf axils.⁵ The flowers are compound and in bunches (racemes). They have a pot-shaped crown (hypanthodium), whitish yellow color, and androgynous

with a diameter of 2 to 3 cm. A single flower has four irregular petals, many stamens, and a shield-shaped pistil.^{7,8} Nyamplung can be multiplied vegetatively (cuttings) and generatively (seeds). However, generative reproduction is commonly used in the multiplication of this plant. Nyamplung belongs to the Dicotyledone class and the Gutiferae family. It has thick taproots, which makes it an excellent coastal abrasion controller. Nyamplung produces fruit throughout the year after the age of 5-20 years, with a peak being in May-June and the amount reaching 20 tons/ha/year or even 250 kg/tree/year. The fruit of nyamplung has a round shape and green color that turns yellowish as it ages. The fruit's thin skin will gradually peel off, and its seed has a hard shell that protects the flesh. The dried seed produces oil with a yield of 50-75%.⁵ This high oil content makes it a good source of vegetable oil. The oils have various biological activities, such as antiviral, anti-inflammatory, UV protection, wound healing, anticancer, and antioxidants.^{5,9} The oil has a greenish-brown color, thick texture, pungent aroma like caramel, and a significantly high amount of unsaturated fatty acid content.¹⁰ Nyamplung seed oil can be used as a cosmetic product because it is rich in skin antioxidants.¹¹ There have been previous studies conducted on nyamplung seeds, such as the study on physicochemical and antibacterial characteristics of pure nyamplung seed oil¹⁰; nyamplung seed oil as a potential raw material for biodiesel production¹²; extraction of nyamplung oil and resin with a mixture of hexane-ethanol solvents⁹; nyamplung oil and skin-active abilities as traditional and modern cosmetic usage¹³; anti-aging effects of polyphenol-rich fruit extract from nyamplung¹⁴; and antioxidants in cosmetic products containing the nyamplung oil.¹¹ These studies have also shown that nyamplung seeds have plenty of natural antioxidants and can be developed as raw materials for medicines and cosmetics. Even though the nyamplung plants grow well along the coastal areas of Aceh, they are still used for limited purposes due to a lack of information about their use and the advantages of the plants. The yield of nyamplung seed oil from Aceh is 55.5% - 66.6% with an iodine number of 83.53 mg iodine/g and a hydroxy number of 64.29 mg KOH/g. The compounds in nyamplung seed oil include 17.94% palmitic acid, 23.12% linoleic acid, 42.54% oleic acid, and 15.33% stearic acid.¹⁵ The current study aimed to examine the potential of nyamplung seed extracts as antioxidant active compounds. Two extraction methods, maceration and ultrasound-assisted extraction with ethanol and ethyl acetate as solvents were used. The extraction process is important to obtain active compounds from materials and increase the content of active compounds extracted.

EXPERIMENTAL

Materials

The materials utilized were ethanol and ethyl acetate as solvents and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as antioxidant reagents. Each material was analytical grade from Merck (Darmstadt, Germany).

Raw Material Preparation

Nyamplung seeds were collected from a coastal area in Mesjid Raya district, Aceh Besar, Indonesia. Sampling location is shown in Fig.-1. The seeds were then cleaned of dirt, washed, drained, and aerated to reduce the moisture content without damaging other contents in the seeds. Next, the seeds that had been air-dried for 3 days were peeled and crushed.

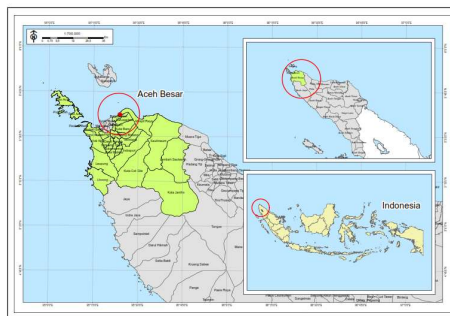


Fig.-1: Sampling Location of Nyamplung Seeds in Aceh Besar

Extraction Process

In the present study, two different extraction processes were applied. For the first sample group, the maceration process of nyamplung seeds was carried out using two kinds of solvents (ethanol and ethyl acetate). One hundred grams of nyamplung seeds were put into a maceration container and given 500 mL

of ethanol or ethyl acetate separately. The extraction process took place at room temperature over 24 hours. After that, it was filtered. Then, the nyamplung seed extract was separated from the solvent using a rotary evaporator (Yamato RE 200). For the second sample group, the extraction process using ultrasonic waves was carried out using the two kinds of solvents (ethanol and ethyl acetate). One hundred grams of nyamplung seeds were placed into an ultrasonic (Branson 8510) container and given 500 mL of ethanol or ethyl acetate separately. The extraction process took place at room temperature over 5 hours with a frequency of 42 kHz. After that, it was filtered, and the nyamplung seed extract was separated from the solvent using a rotary evaporator (Yamato RE 200).

Characterization of Nyamplung Seed Extract

Nyamplung seed extract produced from the maceration and ultrasound-assisted extraction was characterized through phytochemical screening, functional group analysis using Fourier Transform Infra-Red (FTIR) (IR-Prestige-21, Shimadzu, Tokyo, Japan), extract component analysis using Gas Chromatography Mass-Spectrometry (GC-MS) (TRACE 1310 GC and iSQ 7000 single quadrupole MS, Thermo Fischer, USA), and antioxidant strength analysis using DPPH.

RESULTS AND DISCUSSION

Active Compounds in Nyamplung Seed Extract

Nyamplung seeds were extracted using two methods (maceration and ultrasonic) and two solvents (ethanol and ethyl acetate). The two extraction processes produced the same yield of 30-35%. The extract had a greenish-yellow color. The results of the phytochemical screening of nyamplung seed extract are shown in Table-1. From these results, it can be seen that both maceration and ultrasonic methods produced the same phytochemical screening. Ethanol solvent in maceration and ultrasonic processes produced phenolic, flavonoids, tannins, and steroid compounds. Meanwhile, ethyl acetate solvent only produced phenolic, flavonoids, and steroid compounds. Nyamplung oils have several active compounds, such as calophyllic acid, tacamahin, essential oil resins, bitter compounds, glycerin, tannins, tocopherol, lipids, fiber, protein and carotenoids with a calorific value of 6092 cal/g.⁹ The content of the extracted compounds is strongly influenced by the solvent used. The active compounds found in nyamplung seeds have great potential for biological activities. Utilization of nyamplung seeds as a source of natural active compounds can increase their economic value and provide an alternative source of easily available cosmetic ingredients.⁷

Table-1: Phytochemical Screening of Nyamplung Seed Extract

Phytochemical screening	Maceration using ethanol	Maceration using ethyl acetate	Ultrasonic using ethanol	Ultrasonic using ethyl acetate
Phenolic	+	+	+	+
Tannins	+	-	+	-
Flavonoids	+	+	+	+
Terpenoids	-	-	-	-
Steroid	+	+	+	+
Saponins	-	-	-	-
Alkaloids				
- Dragendorff	-	-	-	-
- Mayer	-	-	-	-
- Wagner	-	-	-	-

Functional Group Analysis

The FTIR spectrum of nyamplung seed extract obtained through two methods and two solvents, as illustrated in Fig.-2, shows the presence of several functional groups with low transmittance percentages, such as O-H stretching at 3600 cm⁻¹, C-H Asymmetric stretching at 2900 cm⁻¹, C-H Symmetric stretching at 2800 cm⁻¹, C=O stretching at 1750 cm⁻¹, C=O Aromatic stretching at 1690 cm⁻¹, C-N stretching at 1350 cm⁻¹, C-O stretching at 1290 cm⁻¹, and Ar-C stretching+vibration 850 cm⁻¹.¹⁶ The FTIR spectrum also shows that the maceration and ultrasonic extraction processes did not have a significant difference in shape; however, there is a significant difference in the transmittance percentages, as shown in Table-2 for the use of ethanol and in Table-3 for the use of ethyl acetate. FTIR analysis results showed that the maceration

process resulted in a lower transmittance percentage than the ultrasound-assisted extraction process, which was due to the extraction time. The longer the extraction time, the more active compounds can be drawn by the solvent.

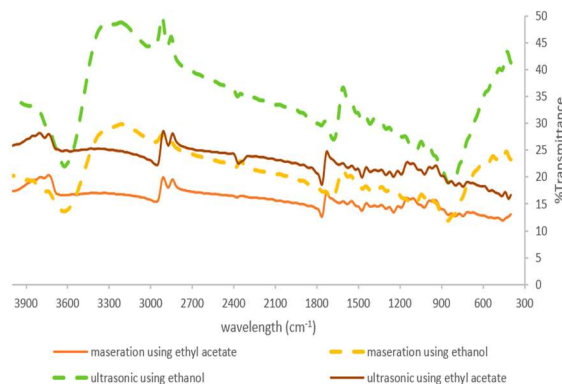


Fig.-2: FTIR Spectrum of Nyamplung Seed Extract

Table-2: The Differences in Transmittance Percentages on the Use of Ethanol Solvent

Functional Groups	Maceration		Ultrasonic	
	λ (cm ⁻¹)	T (%)	λ (cm ⁻¹)	T (%)
O-H stretching	3624	13.639	3623	22.003
C-H Asymmetric stretching	2975	26.530	2985	43.994
C-H Symmetric stretching	2876	26.460	2878	44.528
C=O stretching	1754	17.265	1769	29.631
C=O Aromatic stretching	1692	15.926	1680	26.998
C-N stretching	1354	17.927	1352	29.941
C-O stretching	1298	17.390	1299	28.989
Ar-C stretching+vibration	854	11.901	851	18.992

Table-3: The Differences in Transmittance Percentages on the Use of Ethyl Acetate Solvent

Functional Groups	Maceration		Ultrasonic	
	λ (cm ⁻¹)	T (%)	λ (cm ⁻¹)	T (%)
O-H stretching	3640	16.636	3638	24.844
C-H Asymmetric stretching	2957	15.423	2955	22.221
C-H Symmetric stretching	2874	18.063	2873	25.939
C=O stretching	1764	12.638	1764	18.605
C=O Aromatic stretching	1638	15.167	1640	22.296
C-N stretching	1475	13.747	1474	19.972
C-O stretching	1247	13.431	1247	20.073
Ar-C stretching + vibration	851	12.955	852	18.892

Analysis of GC-MS

Nyamplung is widely used in ethno-medicine, especially its oil for the treatment of various skin diseases. The oil can be used as an ingredient for medicinal and cosmetic products because it has excellent characteristics as a skin antioxidant agent.¹¹ Nyamplung seeds can produce a large amount of vegetable oil and have several active compounds that drive many biological activities in it.⁷ The results of GC-MS analysis using nyamplung seed extract components showed that nyamplung seed extract contains many active compounds, each extract containing 30-40 compounds. The identified compound is part of long-chain fatty acids and terpenoids. The chemical component components and retention times are shown in Table-4. Based on these data it shows that the ten compounds that have the highest percentage of each extract indicate that each extract has the same compound, namely octadecanoic acid, cis-13-octadecenoic acid, n-hexadecanoic acid, butylated hydroxytoluene, hexadecanoic acid, ethyl ester, 2'-(trimethylsilyl)oxy-3,4,4',5-tetramethoxychalcone, trans-13-octadecenoic acid, methyl ester, hexadecanoic acid, phenol, 4,6-di(1,1-dimethylethyl)-2-methyl, methyl ester, and dodecyl acrylate. The terpenoid compounds that appear, namely lupeol (C₃₀H₅₀O) and squalene (C₃₀H₅₀) in the macerated extract of ethyl

acetate are thought to be due to the role of the semi-polar solvent used so that they can extract these compounds. The maceration process using ethanol and ethyl acetate solvents produces a higher percentage of compound content when compared to ultrasound-assisted extraction. This is because the maceration method involves a lower temperature than ultrasound-assisted extraction; thus, they can extract all secondary metabolites that are not resistant to heating. These results are supported by the FTIR results which show that there are more functional groups in the extract produced by the maceration method compared to ultrasound-assisted extraction.

Table-4: Composition of Nyamplung Seed Extract

Compounds	Maceration with ethanol (%)	Maceration with ethyl acetate (%)	Ultrasonic with ethanol (%)	Ultrasonic with ethyl acetate (%)
cis-13-Octadecenoic acid	45.21	30.52	43.58	19.09
Octadecanoic acid	14.4	6.38	11.3	5.34
n-Hexadecanoic acid	11	8.5	14.41	6.25
Butylated Hydroxytoluene	8.52	-	3.53	-
Hexadecanoic acid, ethyl ester	5.7	-	4.55	-
2'-(Trimethylsilyl) oxy-3,4,4',5-tetramethoxychalcone	2.78	-	1.98	-
Phenol, 4,6-di(1,1-dimethylethyl)-2-methyl-	2.71	-	2.34	-
trans-13-Octadecenoic acid, methyl ester	1.56	-	-	-
Hexadecanoic acid, methyl ester	1.08	-	-	-
Dodecyl acrylate	0.94	-	-	-
Butylphosphonic acid, hexyl 4-(2-phenylprop-2-yl) phenyl ester	-	16.93	-	8.21
Lupeol	-	3.74	-	3.68
2,6-Di-tert-butyl-4-methoxyphenol, trifluoroacetate	-	3.53	-	-
2,6-Diisopropyl-naphthalene	-	3.43	-	-
Squalene	-	2.54	-	-
Cannabinol, TMS derivative	-	2.03	-	-
2,4-Di-tert-amylphenol, O-pentafluoropropionyl-	-	1.96	-	-
9-Octadecenoic acid (Z)-,	-	-	5.44	-
Cyclopropanecarboxylic acid, 1-hydroxy-, (2,6-di-t-butyl-4-methylphenyl) ester	-	-	1.94	-
Phenol, 2,4,6-tris(1-methylethyl)-	-	-	1.6	-
Phthalic acid, hept-4-yl isobutyl ester	-	-	-	5.24
Oryzalin	-	-	-	3.28
Diethyl carbitol	-	-	-	2.89
Dibutyl phthalate	-	-	-	2.72
Acetic acid, 2-ethylhexyl ester	-	-	-	2.32

Antioxidant Analysis

There are many sources of antioxidants (natural and synthetic), but the natural ones are preferred because they generally have minimal side effects. Nyamplung seed extract is a potential natural source of antioxidants with an IC_{50} value of <50 g/mL. Table-5 shows the antioxidant values of nyamplung seed extract produced from maceration and ultrasonic processes with two solvents. The extract produced with ethanol has a lower IC_{50} value than that produced using ethyl acetate. This suggests that there are more antioxidant compounds in the polar extract of nyamplung seeds. The compound that is suspected to have antioxidant activity is cis-13-octadecenoic acid ($C_{18}H_{34}O_2$) which is part of long-chain fatty acids. There is a correlation between the increase in the amount of cis-13-octadecenoic acid causing antioxidant activity

with a stronger IC₅₀ value. Phenolic compounds, namely phenol, 4,6-di(1,1-dimethyl ethyl)-2-methyl- (C₁₅H₂₄O) contained in the ethanol extract from two methods, namely maceration and ultrasonic, are also thought to increase the antioxidant activity of nyamplung seeds. Phenolic compounds act as antioxidants by reacting and deactivating various types of free radicals.¹⁷ The mechanism of antioxidant action involved in the transfer of hydrogen atoms, single electron transfer, and transition metal chelation.

Table-5: The IC₅₀ Value of Nyamplung Seed Extract as an Antioxidant

Extraction Process	IC ₅₀ (µg/mL)
Maceration using ethanol	14.68
Maceration using ethyl acetate	46.32
Ultrasonic using ethanol	17.94
Ultrasonic using ethyl acetate	46.61

CONCLUSION

Nyamplung plants are the type of plants that commonly grow in coastal areas and have many benefits. Many studies examined the oil (non-polar) of nyamplung seeds as a source of active compounds; however, the polar extracts of nyamplung seeds are still not widely used. The extract of nyamplung seeds produced using ethanol solvent and the maceration and ultrasonic methods was found to contain phenolics, tannins, and steroids with an IC₅₀ value of < 50 g/mL. Thus, the ethanolic extract of nyamplung seeds is a potential source of antioxidants.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest existed.

AUTHOR CONTRIBUTIONS

All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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