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THE SCREENING OF PHYTOCHEMICAL CONTENTS, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF KALANGKALA PLANT (*Litsea angulata*): LITERATUR REVIEW

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ABSTRACT

Litsea angulata, more commonly known as Kalangkala, is a type of plant that belongs to the Lauraceae family. Empirically, the plant has been used traditionally by local people for the treatment of boils, diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, insect bites, and anti-irritation. This review article aims to find out the results of phytochemical screening of metabolites from parts of Kalangkala plant with various solvents as well as the results of the tests of antioxidant and antibacterial activities that several researchers have carried out. The method used was a literature search on Google Scholar and Pubmed with the keywords kalangkala, *Litsea angulata*, phytochemical screening, antioxidant, and antibacterial. The results of this review showed that various solvents, such as water, ethanol, methanol, N-hexane, and ethyl acetate in seed, fruit flesh, bark, stem, and leave parts of Kalangakala plant produces different amounts of secondary metabolites. Secondary metabolites such as alkaloids, flavonoids, saponins, and tannins extracted well using etanol as a solvent due to their polarity. The results of the antioxidant activity showed that the part of kalangakala plant such as the bark of the tree, has a very good antioxidant activity of 2.41 ppm when tested using ethyl acetate solvent. Furthermore, the results of the antibacterial activity test show an inhibition of 50 mm on *S. mutans* bacteria when tested using ethanol solvent. In conclusion, kalangkala plant has secondary metabolites which act as antioxidants and antimicrobials that also potential to developed as traditional medicine.

Keywords: Kalangkala, *Litsea angulata*, phytochemical screening, antioxidant, antibacterial

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INTRODUCTION

Litsea, an important genus of the Lauraceae family, is often found in areas such as tropical and subtropical Asia (Tanaka et al., 2009). Kalangkala (*Litsea angulata* Bl) is a species of the genus *Litsea* belonging to the Lauraceae family. Traditionally, the people of Kalimantan use Kalangkala plant (*Litsea angulata*) to treat diseases such as diarrhea, stomachache, dyspepsia, gastroenteritis, diabetes, and so on (Rohama and Melviani, 2021). Some people in South Kalimantan have traditionally used the seeds of the Kalangkala fruit for the treatment of boils (Mustikasari and Ariyani, 2010). Other parts of Kalangkala plant such as young stem bark are empirically efficacious for anti-bacteria and reduces irritation of insect bites (Kusparidin et al., 2018).

The composition of chemical compounds in plants is mostly determined by a region's soil fertility, temperature, and geographic location. Regional variations exist in the chemical component content of plants belonging to the same species. The plant samples utilized in the phytochemical test may be leaves, stems, fruits, flowers, or roots; all of these parts of the plant have therapeutic qualities and are used as raw materials to make both conventional and modern medications. (Agustina, Wiraningtyas and Bima, 2016).

The method for determining a secondary metabolite from plant is phytochemical screening. An overview of the specific components present in the natural product under investigation can be obtained through the preliminary step of phytochemical screening. Depending on the intended outcomes, phytochemical screening can be done in a qualitative, semi-quantitative, or quantitative manner. A color reaction with a specific reagent can be used to perform a qualitative phytochemical screening approach. The selection of the extraction method and solvent has a significant impact on the phytochemical screening procedure. The required active ingredient may not be entirely and correctly attracted when using the wrong solvent.

One method for determining a natural product's secondary metabolite content is phytochemical screening. An overview of the specific components present in the natural product under investigation can be obtained through the preliminary step of phytochemical screening. Depending on the intended outcomes, phytochemical screening can be done in a qualitative, semi-quantitative, or quantitative manner. A color reaction with a specific reagent can be used to perform a qualitative phytochemical screening approach. The selection of the extraction method and solvent has a significant impact on the phytochemical screening procedure. The required active ingredient may not be entirely and correctly attracted when using the wrong solvent..

Secondary metabolites are compounds synthesized by plants, microbes, or animals through biosynthetic processes that are used to support life. Some of the secondary metabolites are alkaloids, flavonoids, terpenoids, steroids, saponins, and tannins. Secondary metabolites function as antimicrobial, antioxidant, anticancer, and chemopreventive agents for various degenerative diseases. One way to find out whether the content of these compounds has efficacy is by carrying out several tests, one of which is testing antibacterial and antioxidant activities.

Bacteria and viruses are microorganisms that are harmful and capable of infecting both humans and animals, causing mild infections to death. Chemical and biological substances, both synthetic and natural, that have the ability to stop bacteria from growing and functioning are known as antibacterial chemicals. The dilution method, the agar diffusion method, and the dilution diffusion method are three techniques that can be used to study antibacterial activity. A chemical's antibacterial compound capacity can be assessed both subjectively and quantitatively using the dilution method and quantitatively using the special diffusion method (Jawetz, Melnick and Aldebergs, 2005).

Antioxidants are substances that can be utilized to prevent the free radical reactions that lead to a number of diseases, including cancer, heart disease, and premature aging. According to Choi et al. (2014), antioxidants derived from plants include a broad class of bioactive substances that include flavonoids, phenolic compounds, compounds containing sulfur, tannins, alkaloids, phenolic diterpenes, and vitamins. A spectrophotometric technique called DPPH (1,1-diphenyl-2-picrylhydrazyl) is one of the methods used to measure the antioxidant activity of a plant extract (Gulcin et al., 2010). A free radical called DPPH donates hydrogen in order to react with antioxidants. The DPPH solution's color can vary from purple to pale yellow when free radical scavenging activity is measured using the DPPH method (Krishnaiah et al., 2011). Based on the background above, this review article aims to find out the comparison between what metabolites are found in Kalangkala plant using various solvents and their activity as antioxidants and antibacterials.

RESEARCH METHODS

The inclusion criteria in this review article were original articles or studies through searches from the Google Scholar and Pubmed databases. The research subject was kalangkala plant (*Litsea angulata*) with the study results of phytochemical screening of secondary metabolites, antioxidant and antibacterial activities. The keywords used in searching for article references from several relevant studies were kalangkala, *Litsea angulata*, phytochemical screening, antioxidant, and antibacterial.

Literature publications within the last 13 years, as well as studies with phytochemical screening of secondary metabolites, antioxidant and antibacterial activities, were selected. The total references selected were 25 articles. Five articles were excluded first because they discussed different *Litsea* species. However, 13 articles were excluded because they do not discuss phytochemical screening of secondary metabolites, antioxidant and antibacterial activities, and the activity of kalangkala plant. It was then sorted into only 7 articles which included the study results of phytochemical screening of secondary metabolites, antioxidant and antibacterial activities of kalangkala plant. The database search flowchart can be seen in Figure 1.

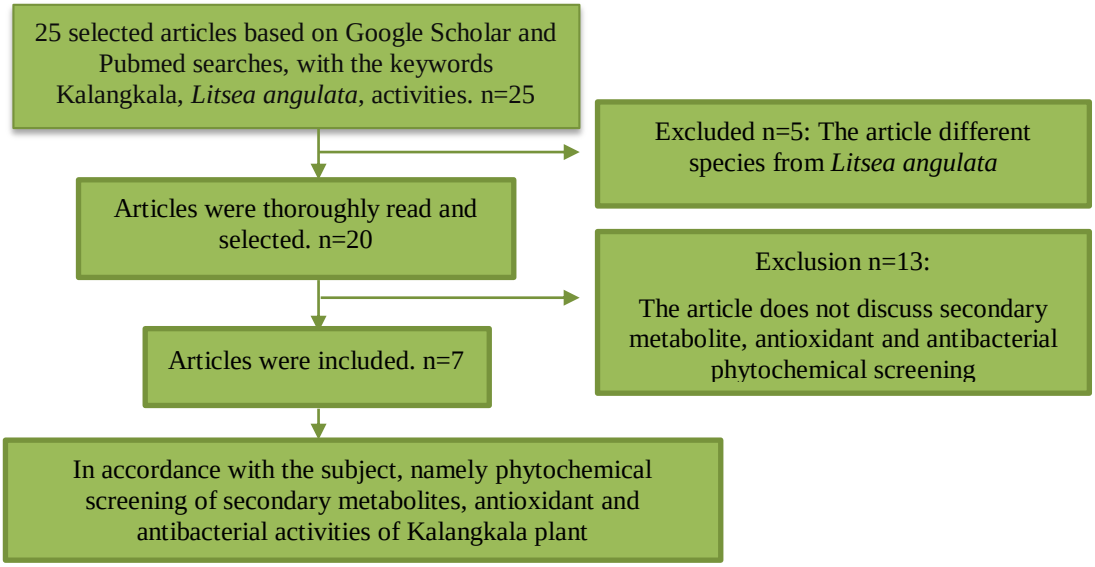

Figure 1. Article database search

RESULTS AND DISCUSSION

The results of 7 articles related to phytochemical screening of secondary metabolites, antioxidant and antibacterial activities of Kalangkala plant (*Litsea angulata*) are presented in Table I.

Table I. The result of articles articles related to phytochemical screening of secondary metabolites, antioxidant and antibacterial activities of Kalangkala plant

No	Study Title, Author, and Year	Study objective	Sample	Method	Result
1	Phytochemical, antioxidant and antimicrobial properties of *Litsea angulata* extracts (Kuspradini et al., 2018)	To determine the antioxidant and antimicrobial activities and phytochemical constituents of *Litsea angulata* branches, bark, and leaves.	The plant samples were obtained from the Faculty of Forestry, Laboratory of Forest Education, Universitas Mulawarman, East Kalimantan. The plant samples were later identified at the Laboratory of Dendrology and Forest Ecology, Faculty of Forestry, Universitas Mulawarman, East Kalimantan.	Extraction was carried out using the successive maceration method with hexane, ethyl acetate, and ethanol solvents. Antioxidant activity was evaluated using DPPH test. Antimicrobial activity against Staphylococcus aureus and Streptococcus mutans was assessed using a 96-well-plate microdilution broth technique.	Litsea angulata extract contains alkaloids, flavonoids, tannins, terpenoids, and coumarins, according to the results of the phytochemical examination. All plant sample extracts have the capacity to prevent the production of DPPH free radicals and all tested bacteria, according to the results.
2	Phytochemical Screening of Methanol Extract of Kalangkala (*Litsea angulata*) Seeds (Mustikasari and Ariyani, 2010)	To determine the chemical compounds of Kalanga seeds. The chemical compounds tested in this study were alkaloids, triterpenoids, steroids, flavonoids, tannins, and saponins	The samples used were Kalangkala Seeds from South Kalimantan	Kalangkala seed powder was extracted with 80% methanol, filtered, and concentrated with a rotary evaporator. Secondary metabolites were discovered in a methanol extract of Kalangkala seeds. Secondary Metabolite Test (alkaloids, triterpenoids, steroids, flavonoids, tannins and saponins.)	The results of phytochemical screening using 80% methanol solvent show that the seeds contain secondary metabolites of alkaloids and tannins.

3	Phytochemical Screening and Antibacterial Activity Test of 70% Ethanol Extract of Kalangkala (*Litsea angulata* Bl.) Seeds against Acne-causing Bacteria *Propionibacterium acnes* (Ramadhan et al., 2020)	To determine the content of secondary metabolites of 70% Ethanol Extract of Kalangkala seeds and their activity as an antibacterial against *Propionibacterium acnes*	The samples used were Kalangkala seeds from South Kalimantan	Kalangkala seeds (Litsea angulata Bl.) were extracted using the cold method, namely maceration in 70% ethanol. The well diffusion method was used to test its antibacterial activity.	The secondary metabolites of alkaloids, flavonoids, saponins, and tannins were detected in a 70% ethanol extract of Kalangkala (Litsea angulata Bl.) seeds. The MIC (Minimum Inhibitory Concentration) results of 70% ethanol extract of Kalangkala seeds, namely, concentration of 25% is included in the moderate category, while concentrations of 50% and 100% are included in the strong category. These results indicate that 70% ethanol extract of Kalangkala seeds has potential as an antibacterial against *P. acnes* but is not more potent than the positive control of 0.1% clindamycin with a very strong antibacterial activity.
4	Identification of Kalangkala (*Litsea angulata* Bl) Bark Macroscopically, Microscopically, and Phytochemical Screening (Fitriyanti, Qalbiyah and Sayakti, 2020)	To provide scientific data regarding a qualitative pharmacognostic picture with several examination stages of organoleptic, macroscopic, microscopic, extraction, and phytochemical screening.	The samples of young green bark of Kalangkala in Madurejo, Sambung Makmur Sub-district, Banjarmasin	The screening was done by organoleptic examination, macroscopic and microscopic also phytochemical screening	The results of the organoleptic examination, fresh Kalangkala stem bark is green in color, tastes slightly bitter, and has a weak characteristic odor. Meanwhile, simplisia powder is light brown, tastes slightly bitter, and has a slightly pungent odor. The results of the microscopic examination, there are epidermis, vacuoles, chloroplasts, stone cells, calcium oxalate crystals, lumen fibers, and cork tissue. The sample also positive contains alkaloids, flavaonoids, saponins and tannin

5	Antioxidant Activity Test of Ethanol Extract of Kalangkala (*Litsea angulata*) Fruits and Seeds from South Kalimantan (Saputri and Susiani, 2018)	The antioxidant activity of an ethanol extract of Kalangkala fruits and seeds (fruit seeds) from South Kalimantan was investigated.	Kalangkala fruits and seeds (fruit seeds) from South Kalimantan were used as examples.	The DPPH technique was used to measure antioxidant activity.	On the TLC plate, the antioxidant activity test results of ethanol extract of Kalangkala fruits and seeds show yellow dots with a purple background. The antioxidant activity test of ethanol extract of Kalangkala seeds yielded a quantitative IC50 value of 48.78 ppm, while Kalangkala fruits yielded a quantitative IC50 value of 152.39 ppm.
6	Antioxidant Activity Test of Ethanol Extract of Kalangkala (*Litsea angulata*) Leaves and Stem Bark from South Kalimantan (Susiani and Saputri, 2020)	The antioxidant activity of an ethanol extract of Kalangkala (Litsea angulata) leaves and bark from South Kalimantan was investigated.	Kalangkala leaves and bark from South Kalimantan were used as samples.	The DPPH method was used to measure antioxidant activity both qualitatively and quantitatively.	The results of the antioxidant activity test of ethanol extract of Kalangkala (*Litsea angulata*) leaves and bark qualitatively show yellow spots with a purple background on the TLC plate. Whereas, the results of the antioxidant activity test of ethanol extract of Kalangkala leaves obtain an IC50 value of 152.39 ppm, while Kalangkala bark obtain an IC50 value of 85.33 ppm.
7	Toxicity, antioxidant ability and inhibition of oral pathogens by monoterpene-rich essential oil of *Litsea angulata* Blume (Kuspradini, Putri and Diana, 2020)	The chemical composition, toxicity, antioxidant activity, and in vitro antibacterial activity of essential oil extracted from Litsea angulata leaves were investigated.	*Litsea angulata* leaves were collected from the botanical garden of Universitas Mulawarman, East Kalimantan, Indonesia.	Gas chromatography -mass spectrometry (GCMS) was used for the qualitative analysis. Inhibitory activity against oral pathogens (*Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus sobrinus*, and *Candida albicans*)	The GC-MS analysis results indicate that 85.28 percent of the substances were monoterpenoids, with β-pinene and cis-verbenol being the most prevalent ones. The four studied bacteria are susceptible to the antimicrobial activity of the essential oil, which produces an inhibition zone measuring 11.44–50.00 mm in diameter. The highest inhibitory activity was obtained against *S. mutans* and *S. sobrinus*.

Phytochemical Screening of Metabolites of Kalangkala (*Litsea angulata*)

One method used to identify the compound content of a plant is phytochemical screening. Phytochemical screening is a preliminary stage that can provide an overview of the content of certain compounds in the natural ingredients to be studied. The classes of compounds contained in plants will be illustrated from the results of phytochemical screening by visually observing color changes. The main secondary metabolites content was obtained by optimizing the extract manufacturing process. The solvent used in extraction is one optimization that can be done to produce the main secondary metabolite content. The amount of substances that can be extracted can be seen by optimizing the extraction solvent.

The findings indicate that, in comparison to other extracts, the ethanol extract of *Litsea angulata* yields a higher amount of secondary metabolites. Depending on the kind of chemical structure, different polarity solvents can be used for the extraction of secondary metabolites. Compared to ethyl acetate and n-hexane, which are semi-polar and non-polar solvents, ethanol (polar) solvents are observed to be more effective in extracting different phytochemicals (Kuspradini et al., 2018). These findings demonstrate that macerating kalangkala seeds in a 70% ethanol solvent can yield more secondary metabolites than macerating the seeds in a methanol solvent, which solely yields alkaloids and tannins (Mustikasari and Ariyani, 2010).

The use of 70% ethanol solvent in the maceration process is effective due to its ability to dissolve almost all polar, semi-polar, and non-polar substances. Ethanol is the best solvent for the extraction of phenolic compounds in almost all plants, when compared to other solvents, because it is polar which extracts phenolic compounds from a plant. Ethanol can also produce more chemical compounds compared to water and methanol. Ethanol solvent produces secondary metabolites of alkaloids, flavonoids, saponins, and tannins. In conclusion, polar solvent (ethanol) can produce more secondary metabolites than semi-polar and non-polar solvents (ethyl acetate and n-hexane).

Antibacterial Activity Test

The kind and quantity of chemicals that can be extracted from the material depends on the solvent's polarity level; it will only extract substances that share its polar characteristics (Lestiani, 2008). Ethanol extract containing polar compounds (alkaloids, flavonoids, saponins and tannins) also provided an inhibition zone against all tested bacteria. Based on the observation results, it was found that the greatest inhibitory power was found in the ethanol extract which contains alkaloids, flavonoids, saponins and tannins against bacteria. Antibacterial activity be affected by the polarity of the compound extracted by each solvent and the ability of the substance to disperse in different media used in testing antibacterial activity (Parekh, Jadeja and Chanda, 2005). that each group of compounds can provide different effects in inhibiting bacterial growth. The differences in activity that occur are caused by the secondary metabolites contained which have different synergistic effects depending on the nature and morphology of the bacteria (Schlegel, 1993).

The inhibition zone produced by ethanol extract of Kalangkala is related to the results of phytochemical screening test which shows the content of flavonoids, alkaloids, saponins, and tannins which play a role in producing antibacterial activity. Flavonoids and tannins are phenolic group compounds that have an antibacterial mechanism by changing the permeability of the cytoplasmic membrane, causing cell leakage. This class of compounds also denatures and inactivates proteins/enzymes. Mechanisms of flavonoids are specifically able to stop the formation and release of substances that cause inflammation (Setyopuspito, 2017). Tannins have pharmacological activity as anti-inflammatory, astringent, anti-diarrheal, and antiseptic (Dwika et al., 2016).

Whereas, mechanisms of alkaloids interfere with the peptidoglycan component, so the cell wall layer is not formed completely and causes cell death (Hafsari et al., 2015). Saponins are also positively identified in 70% ethanol extract of kalangkala seeds. Saponins work effectively on Gram-positive bacteria. The antibacterial properties of saponins are due to their mechanisms which can increase the permeability of cell membranes, so they become unstable and hemolysis of cells occurs (Rahman, Haniastuti and Utami, 2017). Saponins also have anti-inflammatory activity (Dwika et al., 2016). Alkaloids can affect the preparation of the bacterial cell wall, which interferes with the formation of peptidoglycan, so the cell wall does not form completely. Without a cell wall, bacteria cannot withstand external influences and soon die. Alkaloids have effects in the form of antimicrobials, reducing pain, accelerating wound healing, and so on (Aksara, Musa and Alio, 2013).

Antioxidant Activity Test

Antioxidant testing with DPPH is a simple method using 1,1-diphenyl-2-picrylhydrazil (DPPH) as a detection compound, the antioxidant testing method with DPPH is foolproof to apply because this method is simple, practical, accurate and fast in capturing free radicals with other compounds. DPPH is a free radical compound that is stable till it can react with hydrogen atoms originating from an antioxidant to form reduced DPPH, a compound is said to be a very strong antioxidant if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value

is 50-100 ppm, moderate if the IC_{50} value is 100-150 ppm, weak if the IC_{50} value is 150-200 ppm, and very weak if the IC_{50} value is > 200 ppm (Molyneux, 2004).

A quantitative test of the antioxidant activity of ethanol extract of kalangkala leaves were included in the weak category (IC50 152.39 ppm), while results of the antioxidant activity of ethanol extract of Kalangkala stem bark are included in the strong category with an IC_{50} value of 85.33 ppm. The smaller the IC50 value, the greater the antioxidant capacity. When compared to quercetin, the antioxidant activity of ethanol extract of Kalangkala leaves and stem bark is still much lower (Susiani and Saputri, 2020)

DPPH radical scavenging activity of *Litsea angulata* essential oil shows the ability to scavenge DPPH radical. However, the activity is much lower than the reference standard. *Litsea angulata* essential oil shows a concentration-dependent increase in DPPH up to a certain concentration and decreases thereafter. *Litsea angulata* essential oil can reduce DPPH radical without reaching 50% inhibition of DPPH radical with DPPH activity of 6.14–13.72% for the concentration range of 6.25–100 µg/mL (Kuspradini, Putri and Diana, 2020). This indicates that essential extract of *Litsea angulata* leaves is a weak antioxidant at concentrations ranging from 6.25–50 µg/mL. The highest scavenging is observed at 12.5 µg/mL with only 13.96 % inhibition. The weak antioxidant activity of *Litsea angulata* oil is possibly related to the high content of monoterpenes (non-phenolic compounds) and the presence of y-pinene and cis-verbenol as major components.

Different antioxidant activities are shown from the results of kalangkala extraction using different solvents, that the type of solvent influences the antioxidant activity based on the IC_{50} value of the extract. The use of solvent will determine the level of antioxidant activity obtained in an extraction because antioxidant activity will be shown differently with the polarity of different compounds (Ismail and Hong, 2013). Extraction using ethanol as a solvent produced the highest antioxidant activity compared to water, methanol, n-hexane and ethyl acetate. The research results showed that the ethanol solvent afford of produce phenolics, flavonoids and tannin so this also affected the antioxidant activity of kalangkala extract. Antioxidant activity is influenced by the increase in phenols and flavonoids in the ingredients so the higher the total phenols and flavonoids, the higher the antioxidant activity.

CONCLUSION

The Kalangkala plant (*Litsea angulata*) contains chemicals that are secondary metabolites, including tannins, carotenoids, alkaloids, flavonoids, tannins, saponins, triterpenoids, and coumarin. Those secondary metabolites can be extracted with ethanol as the solvent. The use of ethanol as a solvent has strong antibacterial and antioxidant properties as well. Therefore, it is possible to recommend additional standardization of the plants and extraction technique in the studies in order to formulate a topical or oral preparation as an antioxidant.

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