

Stigmasterol As A Secondary Metabolite Of *Peronema Canescens* Jack. Leaves And Its Efficacy As An Immunostimulant

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ABSTRACT

Introduction: Sungkai plants (*Peronema canescens* Jack) have many benefits, one of which is as an immunostimulant. secondary metabolite content contained in sungkai leaves, one of which is stigmasterol. **Objective:** to identify the efficacy of stigmasterol which is a secondary metabolite of sungkai leaves that can act as an immunostimulant. **Methods:** Literature Review. Data sources came from research journals on secondary metabolites produced by sungkai plants that can improve the body's immune system and play a role in traditional medicine from various national and international sources. The data search strategy used was to search directly for literature through the Google search engine and use the Google Scholar, Pubmed, and Science Direct databases with the keywords "Peronema canescens Plant", "Secondary Metabolites of Sungkai Leaf", or "Efficacy of Sungkai Leaf". Inclusion criteria included national journals on the efficacy of sungkai plants with Sinta accreditation 1-6, and Scopus indexed international journals with Q1-Q4 rankings. Exclusion criteria included journals that did not focus on the efficacy of sungkai plants, journals that were not accredited, and research journals with a review method. **Results:** From national and international journals that have been reviewed, it was found that the secondary metabolites of sungkai plants have properties as immunostimulants, fertility, and have been tested for safety in animal trials and from generation to generation by the community. besides that one of the secondary metabolites contained in sungkai leaves, namely stigmasterol, has been shown to increase the concentration of CD8 + T cells. **Conclusion:** Based on the literature that has been collected, it can be concluded that there have been many studies on sungkai leaf extract which is effective for maintaining endurance and proven safe, but for the secondary metabolites found are still relatively fewer, so it is hoped that in the future a lot of research will be carried out related to the active compounds of sungkai leaf.

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I. INTRODUCTION

The compound stigmasterol is employed in various chemical manufacturing processes intended to generate various semi-synthetic and synthetic components for the pharmaceutical industry. Within the European Union, it is listed as a food additive under the number E499, which may be used to boost phytosterol levels in the production of foods, thereby helping to improve low-density lipoprotein cholesterol (LDL-cholesterol) levels. This phytosterol is C24 alkylated cholesterol operating primarily as a common constituent of the cell membrane and playing a central role in membrane stability. Increased interest is focused on essential oils, algae, and plant extracts as a source of promising bioactive compounds for drug development. There are currently around 250 plant sterols that differ in function and accessibility, but stigmasterol has become a unique compound due to its diverse pharmacological properties. Indeed, this phytosterol has been widely studied for its anti-diabetic, antioxidant, anticancer, anti-inflammatory, antiviral, antiparasitic, anti-osteoarthritis, antibacterial, neuroprotective, and immunomodulatory properties (1).

Stigmasterol rich plant extracts exhibited effective anti-inflammatory and immunomodulatory activities in vivo. It was able to ensure the decrease in the release of tumor necrosis factor- α (TNF- α), nitric oxide (NO), and pro-inflammatory cytokines, as well as the inhibition of cyclooxygenase-2 (COX-2). Traditional Chinese

medicine values stigmasterol containing drugs in immune inflammatory response control, such as Smilacis Glabrae Rhixoma (SGR) for the management of myasthenia gravis and xiaoqinglong (XQLD) against severe coronavirus disease 2019 (COVID-19) (1).

The Indonesians see several herbal plants as natural fertility enhancers. One such herb is pegandew which contains isoflavone flavonoids, saponins, and alkaloids that promote mammal estrogen production. Pegagan dew leaf extract effectively increases the number of ovarian follicles at low doses. According to studies, gandarusa leaf, which contains flavonoid compounds in the form of isoflavones, can increase the endogenous hormone estradiol and the number of secondary follicles. These plants contain secondary metabolites, almost the same as the sungkai plant. The results of the phytochemical test on Sungkai revealed that the plant contains various secondary metabolites, including alkaloids, steroids, terpenoids, tannins, and saponins. Another research shows that the ethanol extract of sungkai leaf also contains phenolics, anthraquinones, and flavonoids. Flavonoids are polyphenols found in many plants (2).

Sungkai plants (*Peronema canescens* Jack) are widely used as immunostimulants. Research conducted by Dillasamola (2023) found that the toxicity of ethanol extract of Sungkai leaves on the observation of the ratio of kidney organs showed that there was a significant effect of dose variation and duration of administration on the ratio of kidney organs (3).

Sungkai leaf decoction is also used by local residents in the Curup area, Bengkulu Province as a cure for malaria. In the treatment of the Dayak Tunjung tribe in East Kalimantan, young sungkai leaves are used as a fever medicine while the roots are used as a diuretic and body aches medicine (4).

Dayak people in East Kalimantan still use sungkai in the treatment of diseases. The young leaves are used as mouthwash to prevent toothache and fever. According to Yusrin (2008), in the treatment of the Serawai tribe in South Bengkulu, sungkai leaves are pounded and given to bruises. The Suku Anak Dalam (SAD) tribe in the Bukit Duabelas National Park (TNBD) area of Jambi uses sungkai bark for external wounds, internal wounds, and bloody diarrhea. The utilization of sungkai bark for traditional medicine is knowledge and skills that have been passed down from generation to generation by the tribe. (3).

The use of traditional medicine has been known long before the existence of formal health services by considering its benefits empirically. Nowadays, herbs are used as a complement to primary treatment. In the last decade, many have turned to traditional medicine products and practices with the assumption that natural means safe, which is not necessarily true. All effective drugs can have adverse reactions, including herbal medicines. For this reason, in the use of herbal medicine, it is important to consider the dose, time of use, method of use, and selection of drugs for the disease. The immune system can be divided into two types: the nonspecific immune system (innate immunity) and the specific immune system (adaptive immunity). The nonspecific immune system has faster activity because it does not involve the memory cells. Several components that are involved in the nonspecific immune system are macrophage cells (phagocytic white blood cells) and natural killer cells, which protect the body from pathogen attacks so that the body eventually builds its defence system. The body's defence system can be activated by providing compounds that can increase the body's immune response (1).

Sungkai skin can be used as a natural antioxidant and sungkai leaves can boost the immune system. So that this traditional treatment can be used in the health care system and in accordance with the rules of formal health services, which must be medically accountable. In addition, sungkai skin also has potential as a natural antioxidant (4).

The literature study of this Sungkai plant extract is as follows: Yani's research (2014), *Peronema canescens* Jack dose of 0.5625 mg/kgbb can increase the number of leukocytes by 36%. The results of Ningsih's research (2013), that the ethanol extract of *Peronema canescens* Jack leaves has antiplasmodium activity in vivo with an ED50 value of 102.88 mg / kgbb. Arna Ningsih's research (2013) examined the n-hexane extract of Sungkai leaves to provide growth inhibition against all test bacteria at a level of 1 mg/ml. Research on the activity of hand antiseptic gel preparations made from ethanol fraction extract of Sungkai leaves (*Peronema canescens* Jack) against several pathogenic bacteria (5). Research on Antiplasmodium Activity Test of n-hexane Fraction of Sungkai Leaf (*Peronema canescens* Jack) against *Mus musculus* (5). Research on Antiplasmodium Activity Test of n-hexane Fraction of Sungkai Leaf (*Peronema canescens* Jack) against *Mus musculus* (6).

From the results of immunostimulant testing of 3 sungkai leaf isolates, it was found that (S)-4-methylheptyl 1H-imidazole-4-carboxylate had an immunostimulant effect in increasing Natural Killer cells (NK cells) higher than stigmasterol and bis (2-ethylhexyl) phthalate. Increasing the dose given also increases the effect on the immunostimulant effect of each isolate. In the results of Cluster of Differentiation 8 + T (CD8 + T) showed the highest concentration at a dose of 100 Mg / kgbb on stigmasterol isolate (7).

Determination of total flavonoid content was carried out by UV-Vis spectrophotometry using the collometric method with AlCl₃ complex reagent and antioxidant activity test of sungkai leaf extract determined by DPPH (2,2- diphenyl-1-picrylhydrazyl) method showed total flavonoid content of ethanol extract of

Peronema canescens Jack leaves of $1,057 \pm 0.002$ mg EK/g extract and has antioxidant activity with IC₅₀ value of 44,933 ppm so that it is included in the category of highly active antioxidants (8).

Another study also highlighted the antihyperuricemia activity of sungkai extract through a decrease in blood uric acid levels in rats. This extract was also reported to show antibacterial activity against the growth of *E. coli* (9,10).

The anti-inflammatory and anti catabolic effects of stigmasterol have been reported to be associated with the inhibition of the NF- κ B pathway. Stigmasterol protects hypoxia/reoxygenation-induced excitotoxicity by suppressing reactive oxygen species (ROS) generation and modulating mitophagy, which indicates that stigmasterol has neuro protective properties against ischemic stroke. Stigmasterol also exhibits anti-cancer activity in various cancer cells, such as breast, liver, gastric, ovarian, gall bladder, and cholangiocarcinoma. It affects cancer cell proliferation, growth, and apoptosis via inducing cell cycle arrest, upregulating apoptotic protein expression, inducing endoplasmic reticulum-mitochondrial dysfunction, inhibiting cell migration and invasion, and suppressing angiogenesis. Recently, Liao and colleagues showed that stigmasterol overcame cisplatin resistance in EC cells by repressing the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway. However, the effect of stigmasterol in cancer stem cell (CSC) activity, which is the key characteristic for resistance and metastasis in malignancies, remains unclear (11).

Sungkai plant (*Peronema canescens* Jack) is one of the medicinal plants that has been widely used as medicine such as medicine for malaria, antiplasmodium, pesticides, antipyretics, immunity, and teratogenicity. The content of secondary metabolite compounds contained in sungkai leaf extract is such as alkaloid, terpenoid, steroid, flavonoid, and tannin compound groups, and there are seven types of clerodane diterpenoid compounds contained, namely peronemin A2, A3, B1, B2, B3, C1, and D1 (12).

According to Pindan 2021, ethanol extracts from sungkai leaves have been shown to contain alkaloids, flavonoids, steroids, triterpenoids, phenolics and saponins. The flavonoid compound here acts as an analgesic by reducing the production of prostaglandins, where the mechanism of the flavonoid compound is by inhibiting the work of the cyclooxygenase enzyme so that it can reduce pain (2). In sungkai leaves there are bioactive compounds such as triterpenoids, alkaloids, flavonoids, phenolics, steroids and saponins, where these compounds are believed to have antioxidant activity (13).

The types of secondary metabolites found in sungkai leaves in crude extracts are alkaloids, flavonoids, phenolics, steroids and saponins. In the n-hexane fraction there are compounds such as steroids, flavonoids and triterpenoids. Furthermore, the ethyl acetate fraction contained compounds such as alkaloids, triterpenoids and steroids and the remaining ethanol fraction was alkaloid, flavonoid, phenolic, steroid and saponin (4).

In this literature review, examine the efficacy of sungkai leaves that can play a role in maintaining the body's immune system, especially when attacked by the Covid-19 virus. In various conditions that can reduce the human body's defense system so that with the existence of sungkai leaves which contain secondary metabolites that play a role in increasing the immune system, there is a need for more studies on the efficacy of sungkai leaves.

II. METHODOLOGY

The method used is literature review. Data sources came from research journals on secondary metabolites produced by sungkai plants that can improve the body's immune system and play a role in traditional medicine from various national and international sources. The data search strategy used was to search directly for literature through the Google search engine and use the Google Scholar, Pubmed, and Science Direct databases with the keywords “*Peronema canescens* Plant”, “Secondary Metabolites of Sungkai Leaf”, or “Efficacy of Sungkai Leaf”. Inclusion criteria included national journals on the efficacy of sungkai plants with Sinta accreditation 1-6, and Scopus indexed international journals with Q1-Q4 rankings. Exclusion criteria included journals that did not focus on the efficacy of sungkai plants, journals that were not accredited.

III. DISCUSSION

Indonesia is an archipelagic country which has various kinds of natural resources. One of the wealth owned by Indonesia is its flora and fauna. The wealth of flora owned by Indonesia is included in the category of medicinal plants. Indonesia has about 30,000 types of plants and 7,000 other types of plants that can be used as medicinal. One example of a plant that can be used as traditional medicine and whose efficacy has not been scientifically proven is sungkai (*Peronema canescens* Jack). Sungkai is a plant that contains compounds such as flavonoids, tannins, phenolics, saponins, steroids, and terpenoids, which have the potential to develop native Indonesian herbal medicines to treat the prevention and treatment of various diseases. (14).

The natural resources in Indonesia stores various types of plants, including medicinal plants. The use of plants as medicines is still ongoing today. The use of medicinal plants is often used by the community because those are believed to have relatively mild side effects, easy to obtain, and cheaper than synthetic drugs. The plant that is often used as a medicinal plant is sungkai. The root bark is used to cure infectious diseases.

Infectious diseases are diseases caused by infectious pathogens. Infectious disease is one of the diseases that greatly impacts the entire population and economy globally. Corona virus, one of the infectious diseases, is a problem that is still being faced by the global community today. To prevent its spread, foods or substances that contain antioxidant and antibacterial compounds are needed to increase immunity. Immunostimulants are closely related to antioxidant and antibacterial activity (15).

There was an effect of dose variation and duration of administration of ethanol extract of sungkai leaves on the histology of kidneys, with increased kidney histology damage. The highest increase occurred at 21 days of administration at a dose of 560 mg/kg bw. The study concluded that there is a need for caution in the prolonged use of sungkai leaf extract, especially at a dose of 560 mg/kg bw and beyond (1).

The ethanol fraction of Sungkai leaves is proven to be able to inhibit the growth of *Plasmodium berghei* parasites in the red blood cells of male white mice, where at a dose of 0.084 g / kgbb has the greatest percentage of inhibition compared to other treatment groups, reaching 54.06%. It can be concluded that the ethanol fraction of Sungkai leaves at a dose of 0.084 g / kgbb is the most effective dose and can have potential as an antimalarial. The results showed that the ethanol fraction of Sungkai leaves has the ability to inhibit the growth of *Plasmodium berghei* parasites in the blood of male white mice better than the synthetic drug chloroquine (12).

The antiplasmodium activity test of the n-hexane fraction of Sungkai leaves against male white mice showed a decrease in the number of parasitemia in male white mice infected with *Plasmodium berghei*. At a dose of 0.084 g / kgbb has an average value of a more significant decrease compared to doses of 0.028 and 0.056 g / kgbb. This shows that there is a relationship between increasing the dose of the n-hexane fraction of Sungkai leaves on the decrease in % parasitemia of male *M. musculus* directly proportional to the increase in sample dose (12).

Different doses of ethanol leaf extract of *Peronema canescens* have been shown to increase the number of ovarian follicles, corpus luteum, and ovulation rates of both ovaries of female Wistar mice (*Mus musculus* L). This increase was optimal at an oral dose of 200 mg/kg bw. The study concludes that the leaf extract of *Peronema canescens* is a potential natural product that could be used in treating female infertility (2).

Other information states that flavonoids are known as antibacterial agents against various pathogenic microorganisms. With the increasing prevalence of untreatable infections caused by antibiotic-resistant bacteria, flavonoids have been shown to be potential substitutes for antibiotics. The hydroxyl groups in the aromatic ring of flavonoids increase their bioactivity, both antioxidant and antibacterial. However, methylation of active hydroxyl groups generally decreases bioactivity. In addition, the lipophobicity of the A ring is critical for the activity of the chalcones. Hydrophobic substituents such as prenyl groups, alkylamino chains, alkyl chains, and heterocyclic groups containing nitrogen or oxygen usually increase their bioactivity (15).

Plants containing epifriedelinol, lupeol, stigmasterol, triacontane-1-ol, dotriacontane-1-ol, lupeol acetate, deoxyelephantopin, isodeoxyelephantopin, polyphenol luteolin-7, as well as various flavonoids and glucosides can act as immunostimulants because stigmasterol is a compound that has been shown to have health benefits that include lipid-lowering, anti-cancer, anti-inflammatory and anti allergic effects. Previous studies reported that stigmasterol acts as an anti-inflammatory and anti-arthritis and stigmasterol has the potential to modulate the immune response. Stigmasterol is an unsaturated phytosterol belonging to the class of tetracyclic triterpenes. It is one of the most common plant sterols, found in various natural sources, including vegetable fats or oils from many plants. Currently, stigmasterol has been examined through in vitro and in vivo assays and molecular docking for its various biological activities on different metabolic disorders. Findings show strong pharmacological effects such as immunomodulators in enhancing the specific immune system (7).

IV. CONCLUSIONS

Based on the literature that has been collected, it can be concluded that there have been many studies on sungkai leaf extract which is effective for maintaining endurance and proven safe, but for the secondary metabolites found are still relatively fewer, so it is hoped that in the future a lot of research will be carried out related to the active compounds of sungkai leaf.

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