Chemical Profiling of Kalangkala (Litsea angulata) Seed Using LC-HRMS as Antioxidants

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KEYWORDS
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ABSTRACT
Kalangkala (Litsea angulata) is one of the most frequent florae utilized in traditional medicine in the Kalimantan area, and this showed significant antioxidant activity on the part of the seeds. Ability antioxidants it has seed sometimes become potency development material natural as material standard medicine, so need did its identification compounds present in seeds sometimes. Research purposes: this is to identify compound potential antioxidants as candidate materials for active pharmacy. The method used is to test the activity of antioxidants using the DPPH technique from the n-hexane, ethyl acetate, and methanol kalangkala seed fractions, achieved by identifying components using LC-HRMS. Research results show that the IC50 values from the n-hexane, ethyl acetate, and methanol kalangkala seed fraction are, respectively, 52.05, 46.10, and 32.84 ppm. Identification results compound from methanol fraction with activity antioxidant best show 5 major compounds with abundant more than 3% are 3-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-4-hydroxy-5-methoxybenzoic acid, 2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone, Betaine, Choline, and (4E,6E)-4,6-Tetradecadiene-8,10,12-triyn-1-yl acetate.

1. Introduction

Nowadays, cancer, inflammation disorders, heart diseases, and diabetes are a number of degenerative diseases suffered by Indonesian people. Many influencing factors contribute to the emergence of disease, and free radicals are one of the possible factors that trigger diseases (Matheos et al., 2014). Therefore, free radicals in normal amounts, neutralized by antioxidants in the body, give electrons in compounds that are radical-free so that they can hinder activity; however, at high concentrations, antioxidants in the body cannot neutralize them and need antioxidant externals (Wayan et al., 2014).

Figure 1. Kalangkala (Litsea angulata)

One of the florae that have antioxidant activity is Kalangkala (Litsea angulata) (figure 1). Kalangkala is a plant that can be found in several areas of Kalimantan. The people of Kalimantan use it in their daily lives as an additions food and to treat various diseases like antibacterials, pain joints, and antidiabetics (Kuspradini et al., 2018). Many research that has been done on fruit, seeds, leaves, and skin stems sometimes mentions that test samples have potency as antioxidants, with IC_{50} values of 243.14 ppm, 48.78 ppm, 152.39 ppm, and 85.33 ppm, respectively. From several tested samples, section seed sometimes has the strongest antioxidant activity (Saputri & Susiani, 2018; Susiani & Saputri, 2020).

Looking at the potential antioxidant activity of kalangkala seeds, in this research the compound components contained in kalangkala seeds were identified. In an era of progress in technology and science, this is innovation, invention, drug, material, natural potential, and increased success in group therapy. Invention drug product experience stands as a main contributor to solving the challenge of global health and achieving objective, sustainable development in the field of health (Thomford et al., 2018). Identification of compounds from Kalangkala seeds has not been carried out so that the information from this study is a novelty on the content of compounds in Litsea angulata. Identification of compound was carried out using the LC-HRMS method in which the samples were separated into fractions and the antioxidant activity was evaluated using the DPPH technique.

2. Materials and Methods

Extraction

Kalangkala seeds were obtained from Banjarmasin, East Kalimantan which were then determined at Lambung Mangkurat University. The process of washing, chopping, drying and grinding the kalangkala seeds is carried out to obtain simplicia powder, then 2 kg of kalangkala seed simplicia powder was extracted using 6 L of 96% ethanol for 24 hours, the mixture was then filtered to separate the filtrate from the residue, which was re-milled twice. All filtrate was evaporated with a rotary evaporator at 60°C until a thick extract was produced (Ramadhan et al., 2020; Sinaga et al., 2021).

Fractionation

The extract of kalangkala seeds was diffracted using the solid-liquid method using a solvent with increasing polarity. Fractionation was carried out by adding 200 mL of n-hexane, then stirring, separating the soluble and insoluble parts of n-hexane, the insoluble parts of n-hexane were added with 200 mL of n-hexane, this process was carried out 3 times. The n-hexane phase was concentrated with a rotary evaporator and then weighed to determine the n-hexane fraction. Next, the insoluble phase was partitioned successively using ethyl acetate and methanol in a similar manner. (Fauziyya et al., 2017; Ochoa-Pacheco et al., 2017).

Antioxidant Activity Assay

The inhibition DPPH was calculated based on IC_{50} to evaluate antioxidant activity. Various concentrations of kalangkala seed fraction by ranging between 20-100 ppm. 1 ml of each sample was pipetted and 1 ml of 0,1 mM DPPH was added and homogonised for 1 minute then incubated in a place protected from light for 30 minutes at 37^{o}C. The samples were tested using UV-Vis spectrophotometry at a maximum wavelength (Hasanah et al., 2017; Husnayanti et al., 2017; Martiningsih et al., 2016; Nurkhasanah et al., 2016). The vitamin C as a material standard in this testing.

Analysis of LC-HRMS

LC-HRMS analysis was carried out on the kalangkala seed fraction which was most active in counteracting DPPH free radicals. Analysis was carried out by inserting the sample into a column (Accucore TM phenyl hexyl 100 mm x 2.1 mm ID x 2.6 ppm), and eluting it in stages with water (0.1% formic acid), and methanol (0.1% formic acid) as the mobile phase for 25 minutes at a flow rate of 0.30 mL/minute. Data were detected using an orbitrap mass spectrometer MS/dd-MS acquisition mode to collect untargeted metabolites in positive and negative ionization polarities. Data analysis was processed on Xcabur version 44 software (Thermo Scientific, Bremen, German).

3. Results and Discussion

Extraction and Fractionation

Extraction is the separation or taking of components in a material with the help of a solvent. Separation occurs based on the solubility of the components in a combination of solvent and solute (Martinus et al., 2015). While the separation of molecules from a sample that has been extracted based on its polarity is called fractionation. Based on table I, the yield results of fractions obtained from kalangkala seeds show that polar compounds are more dominant than non-polar and semi-polar compounds. Compounds that are attracted to non-polar fractions such as fats, steroids, terpenoids. Compounds with semipolar properties such as flavonoid aglycones, alkaloids, polyphenols, while polar segments such as flavonoid glycosides, saponins, tannins, etc. (Pratiwi et al., 2021).

Table I. Yield of kalangkala seed fractions

Sample	Yield (%)
N-hexane fraction	6.19
Ethyl acetate fraction	12.52
Methanol fraction	45.35

Table I reveals that the methanol fraction of kalangkala seeds produces the highest yields compared to other fractions. The methanolic fraction of this herb leads to over three times as much as the n-hexane and ethyl acetate fractions. A previous study reported that polar solvents can lead to higher yields obtained (Ghasemzadeh et al., 2011).

Antioxidant Activity

This study reports the ethanolic extract and its fractions of kalangkala seeds as well as the positive control in the form of ascorbic acid which was reacted with DPPH. The DPPH technique quantitatively evaluates antioxidant activity by measuring changes in the intensity of the deep violet color of DPPH, which is directly related to the concentration of the DPPH solution. DPPH free radicals, which have unpaired electrons, exhibit a violet color. When electrons pair up, the color changes to yellow. This color change occurs because the reaction between DPPH molecules and hydrogen atoms released from the test compounds weakens the free radicals, resulting in the formation of a 2,2-diphenyl-1-picrylhydrazyne (DPPH-H) compound, which causes the DPPH to turn yellow (Rizkayanti et al., 2017; Sirivibulkovit et al., 2018).

The remaining DPPH radicals were measured using a UV-Vis spectrophotometer at a wavelength of 515-520 nm to quantify antioxidant activity (Celiz et al., 2020), In this study, a wavelength of 515.70 nm was used, which corresponds to the maximum absorbance. This allowed for the expression of free radical scavenging activity as the IC_{50} value, defined as the concentration of the test substance required to reduce free radicals by 50%. As the IC_{50} value decreases, the antioxidant activity of the substance increases (Suena & Antari, 2020).

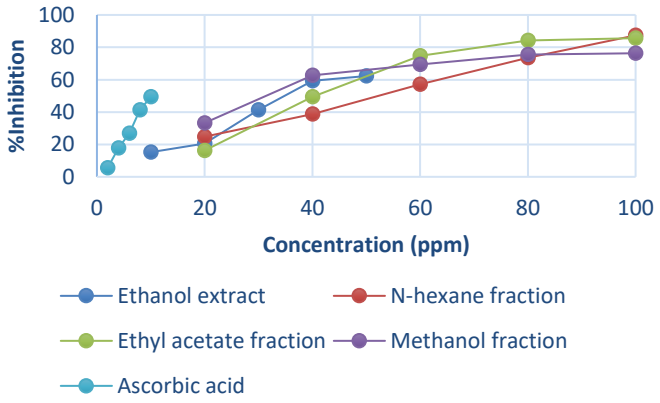


Figure 2. Percent inhibition of DPPH againt different concentration of samples

Table II. Antioxidant activity of samples with the DPPH method

Sample	IC_{50} (ppm)±SD
Ethanol extract	37.69±0.34
N-hexane fraction	52.05±1.33
Ethyl acetate fraction	46.10±0.16
Methanol fraction	32.84±1.51
Ascorbic acid	9.93±0.03

Several parts of this plant have a good potency as natural antioxidant source. Kuspradini et al., (2019) reported that the ethanolic extracts of kalangkala from bark, branch, and leaves have IC_{50} value of 14.69, 26.81, and 14.58 ppm, respectively. The antioxidant activity of a substance is classified as very strong (IC_{50} value <50 ppm), strong (IC_{50} value 50-100 ppm), medium (IC_{50} value 101-150 ppm), moderate (IC_{50} value 151-200 ppm), and very weak (IC_{50} value > 200 ppm) (Bahriul et al., 2014; Islamiati et al., 2022).

Based on the IC_{50} results obtained (table II), ascorbic acid as a standard and widely used antioxidant source, shows very strong antioxidant activity compared to the test sample, ascorbic acid removes DPPH color through its ability to donate hydrogen to DPPH radicals (Yokozawa et al., 1998). Apart from ascorbic acid, ethanol extract also has very strong antioxidant activity which has IC_{50} of range 37.69 ppm. This study is similar to

previous research conducted by Saputri & Susiani (2018) which stated that 96% ethanol extract of kalangkala seeds had antioxidant activity with an IC_{50} value of 48.78 ppm. Another study that used 80% methanol and water as solvents in the extraction of kalangkala seeds, produced EC_{50} values of 17.3 and 22.7 mg/mL, respectively (Hassan et al., 2013). This finding shows a high potency of the use of kalangkala seed extract as a natural antioxidant source.

Generally, polyphenol compounds correlated positively with free radical scavenging (Akhtar et al., 2018; Moukette et al., 2015). As a result, the phenolic and flavonoid contents of kalangkala seeds contribute to their strong antioxidant activity. According to a previous study, the percentage of DPPH free radical scavenging activity showed a positive correlation with the phenolic and flavonoid content in the 80% methanol extract of kalangkala seeds, which have total phenolic amounts of 8.09 mg GAE/g and total flavonoids of 5.73 mg RE/g, whereas water extract of kalangkala seeds has total flavonoids of 3.54 mg GAE/g and 2.63 mg RE/g, respectively (Hassan et al., 2013). Phenolic compounds and flavonoids possess redox properties that enable them to act as reducing agents, donate hydrogen, and quench singlet oxygen (Croft, 1998; Olszowy, 2019).

According to Table II, the methanol fraction of kalangkala seeds is the most potent antioxidant source, with an IC_{50} range of 32.84 ppm. The use of a polar solvent during the extraction process contributed to the presence of phenolic compounds. In fact, phenolic and some flavonoids are polar because they contain a large number of hydroxy groups. This proposed that the methanol fraction of kalangkala seed could be chosen as an antioxidant candidate.

Identification of Compound using LC-HRMS

The methanol fraction with the highest antioxidant activity was analyzed using LC-HRMS to identify potential antioxidant compounds in kalangkala seeds. Table III shows the identification results for components with a percentage greater than 1% in the methanol fraction. Figure 2 shows the structures of the identified compounds.

Table III. Results of compound analysis of the methanol fraction of kalangkala seeds using LC-HRMS

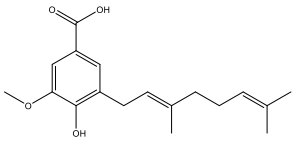
RT	Compounds name	Formula	Compound Groups	%Area
13.069	3-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-4-hydroxy-5-methoxybenzoic acid	$C_{18}H_{24}O_4$	Phenol group	17.20
12.87	2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone	$C_{18}H_{24}O_4$	Phenol group	14.86
0.837	Betaine	$C_5H_{11}NO_2$	Amino acid	9.96
0.804	Choline	$C_5H_{13}NO$	B Vitamin	4.81
12.871	(4E,6E)-4,6-Tetradecadiene-8,10,12-triyn-1-yl acetate	$C_{16}H_{16}O_2$	PUFAs	3.87
14.344	2,2,4-Trimethyl-1,3-pentadienol diisobutyrate	$C_{16}H_{30}O_4$	Isooctane	2.99
5.2	Sinomenine	$C_{19}H_{23}NO_4$	Alkaloids/phenol group	2.94
0.815	N-(3-Carboxypropanoyl)-5-hydroxynorvaline	$C_9H_{15}NO_6$	Amino acid derivate	2.68
13.127	3-Phenyl-7-chromanol	$C_{15}H_{14}O_2$	isoflavone/phenol group	2.63
11.171	2-(2-Carboxyethyl)-4-methyl-5-pentyl-3-furoic acid	$C_{14}H_{20}O_5$	Heterocyclic fatty acid	2.09
14.344	δ-Dodecalactone	$C_{12}H_{22}O_2$	Lactone	1.71
4.654	Laurolistine	$C_{18}H_{19}NO_4$	Alkaloids/phenol group	1.66
6.002	Dipropylene glycol dimethyl ether	$C_8H_{18}O_3$	Eter	1.49
11.171	Asarone	$C_{12}H_{16}O_3$	Phenylpropanoid	1.30
11.359	5,5′-Dihydroxy-4,4′,8′,8′-tetramethyl-4,5-dihydro-2′H,3H-spiro[furan-2,6′-[7]oxabicyclo[3.2.1]oct[3]en]-2′-one	$C_{14}H_{20}O_5$	Furan	1.27
0.805	Piracetam	$C_6H_{10}N_2O_2$	Racetams	1.26
14.344	Octadiene	C_8H_{14}	Alkadiene	1.09
12.906	1-[(11Z)-octadecenoyl]-sn-glycero-3-phosphocholine	$C_{26}H_{52}NO_7P$	Fatty acyl	1.02
0.824	5-Hydroxymethyl-2-furaldehyde	$C_6H_6O_3$	Furan	1.02
6.281	Palaudine	$C_{19}H_{19}NO_4$	Isoquinolines/phenol group	1.00

The primary constituent, 2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone is classified as a ubiquinone. This compound is a derivative of coenzyme Q, featuring a 5,6-dimethoxy-3-methyl(1,4-benzoquinone) structure along with an isoprenyl group attached at the 2 (or 6) position of the ring. MTT testing revealed that treating HepG2 cells with 350 and 335 μM ubiquinone for 24 and 48 hours resulted in reduced oxidative stress, increased antioxidant capacity, and increased redox hemostasis, which decreased cell viability (Heidari-Kalvani et al., 2023).

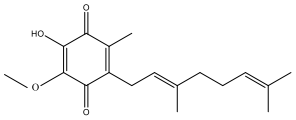
Other phenol-containing compounds include 3-Polyprenyl-4-hydroxy-5-methoxybenzoate, Sinomenine, 3-Phenyl-7-chromanol, Laurolistine, and Palaudine. 3-Polyprenyl-4-hydroxy-5-methoxybenzoate is a phenol found in vanillic acid derivatives. A previous study found that vanillic acid has lower antioxidant activity than gentisic acid because the methoxyl group of vanillic acid significantly reduces its antioxidant activity (Chandrasekar et al., 2016). Furthermore, Sinomenine belongs to the alkaloid group and can be used as an antioxidant and anti-inflammatory (Ahmed A.j. Jabbar et al., 2024). Kayalı A et al., (2024) shown that sopomenine shields hepatocellular cells from oxidative stress and injury. 3-Phenyl-7-chromanol is an isoflavone group with numerous disease-prevention benefits. Pejčić T et al. (2023) found that isoflavone groups can prevent breast cancer by reducing oxidative stress. Furthermore, Laurolistine, an aporphine alkaloid, exhibits potent antihyperglycemic and antihyperlipidemic properties (Tan et al., 2021). Palaudine, a member of the isoquiloline family, requires additional studies to demonstrate its ability to function as an antioxidant. Therefore, this study successfully identified some phenolic groups that regulate oxidative stress.

Other compounds that have been extensively studied for their antioxidant activity, such as betaine and choline. The impact of betaine on oxidative stress due to asthma in the liver and kidneys in mice shows that betaine has an antioxidant effect which not only prevents oxidative stress but also improves airway inflammation in lung tissue (Pourmehdi et al., 2020). In addition, betaine can protect against cadmium nephrotoxicity by lowering lipid peroxidation, boosting total antioxidant status, and decreasing caspase signaling cascades in renal tissue (Hagar & Al Malki, 2014).

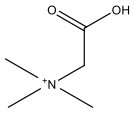
Choline compounds administered via oral or intranasal routes can limit oxidative stress and attenuate immunological responses in mouse models of allergic airway disease (Mehta et al., 2009). Choline is not only harmless but also capable of protecting against DNA damage produced by oxidative genotoxins, the outcome of choline's genotoxic inhibitory capacity in vivo due to its antioxidant activity against peroxides created by its hydroxyamine group (Merinas-Amo et al., 2017).



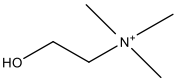
3-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-4-hydroxy-5-methoxybenzoic acid



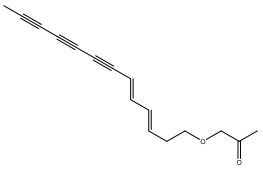
2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone



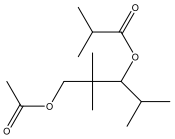
Betaine



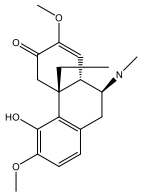
Cholin



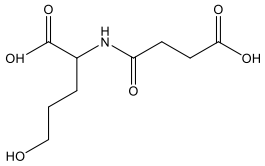
(4E,6E)-4,6-Tetradecadiene-8,10,12-triyn-1-yl acetate



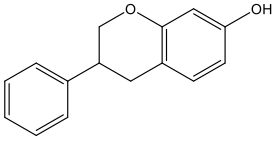
2,2,4-Trimethyl-1,3-pentadienol diisobutyrate



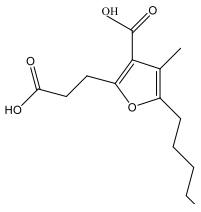
Sinomenin



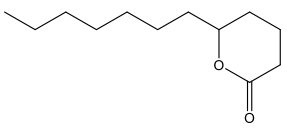
N-(3-Carboxypropanoyl)-5-hydroxynorvaline



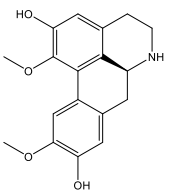
3-Phenyl-7-chromanol



2-(2-Carboxyethyl)-4-methyl-5-pentyl-3-furoic acid



δ-Dodecalactone



Laurolistine

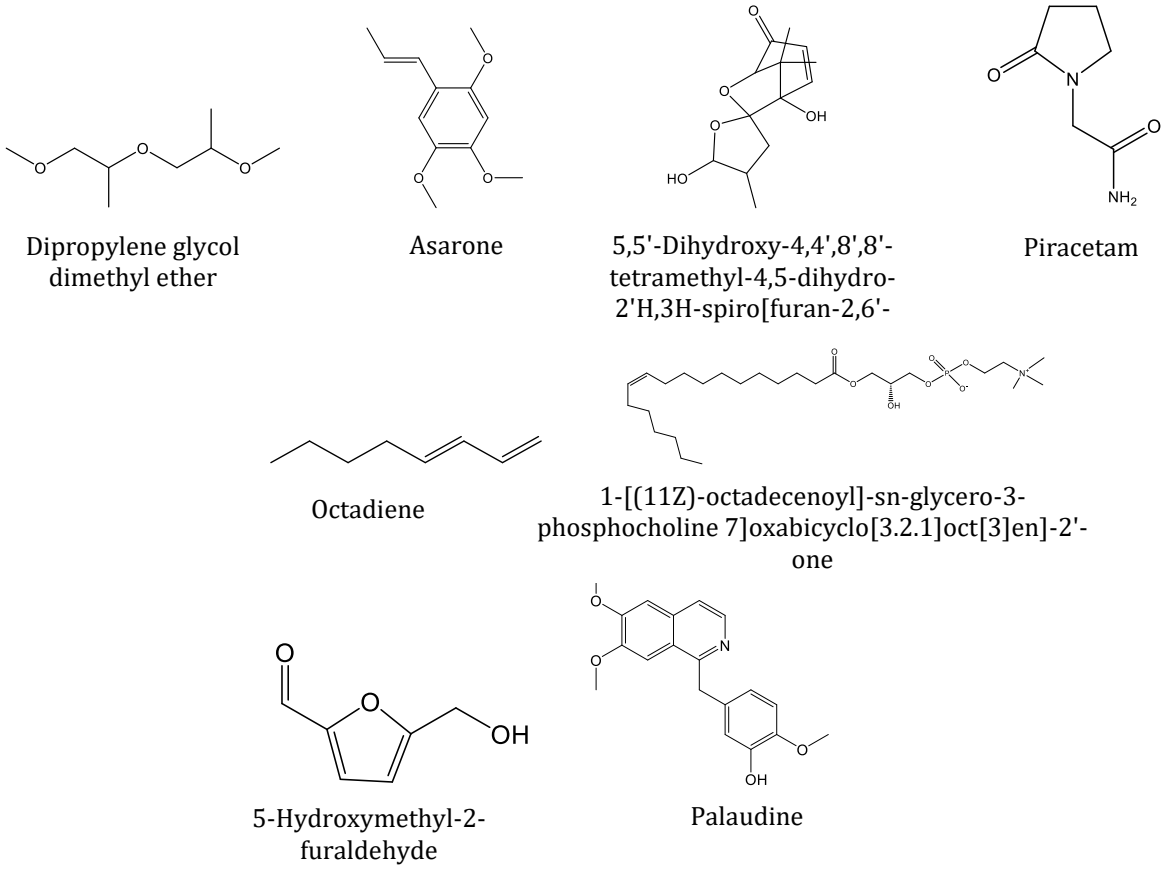


Figure 3. The structure of compound identified from Litsea 1650ngulate seed using LC-HRMS

4. Conclusion

Kalangkala seed is a natural substance utilized in traditional medicine to cure a wide range of ailments. The study found that kalangkala seed extract and its fractions exhibit high antioxidant activity (IC_{50} value <50 ppm). The methanolic fraction of kalangkala seeds demonstrated the highest antioxidant activity. Several phenolic compounds were identified using LC-HRMS, including 3-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-4-hydroxy-5-methoxybenzoic acid, 2-[(2E)-3,7-Dimethyl-2,6-octadien-1-yl]-5-hydroxy-6-methoxy-3-methyl-1,4-benzoquinone, Sinomenine, 3-Phenyl-7-chromanol, Laurolistine, and Palaudine. These compounds could be responsible for inhibiting DPPH-free radicals.

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