

Review

An in-vogue plant, *Peronema canescens* Jack: traditional uses and scientific evidence of its bioactivities

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Received: 2 July 2024 / Accepted: 14 November 2024

Published online: 17 December 2024

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Abstract

Peronema canescens Jack (sungkai) has been utilized to treat numerous medical conditions in the traditional healing practices of people in Sumatra and Borneo for generations. The diverse secondary metabolites in the plant give preliminary scientific evidence of the validity of its traditional uses and further studies have confirmed its various bioactivities. The plant's pharmacological properties have been studied occasionally before 2020, leading to its antimicrobial, cytotoxic, and antioxidant activity discoveries. In 2020, sungkai was rumored to be able to treat coronavirus disease 2019 (COVID-19) which made it gain popularity that changed the history of its pharmacological studies dramatically. Since then, research on sungkai has been pursued exponentially in more diversified and specialized bioactivities. Recent studies over the past three years revealed that the plant further has anti-inflammatory, immunomodulatory, and antidiabetic activities. Reports continue to emerge suggesting the presence of other sungkai's bioactivities. A comprehensive review was presented covering the topic, starting from the discussion of traditional uses of sungkai to its bioactivity studies. The review is integrated with the key events of its bioactivity studies development in history and its link to the current studies. Most recent scientific evidence of sungkai bioactivities is discussed and critically analyzed to give insight into its future development direction. Additionally, the attempt to develop numerous derivative products from sungkai is also briefly introduced. Scientific studies evince that sungkai is a promising plant as a source of bioactive compounds that could be developed for modern medicine.

Keywords *Peronema canescens* · Sungkai · Medicinal plants · Ethnobotany · Phytochemistry

1 *Peronema canescens* Jack: introduction

Peronema canescens Jack was first scientifically described in 1822 by William Jack [1]. The plant is natively distributed on the west side of the Wallace line, including Thailand, the Malay Peninsula, Sumatra, Borneo, and West Java. Local names for *Peronema canescens* Jack include sungkai (Sumatra, Borneo, and the Malay Peninsula),urus (Kalimantan, Banjarmasin), and jati sabrang (Java, Sunda) [2]. Since the plant is widely known under the name sungkai, sungkai will be used as the name to refer *Peronema canescens* Jack throughout the remainder of this article.

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Sungkai, as shown in Fig. 1, is a woody tree that can grow up to 35 m tall. It belongs to the Lamiaceae family and is the only known species in the genus *Peronema* [3]. Its characteristics include having grey-brown, fissured bark and compound leaves with winged rachis, often purplish when young. The leaflets are elliptical and have serrated edges that smoothen as they mature. Sungkai produces small — white or greenish-purple flowers in clusters and dry — capsular fruits. The tree thrives in tropical rainforests, often near streams, and is known for its durable timber used in construction. The wood of sungkai has been widely used to construct houses as well as create numerous pieces of furniture. Besides its constructive material use, in some regions of its native distribution, sungkai has also been used in traditional healing practices for generations. Local communities of the islands of Sumatra and Kalimantan believe the consumption of brewed sungkai leaves could prevent diseases and enhance the body's immune system [4, 5].

In recent years, sungkai's medical potential has gained more attention from the scientific community. Early phytochemical screenings have revealed several important secondary metabolites, such as alkaloids, flavonoids, terpenoids, and phenolics. Such compounds are known for their bioactive properties, including antimicrobial effects, anti-inflammatory qualities, and antioxidant activities. With them in mind, researchers found the pharmacological properties of the plant well worth studying further to confirm current uses and to provide modern scientific evidence.

A review of recent sungkai pharmacology research is necessary to highlight the widespread interest in this traditional medicine and growing scientific evidence of its bioactivities. The urge to review the topic is also driven by the spotlight put on the plant during the COVID-19 pandemic in 2020, after rumors surfaced about its ability to cure the diseases. Although that information was subsequently proven false, the attention still led to an exponential increase in research on its bioactivity, including antimicrobial, cytotoxic, and antioxidant activities. This growing body of work heightens the need for a comprehensive review to bring together historical uses and today's pharmacological studies on sungkai.

2 Ethnobotany: traditional uses

Sungkai has a long history and has been integrated with the culture of Malayic tribes in Sumatra and the Malay Peninsula as well as those under their heavy influence. The name sungkai has been used to name numerous places in the region and mentioned in their oral and written traditional literature [6]. Various utilizations of sungkai as a medicinal plant throughout the island have already been reported. As documented by Indriati [7], leaves of sungkai have long been harnessed to alleviate stomachache by the Anak Dalam people and the bark is used to cure burns and bloody diarrhea in their culture [8]. Meanwhile, East Lampung indigenous people use it as a cure for jaundice and as a body freshener [9]. Furthermore,

Fig. 1 Sungkai tree in the University of Jambi vicinity (plant image is the authors' own documentation)



sungkai leaves are utilized to treat bruises by crushing and patching them on the bruised location by the Serawai tribe in Bengkulu [10]. The prescription of sungkai for the treatment of malaria by the locals in the same region also brought Kitagawa and his team to embark on an expedition in 1990 and found 2 compounds exhibiting antimalarial activity from its leaves [11]. The plant is also widely used across the Malayic tribes in the region to address wounds.

In Borneo, the Dayak tribe of East Kalimantan uses leaves of sungkai to cure colds, fever, and worm infections. Brewed sungkai water is also used as a mouthwash to treat toothache [12]. The Dayak and Banjar indigenous people of Central Kalimantan likewise use brewed sungkai water to boost the body's immune system and to cure fever. In the tribes' tradition, the leaf is harnessed to address bruises by directly attaching the crushed leaves to bruised parts. Sungkai leaves are also mixed with other traditional medicines to prepare traditional postpartum mother's bath ritual by the tribe, by steaming the potion vapor on the postpartum mother's body in a closed room [13].

The notion of sungkai's medicinal properties that are distributed among local people of Sumatra and Borneo is not yet known whether was developed independently, inherited from their common ancestors, or resulted from information exchange in later contact. However, it is clear that the name sungkai for the plant is distributed throughout Malayic people in remote areas of Sumatra, Borneo, and the Malay peninsula that have limited mutual contact in recent times, indicating that the name of the plant might be inherited from their common ancestor. Assuming that the knowledge about the plant exists along with its medicinal use, it implies that sungkai may have been used for millennia since the separation of the Malayic tribe from their common ancestor occurred around 2000 years ago [14]. This precious ancient wisdom is important both in cultural and medicinal perspectives. Therefore, to ensure the continuity of the knowledge as well as to validate the efficacy of sungkai medicinal utilizations, scientific evidence must be provided.

3 Phytochemistry

Although pharmacological research on sungkai started in 1990, the first available complete conventional phytochemistry screening of sungkai extract to date was from a publication by Ibrahim et al. in 2012 [15]. The screening was conducted on the methanol extract of sungkai leaves and gave positive results for the presence of phenolics, tannins, flavonoids, alkaloids, and steroids. Other phytochemistry studies over the following years are summarized in Table 1.

Table 1 Phytochemical screening of sungkai extracts

Plant part	Solvent	Secondary metabolite class							Reference
		Alkaloids	Flavonoids	Terpenoids	Steroids	Phenolics	Saponins	Tannins	
Dried leaf	Ethanol	+	+	+	+	+	+	+	[20]
	Methanol	+	+	+	+	+	-	+	[15]
	n-Butanol	+	+	N/A	+	+	+	+	[21]
	Ethanol	+	+	N/A	+	N/A	+	+	[22]
	Ethanol	+	+	+	+	+	+	N/A	[23]
	Ethanol	+	+	N/A	N/A	+	+	+	[24]
	Water (infusion, brewed at 90 °C)	+	+	+	-	+	+	+	[16]
Fresh leaf	Hexane	+	-	+	-	-	-	-	[18]
	Ethyl acetate	-	+	-	+	+	+	+	[18]
	Methanol	-	+	+	+	+	+	+	[18]
	Ethanol	+	+	N/A	+	+	+	N/A	[25]
Dried bark	Ethanol	+	-	-	+	+	+	+	[20]
	Ethanol	+	+	N/A	N/A	+	+	N/A	[26]
	Methanol	N/A	+	+	+	+	N/A	N/A	[19]
	Ethyl acetate	N/A	+	N/A	+	+	N/A	N/A	[19]
	Hexane	N/A	N/A	+	N/A	N/A	N/A	N/A	[19]

(+): presence of the secondary metabolite detected
(-): presence of the secondary metabolite not detected
(N/A): the data are unavailable, either because the test was not performed, or the result was not explicitly reported

Phytochemical screening and research on sungkai in general are mostly conducted on its leaves because they are the part used by the indigenous people for medications. However, the bark has also been explored by some researchers. Most of the phytochemical screening results agree with one another that sungkai leaves contain all major secondary metabolite classes namely alkaloids, flavonoids, terpenoids, steroids, phenolics, saponins, and tannins although they may be extracted in different solvents. Ethanol exhibits the ability to extract most of the secondary metabolite classes from sungkai leaves. Extraction of dried sungkai leaves by water-brewing still gives positive results to most of the compound classes except steroids [16]. This result agrees with the low polarity of steroids which makes them harder to dissolve in water. The finding indicates that the use of brewed sungkai water in traditional uses may be less effective if the active compound is a steroid.

Methanol and n-butanol showed a similar ability to ethanol in extracting sungkai leaf secondary metabolites [15, 17, 18]. However, for methanol, there are discrepancies between dried leaves [15] and fresh leaves [18] results. The dried leaves gave a negative result for saponin, meanwhile, the fresh ones gave a positive result. In turn, the result of the dried leaves for alkaloids was positive, while it was negative for the fresh leaves. The difference in water content of the sample should not interfere with the test result since water is miscible with methanol. The discrepancies might be due to random or personal errors. Predicting from the results given by ethanol and n-butanol extractions, methanol is likely to give positive results for both alkaloids and saponins. However, a thorough experimental study needs to be done to confirm.

Hexane exhibits a poor ability to extract polar secondary metabolites from sungkai's leaves and bark yet has a good ability to extract its terpenoids [18, 19]. This ability of hexane to discriminately extract terpenoids can be employed to separate them from the rest of the secondary metabolites. However, Santoni et al. [18] reported that sungkai fresh leaf hexane extract tested positive for containing alkaloids in addition to terpenoids. This report needs to be further confirmed with another experiment considering the limited solubility of alkaloids in non-polar solvents such as hexane.

Besides using conventional phytochemical screening, some studies employed chromatographic phytochemical screening techniques such as thin layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectroscopy (GC-MS) screenings. TLC phytochemical screening on sungkai leaf extracts showed the best separation with a mobile phase consisting of hexane to ethyl acetate to isopropanol (7:3:0.3). Further study with HPLC screening run in 0.1% formic acid and acetonitrile found there were eight compounds can be isolated from sungkai leaf methanol extract [27]. It was confirmed that the ¹H-NMR spectra of the compounds were not similar to 7 clerodane-type diterpenoids found by Kitagawa et al. in 1994 [11]. Thus, further studies are necessary to elucidate the structure of the compounds. GC-MS profiling of sungkai leaf shows that there is a similar chemical composition in young leaves, half-mature leaves, and mature leaves of sungkai although intensity differences were observed. The automatic spectra comparison with the National Institute of Standards and Technologies, Mass Spectra Libraries (NIST) data identified the presence of several organosilicon compounds in the extracts [28].

Several attempts to isolate compounds from sungkai leaf have been made. The earliest attempt was from Kitagawa et al. in 1994 [11] who successfully isolated 7 clerodane-type diterpenoids from its acetone extract namely peronemin A₂, A₃, B₁, B₂, B₃, C₁, and D₁. Other compounds claimed to be successfully isolated from sungkai leaf including betulinic acid [29] and squalene [30] from its n-hexane extract; stigmasterol [29] and β-sitosterol [31] from its ethyl acetate extract; 5,7-dihydroxy isoflavones [31] from its methanol extract; and apigenin [30] from its ethanol extract. Compounds isolated from sungkai leaf are summarized in Fig. 2. The bioactivities of the isolated compound were investigated in the studies and further discussed in subsequent sections.

The detected concentration level of various secondary metabolites in sungkai extracts evinces its ability to bear bioactivity. These secondary metabolites are known to be responsible for numerous pharmacological properties of plant extracts. The information about sungkai's phytochemistry led to further research into its bioactivity.

4 Bioactivities

Based on its traditional use as a medicinal plant, sungkai has attracted the attention of the scientific community. The biological description of sungkai has been recorded as early as 1822. In the period of 1850 – 1990, sungkai occasionally appeared in a few botanical publications describing the biology and ethnobotany of the plant. The phytochemistry evaluation of sungkai did not come to scientific attention until 1994. The work published by Kitagawa et al. [11] identified seven clerodane-type diterpenoid compounds from its leaf acetone extract which two of them showed anti-malarial activity namely peronemin A₁ and peronemin A₃. Since then, scientific evidence of its bioactivity has been being pursued. Reports on sungkai occasionally occurred afterward in publications until its blooming period in 2021—2023. Pharmacological

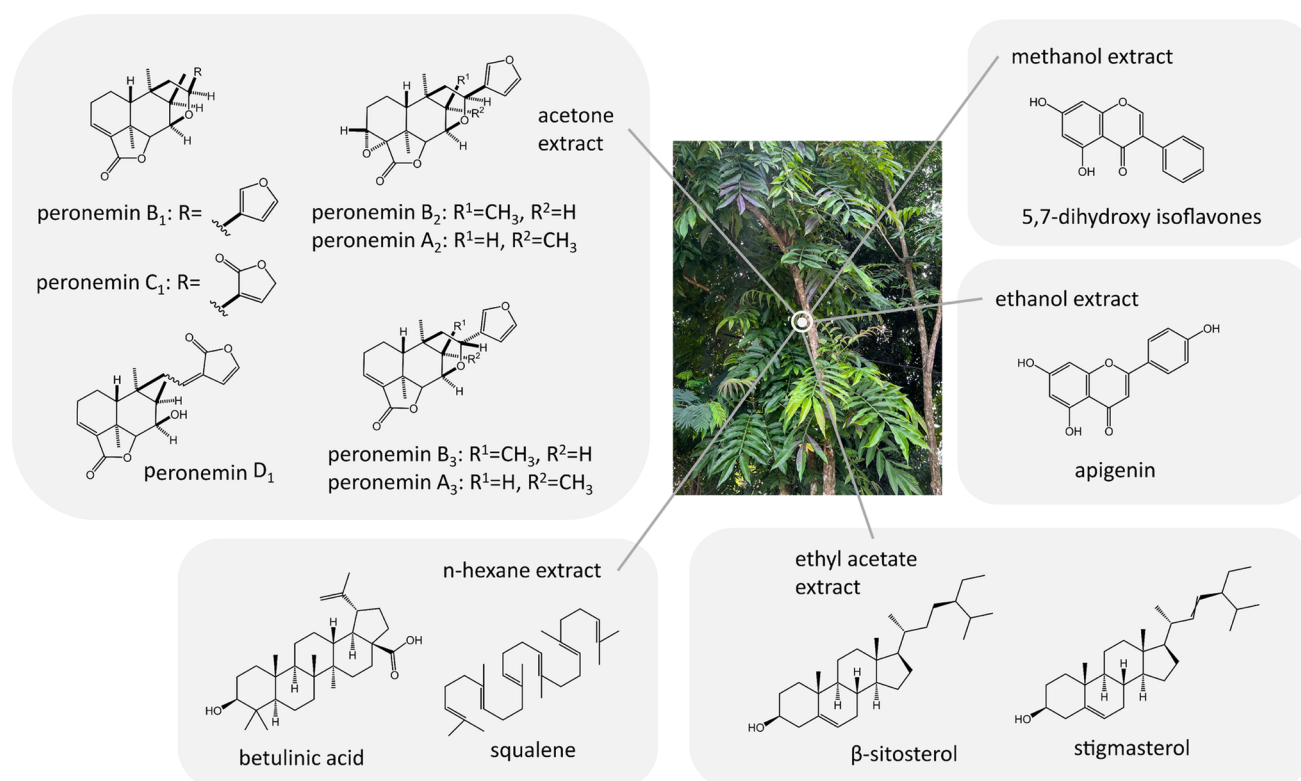


Fig. 2 Compounds isolated from sungkai leaves (plant image is the authors' own documentation)

research on sungkai went from scarcity to trend starting in 2020. The COVID-19 pandemic in the year has given sungkai more attention, especially in Indonesia. The event was initiated by a circulating rumor sourced from a Facebook posting on May 28th, 2020, from an Indonesian user claiming that a paramedic from Jambi successfully cured five COVID-19 patients with sungkai consumption and suggesting its development as an alternative medicine for the disease. However, the rumor was clarified as a hoax by the Ministry of Communication and Information Technology of Indonesia [32]. Although the rumor was a hoax, sungkai has indeed already known for centuries to have medicinal properties by the locals, and the event has triggered research on sungkai ever since. The research on sungkai is blooming in the urge to provide scientific evidence to prove or disprove the claim. The research conducted has mostly centered on bioactivities related to its ability to address COVID-19. However, the studies have also expanded into other sungkai bioactivities.

4.1 Antimicrobial

The traditional utilization of sungkai to address open wounds suggests its antimicrobial activity. The notion is also supported by the presence of secondary metabolites that are often associated with antimicrobial activity in its extract, such as alkaloids, flavonoids, terpenoids, tannins, saponins, and phenolics [33]. The antimicrobial activity of sungkai has been looked over relatively earlier than its other bioactivities. The earliest publication on the study of sungkai against microorganisms was for *Plasmodium falciparum*, which causes malaria. Kitagawa et al. [11] isolated two active compounds against the malaria pathogen in vitro with an IC₅₀ of 13.1 μ M for peronemin C₁ and 83% inhibition at 118 μ M for peronemin A₃. Subsequent research on a microbe with the same genus evinces that the ethanol extract of sungkai inhibits the growth of *Plasmodium berghei* in a Swiss strain of male mice with an ED₅₀ of 102 mg/kg BW [34]. A few years later, the DMSO extract of sungkai was found to have appreciable antimicrobial activity against a dog parasite, *Babesia gibsoni*, in vitro, with an IC₅₀ value of 43.8 μ g/ml [35]. The result reported sparked further research of the plant against other microbes. An antibacterial test of sungkai leaf methanol extract to several pathogenic bacteria, namely *Staphylococcus mutans*, *Staphylococcus thiposa*, and *Staphylococcus aureus* resulted in minimum inhibitory concentration (MIC) value of 20% while for the *B. subtilis* the MIC was 15% [15]. Sungkai leaf n-hexane extract likewise exhibits inhibition of *Bacillus subtilis*, *S. mutans*, and *S. aureus* growth at a concentration of 1 mg/ml [12]. In Separate research, the MIC value of sungkai leaf crude ethanol extract against *S. aureus* was found to be 1024 μ g/ml which implies a similar antibacterial

activity with its n-hexane extract. The ethyl acetate fraction of the crude extract was also found to have the same MIC value. However, higher antibacterial activity was obtained from sungkai leaf methanol extract with a minimum inhibitory concentration of 512 µg/ml both towards *S. aureus* and *Escherichia coli* [20]. In contrast to its leaves, separate studies reported that sungkai bark extracts exhibit low to no antibacterial activity [8, 20]. These scientific findings suggest that antimicrobial active compounds from sungkai are mostly concentrated in the leaves and have a higher polarity that makes them well-dissolved in polar organic solvents such as DMSO and methanol. The functional groups that are often associated with antimicrobial activity are ones containing oxygen, nitrogen, and halides. The polar functional groups disrupt microbes' activities by interacting with cell walls, membranes, proteins, or nucleic acids [36].

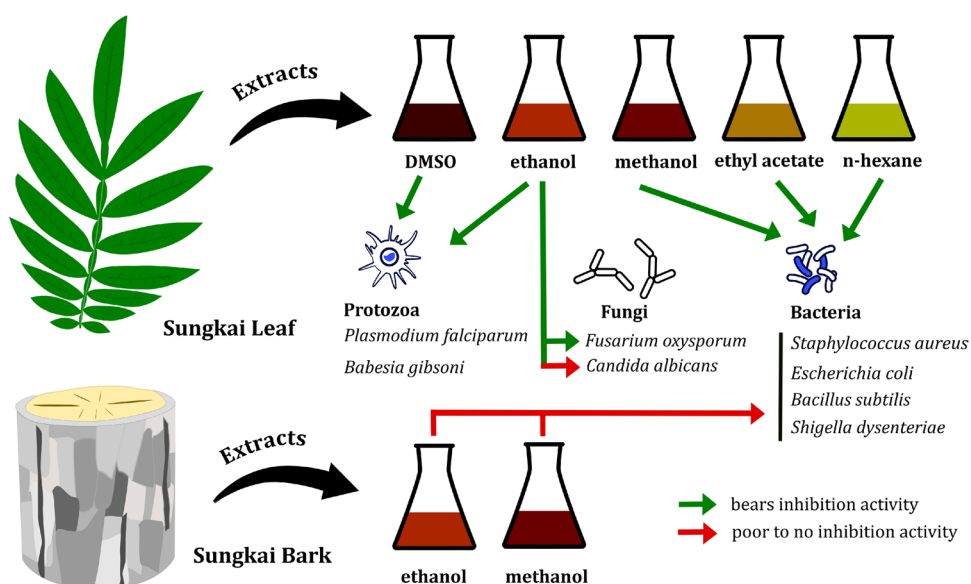
These results are summarized in Fig. 3. The findings highlight an important trend regarding the antimicrobial activity of different sungkai part extracts. The considerable discrepancy in antimicrobial activity found in different parts of the plant, especially leaves versus bark, suggests that active compounds may be distributed differently in the plant. While the extracts of methanol and DMSO from sungkai leaves show a strong antimicrobial effect, as indicated by their low MIC against *Staphylococcus aureus* or *Bacillus subtilis*, there is little or no activity in the bark. This difference implies that the number of active metabolites is much greater in the leaves. These findings suggest what type of metabolite is ultimately responsible for its activity against microbes. Substances like flavonoids or saponins—which are commonly found in polar extracts, could well be pivotal in the antimicrobial activity.

When findings about antibacterial activity compared to those of other research, such as the study from Kitagawa et al. [11] of peronemin A₃ and C₁'s influence on plasmodium species, it becomes apparent that sungkai has a vast potential. In contrast to many antimicrobial agents that specifically attack a particular type of microbes, sungkai most often finds both bacteria and protozoan pathogens susceptible to its attack. A further point to be considered is that the consistently greater effect noted for extracts in polar solvents highlights the importance of solvent choice to boost an extract's potency. These notions suggest future research shall sharply focus on separating and identifying those polar compounds that make this broad-microbe-type-targeting activity possible.

As discussed earlier, since 2020 sungkai has gained popularity, especially among Indonesian scholars, one of the consequences includes more study of its antimicrobial activity. Recent studies include antibacterial activity investigation towards *Shigella dysenteriae* [37]. Recent studies also confirmed the antibacterial activity of sungkai leaf ethanol extract against *E. coli* [38, 39] *S. aureus* [40, 41], and *S. typhi* [42]. Several antifungal tests were also conducted on sungkai leaf extract. It was found that sungkai leaf ethanol extract can significantly inhibit the growth of *Fusarium oxysporum*, a pathogenic fungus on plants [43]. However, various sungkai leaf extracts perform low antifungal activity towards the fungus that causes candidiasis, *Candida albicans* [44, 45].

The antimicrobial properties of sungkai tree leaf extracts have consistently aligned with earlier studies, resulting in expected findings regarding *E. coli*, *S. aureus*, and *S. Typhi*. However, the new addition of *Shigella dysenteriae* substantially broadens its range as an antibacterial agent. The finding that sungkai can inhibit the growth of plants' fungi pathogens but not humans', such as *Candida albicans*, is also worth noting because it may suggest that sungkai is not particularly

Fig. 3 Sungkai extracts' antimicrobial activity on some types of microbes



suited for combating all types of fungi. This selectivity raises questions about the specific mechanisms of action since certain bacteria and fungi are highly sensitive while others show minimal effect. Compared with previous reports, the antimicrobial activity of sungkai is greatly influenced by which pathogen is being targeted and how the compound is obtained. This fact suggests that its compounds' bioactivity may be more selective than was first thought.

The study in sungkai antimicrobial activity now is tending to be developed into practical commodities for community use such as wound bondage additives [40], pesticides [46], and hygiene products [47, 48]. This development shows how these research findings are being translated into real-world applications. However, the toxicity of the plant must be considered prior to its extensive use for safety reasons. This balance between efforts to obtain both efficacy and safety should remain the main theme for future product development and research work.

4.2 Toxicity

To test the safety of sungkai extract use, several acute toxicity studies have been conducted. A subacute toxicity study found that there was no significant change in the serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) blood levels in male mice after daily oral administration of sungkai leaf ethanol extract at doses of 200, 400, and 800 mg/kg BW in 21 days. Elevation of SGPT and SGOT blood levels indicates damage or impaired function of the liver. Thus, the study implies that sungkai leaf extract has no concerning toxicity against the liver [23]. However, a study found that daily oral administration of sungkai ethanol extract over 21-days period at doses of 1400, 2800, and 5600 mg/kg BW significantly affects blood creatinine levels in male rats implying its toxic effects on kidney function at certain doses and time intervals [49]. Another acute study involving both male and female mice revealed that a one-time oral administration of sungkai leaf ethanol extract to the animals over a 24-h period was safe for the male group. Meanwhile, in the female group, mortality was observed, revealing its toxicity with an LD₅₀ value of 6.50 mg/kg BW. Thus, making the toxicity fall into a very toxic and dangerous category. The treatment also showed noticeable impacts on several organs, including the heart, liver, lungs, and stomach of female mice at administration dose above 1250 mg/kg BW. Meanwhile, no noticeable impact was observed on male mice organs up to 5000 mg/kg BW dose [50]. The safer effect of sungkai leaf ethanol extract on male animals was also confirmed by a subchronic study on male rats by Muharni et al. [51]. After daily oral intake of sungkai leaf ethanol extract at doses of 100, 200, 400, and 800 mg/kg BW, there were no significant SGPT, SGOT, or creatinine blood level alterations observed. In addition, significant hepatological impairments of the liver and kidneys were also not observed. The results on liver function agree with Husni et al. [23] study. In contrast, the results on kidney function contradict the results from Fadhilati [49] which show kidney toxicity of sungkai leaf ethanol extract in male rats. The disagreement may be caused by the fact that Fadhilati [49] used higher doses compared to Muharni et al. [51]. This outcome indicates that sungkai ethanol extract is safe for kidneys within a certain range of doses and could alter kidney function when administered above the safe dose.

These toxicity studies resulted in interesting findings concerning dose, duration, and sex-specific effects during the administration of sungkai extract. One of the most remarkable findings was the difference in effect on kidney outcome, which varied from being improved to worsened based on different studies done (Fig. 4). Although Fadhilati [49] found kidney toxicity in higher doses level (1400–5600 mg/kg BW), Muharni et al. [51] found no such toxicity at lower doses (100–800 mg/kg BW), suggesting an apparent dose-dependent effect. This is consistent with the overarching principle of toxicity, which suggests that increased dosage over prolonged durations is more prone to result in adverse effects. Significant sex response differences were also found in acute toxicity studies by Defirson et al. [50], where female mice were sensitized to a level that caused organ damage and death at the dose of 1250 mg/kg BW of extracts, while male mice remained untouched up to the dose as high as 5000 mg/kg BW (Fig. 5). The finding indicates some potential sex-based variation could be present in sensitivity against sungkai extracts effects. These sex-dependent variations in the toxicological responses are especially interesting and deserve additional future research to be performed tightly, as these may have potential associations with hormonal or metabolic differences. It is known that male and female animals can exhibit different toxicity responses towards certain chemicals due to hormonal variation in both sexes [52]. The dissimilarities are influenced by endocrine systems that are responsible for the production of hormones which facilitate communication of cells [53, 54]. Consequently, sex must also be taken into consideration for bioactivity studies. Furthermore, the liver function studies demonstrated a nonsignificant increase in SGPT-SGOT levels, which indicates possible lesser toxicity of moderate doses of sungkai extract to the liver. Together, these findings highlight the need to take into consideration dose, duration of use, and sex, as well as other biological variables, when planning future applications for sungkai extracts, particularly under circumstances where human health safety is a concern.

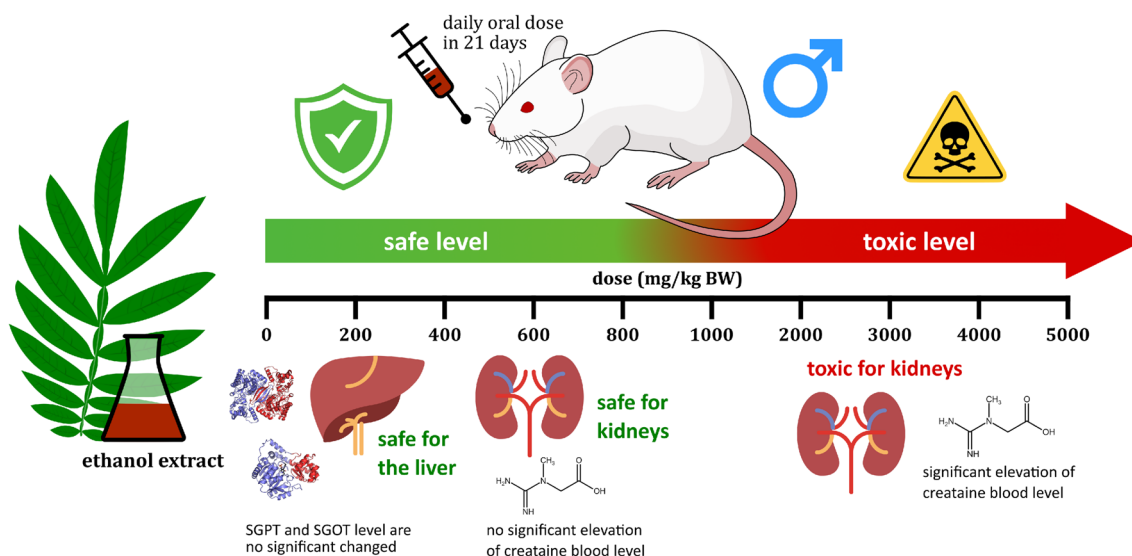


Fig. 4 Oral dose and toxic effect of sungkai leaf ethanol extract on mice

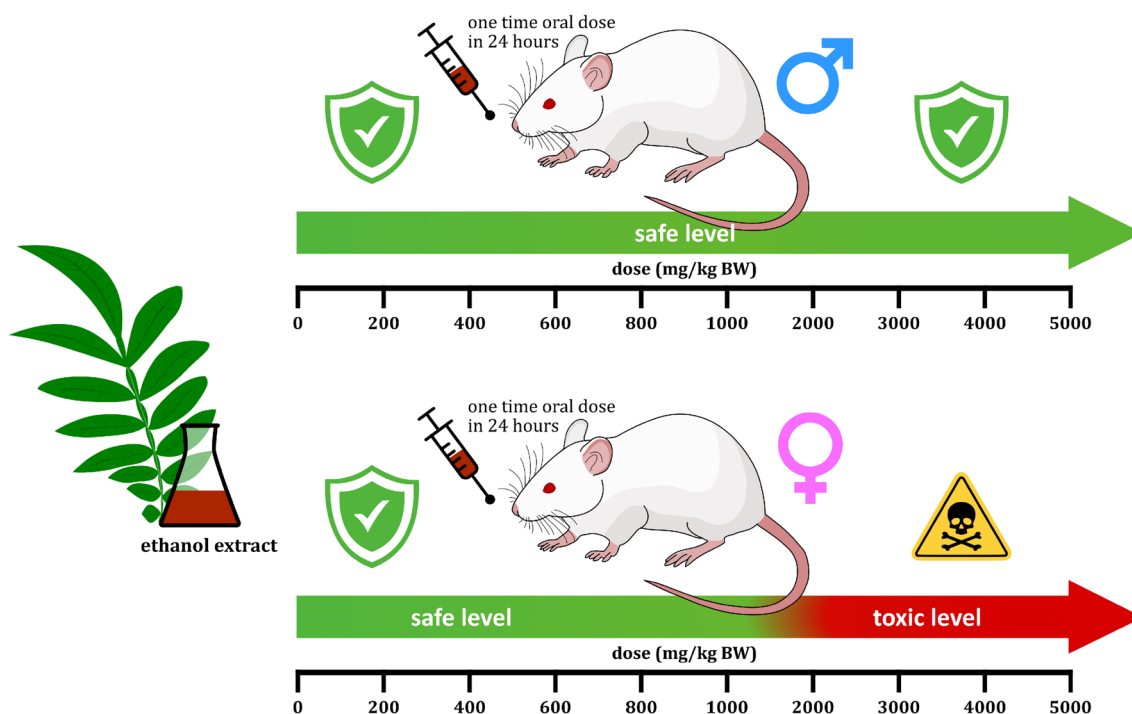


Fig. 5 One-time administration toxicity response of sungkai leaf extract in mice based on sex

4.3 Cytotoxicity and anticancer

Preliminary tests of the methanol extract and the hexane fraction of sungkai leaf against *Artemia salina* larvae indicate the presence of cytotoxicity [55]. A further study revealed that the sungkai chloroform subfraction has cytotoxicity against adenocarcinoma cancer cells, namely AdenoCa pT3N1cM1 cells and HT-29, by inducing apoptosis and necrosis, thus interrupting their cell cycle [56]. Another study has been conducted and found that the chloroform and ethyl acetate fractions of sungkai leaf methanol extract have strong toxicity against HeLa cells (a line of cervical cancer cells) and HT-29 cells. However, moderate cytotoxicity was observed in the ethanol fraction against HeLa cells [57]. Besides in vitro studies, an

in silico study was conducted in our laboratory to investigate the anticancer activity of seven compounds identified by Kitagawa (1994) from sungkai [58]. The target enzyme used was dihydrofolate reductase (DHFR). The study found that the seven compounds can interact with DHFR [58], raising their potential to be developed as DHFR inhibitors. Inhibition of DHFR limits the reduction of dihydrofolate to tetrahydrofolate, which uses NADPH and can eventually disrupt cell proliferation. For that reason, DHFR has been a major anticancer target [59].

These cytotoxic data provide significant information regarding the anticancer potential of sungkai across different extraction methods and cell lines. Figure 6 shows that different cytotoxicity can be observed in the extracts of sungkai against various cancer cell lines, which also indicates the potential of certain methods to optimize extraction methodologies in enhancing its anticancer activities. The cytotoxic effects of chloroform and ethyl acetate fractions on the AdenoCa pT3N1cM1 and HT-29 cell lines are potentially influenced by their significant apoptosis-inducing and antiproliferative properties. This result suggests that specific non-polar compounds found in sungkai hold considerable promise for triggering both apoptotic and necrotic processes. By comparison, the activity observed in an ethanol fraction was more modest. These findings suggest that nonpolar fractions are more promising when attempting to isolate potent cytotoxic compounds from sungkai. Furthermore, the observed cytotoxic effects on HeLa cells suggest that sungkai may also exhibit efficacy against various other forms of cancer. The computational analysis performed in our laboratory offers a further understanding of the anticancer properties of sungkai by investigating seven compounds derived from sungkai that demonstrate potential interactions with DHFR. Despite our current in silico research indicating that sungkai may function as an inhibitor of DHFR expression, additional validation studies are required to utilize these agents in an in vitro and/or in vivo assay system. Comparatively, while the in vitro data by Ibrahim et al. [55–57] have shown potent cytotoxicity, they also highlight heterogeneous activity across cancer cell lines and fractions, suggesting potential for targeted efficacy against specific types of cancer, as well as underscoring the need to refine extraction techniques to obtain active isolates. These results, in combination, demonstrate a potential anticancer effect of sungkai. However, more rigorous pre-clinical studies are warranted to refine its therapeutic efficacy and safety profiles for complex biological systems.

4.4 Antioxidant

Preliminary phytochemical screenings showed that sungkai has antioxidant-active secondary metabolite classes, such as flavonoids, phenolics, terpenoids, and tannins. The results sparked further investigations of its antioxidant activity. Based on the data in Table 2, all recent antioxidant tests support the antioxidant activity of sungkai extracts. Using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay, sungkai ethanol leaf extract exhibits very strong to strong radical scavenging ability, with IC_{50} values ranging from 10.489 to 116.7865 ppm. The leaf methanol extract shows strong activity, with IC_{50} values ranging from 8.667 to 60.2629 ppm. Meanwhile, the ethyl acetate extract shows strong to weak activities, ranging from 12.986 to 320 ppm. In contrast, the hexane extract shows weak to inactive antioxidant activity, as observed in all conducted research with IC_{50} values ranging from 412.401 to 3063.1987 ppm. The trend correlates the relation between

Fig. 6 Cytotoxic activity of sungkai extracts on some cancer cell lines

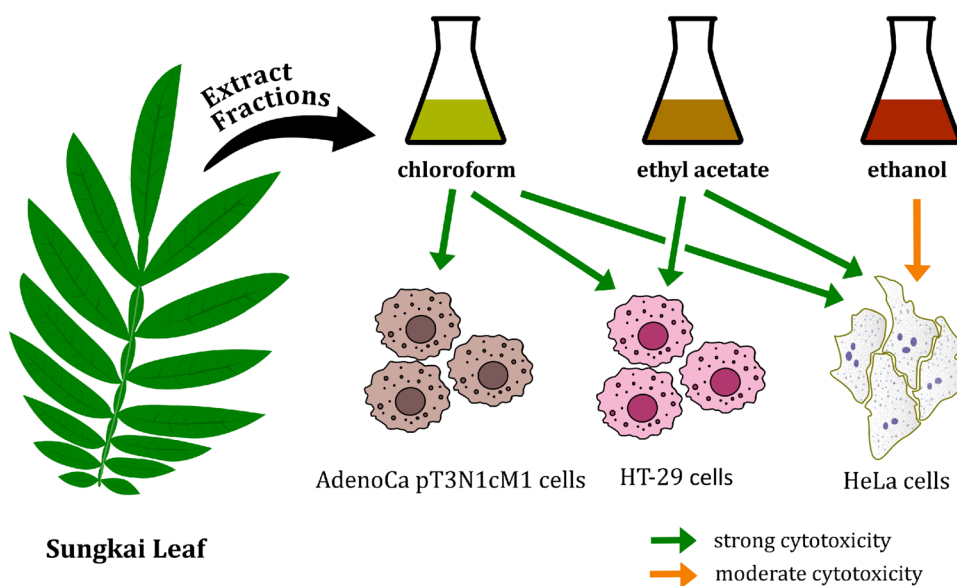


Table 2 Antioxidant tests of sungkai

Sample	Assay	IC ₅₀	Reference
Leaf ethanol extract	DPPH	50.838 ppm (young leaves) 52.835 ppm (mature leaves)	[63]
Leaf ethanol extract from Kalimantan	DPPH	13.340 ppm (pre-dried at 50 °C) 14.610 ppm (wind pre-dried) 16.799 ppm (sun pre-dried) 17.034 ppm (oven pre-dried at 70 °C)	[64]
Leaf extracts from Padang city	ABTS	204.482 ppm (hexane extract) 55.498 ppm (ethyl acetate extract) 13.589 ppm (methanol extract) (Gradual polarity extraction)	[18]
Leaf extracts	DPPH	1567 ppm (n-hexane extract) 320 ppm (ethyl acetate extract) 1281 ppm (methanol extract) (Gradual polarity extraction)	[60]
Leaf extracts	DPPH	29.549 ppm (crude extract) 607.475 ppm (n-hexane fraction) 12.986 ppm (ethyl acetate fraction) 15.766 ppm (ethanol fraction)	[65]
n-butanol leaf extract	DPPH (qualitative)	Positive	[21]
Leaf ethanol extract	DPPH	42.219 ± 19.440 ppm	[66]
Leaf ethanol extract	DPPH	29.50 ppm (ethanol extract) 47.97 ppm (leaf capsule powder)	[67]
Leaf extracts	DPPH	60.2629 mg/L (methanol extract) 201.7494 mg/L (ethyl acetate extract) 3063.1987 mg/L (n-hexane extract)	[68]
Ethanol extracts	DPPH	10.489 µg/mL (leaf extract) 5.103 µg/mL (bark extract)	[69]
Leaf extracts	DPPH	8.667 mg/L (methanol extract) 120.663 mg/L (ethyl acetate extract) 671.417 mg/L (hexane extract)	[70]
Fractions from liquid vacuum chromatography of methanol leaf extract	DPPH	291.557 ppm (fraction F) 23.037 ppm (fraction H) 7.230 ppm (fraction J)	[71]
Leaf ethanol extract	Ferric Reducing Antioxidant Power (FRAP)	132.429 µM/g	[72]
A terpenoid isolated from the leaf	DPPH	128.0743 mg/L	[73]
Leaf ethanol extract	DPPH and ABTS	130.747 mg/L (DPPH method) 47.66 mg/L (ABTS method)	[74]
Leaf extracts from the regency of Agam, West Sumatra	DPPH	12.734 mg/L (methanol extract) 114.124 mg/L (ethyl acetate extract) 412.401 mg/L (n-hexane extract)	[75]

Table 2 (continued)

Sample	Assay	IC ₅₀	Reference
Fresh leaf extract	DPPH	98.02 µg/mL	[76]
Leaf extracts (the solvent was 5/2/2 (v/v/v) of methanol, aquabidest, and chloroform)	DPPH	26.389 mg/L (young leaves)	[28]
		29.874 mg/L (half-mature leaves)	
Leaf powder	DPPH	30.9183 mg/L (mature leaves)	[25]
		73.76 ppm (leaf ethanol extract powder)	
Leaf ethanol extract	DPPH	662.69 ppm (leaf instant powder)	[22]
		116.7865 ppm	

solvent polarity used for the extraction with the extract's antioxidant activity. The lower the polarity of the solvent, the lower the antioxidant activity. A noticeable deviation occurred in Muharni et al.'s [60] study that gave low activity for the methanol extract, with IC_{50} value of 1281 ppm [60].

The investigation of antioxidant activity shows interesting relationships among types of extraction, solvent characteristics, and some mechanisms that may contribute to the antioxidant effects in sungkai extract. The apparent trend in which the polar solvents (ethanol and methanol) generally produce higher antioxidant activities compared to less-polar solvents like hexane demonstrates the polarity property of antioxidants in sungkai (Fig. 7). This trend was also similarly supported by the corresponding IC_{50} values across studies with ethanol and methanol extracts from different plant parts that appeared to possess strong radical scavenging ability compared to their hexane extract. This result indicated that more polar solvents, such as methanol, water, and ethanol, were relatively better at dissolving the antioxidant compounds of sungkai extract (flavonoids and phenolics), resulting in higher activity during the DPPH and ABTS assay. The unusually high IC_{50} value of 1281 ppm for methanol extract reported by Muharni et al. [60] raises important questions. Compared to many other methanol extract studies with IC_{50} values much lower than this outlier, this study's distinctiveness suggests that procedural irregularities, sample variances, and/or human error might have had an impact on its results.

It is worth noting that, under the same conditions, leaf sungkai ethanol extract exhibits higher activity with 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay than in the DPPH assay. The ABTS radical reaction with antioxidants is known to undergo faster kinetics than the DPPH assay, making it more sensitive in identifying antioxidants [61]. An antioxidant trend study in phenolic compounds furthermore found that high results from the ABTS assay were only high for phenolics containing a pyrogallol moiety [62]. The elevated antioxidant activity noted in the sungkai ethanol extract during the ABTS assay, in contrast to the DPPH assay, offers significant insights into the characteristics of its antioxidant constituents. Given the faster kinetics associated with ABTS and the increased sensitivity to phenolics with pyrogallol groups, as highlighted by Platzer et al. [62], this fact indicates that some antioxidant compounds in sungkai may bear a pyrogallol moiety that needs to be confirmed with further studies. Future analyses employing this assay could be optimized by incorporating such substrates to align with these identified characteristics. Since pyrogallol moieties

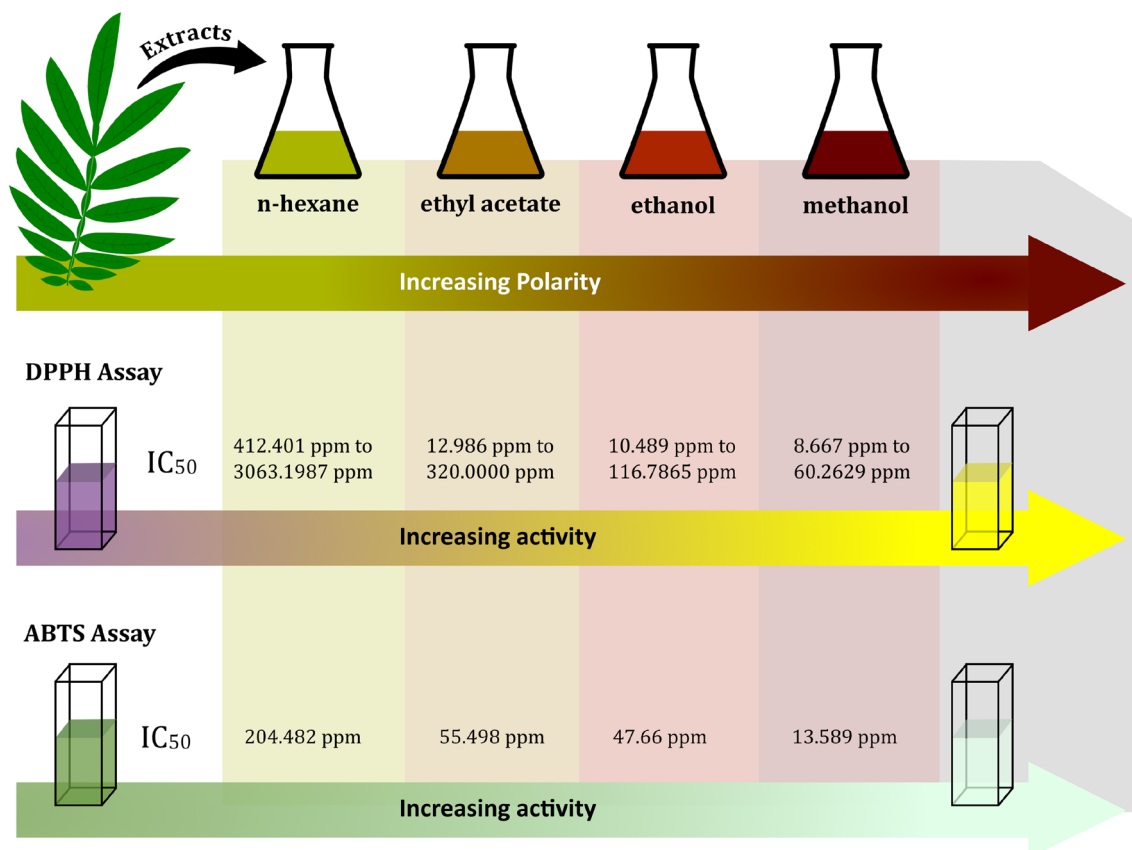


Fig. 7 Antioxidant activity of sungkai leaf extract increases as the polarity of the organic solvent used increases, both in DPPH and ABTS assays

occur in biomolecules and bioactive compounds with known health benefits, the next stage would be to identify organic extracts that contain some or all these entities so they can be isolated and fully characterized for the presence of at least one aromatic core containing three adjacent hydroxyl groups (a pyrogallol moiety). However, other moieties may also contribute collectively to the overall antioxidant efficacy.

Some researchers further attempt to investigate the relationship between sungkai leaf development stages and their antioxidant activity. As shown in Fig. 8, the trend indicates that younger leaves exhibit higher antioxidant activity compared to older leaves. At least two separate studies address this concern. Okfrianti and Irnamera [63] reported that there was a slight difference between the antioxidant activity of extracts from young and mature leaves. A slight difference was also found in the antioxidant activity between sungkai leaf extract prepared with a custom solvent in a study conducted by Rahmi et al. [28] on several different stages of leaf development, namely young, half-mature, and mature. Although the antioxidant activity did not change drastically, the trend that younger leaves show higher activity than older ones agrees in both studies. This fact is important since young and mature sungkai leaves differ in color. Young leaves are purplish, and they become greener as they mature. The color change indicates a change in phytochemical composition in the leaf cells and thus could affect their bioactivities, as proven in the studies.

4.5 Anti-inflammatory

Indigenous communities in regions where sungkai is distributed use the plant to alleviate inflammatory conditions, such as bruises, injuries, and wounds. These practices raise the notion of its potential anti-inflammatory activity. Preliminary phytochemical screenings also confirmed that sungkai has secondary metabolites that can act as anti-inflammatory agents such as flavonoids, terpenoids, alkaloids, tannins, and steroids, amplifying the indication of anti-inflammation compounds presence in sungkai.

The attempt to obtain scientific evidence of the anti-inflammatory activity of sungkai had not been made until 2021. Our laboratory first published the work that shows the ethanol extract of sungkai leaves has anti-inflammatory activity against carrageenan-induced mice [24]. It was found that a 15% ethanol extract of sungkai leaves was able to reduce the animals' exudate volume by $46.67 \pm 5.506 \mu\text{l}$ and inhibit inflammation by 87.78%. The research on various sungkai bioactivities has been blooming ever since. Our work has attracted researchers across Indonesia since sungkai is a native plant to the country and has been rumored to have COVID-19 healing properties previously. The investigation of the anti-inflammatory activity of sungkai has been followed by other researchers from different institutions in the following years. Table 3 summarizes published sungkai anti-inflammatory activity reports over the years.

Over the last three years, it has been found in our laboratory that sungkai leaf extracts have remarkable anti-inflammatory activity. The activity was observed to be high in the ethanol and ethyl acetate crude extracts of the leaf [77, 78]. Meanwhile, the activity of the n-hexane crude extract was relatively lower [30]. These results suggest that the compound(s) responsible for the activity were more likely to be semi-polar. The compound classes that are often associated with anti-inflammatory activity are flavonoids, alkaloids, tannins, steroids, and terpenoids. However, the fact that the anti-inflammatory effects of sungkai were observed to be relatively higher in polar to semi-polar solvents and lower in nonpolar-solvents-derived extracts narrows the notion that most of the active compound(s) are likely to fall in flavonoid and/or alkaloid classes. Alkaloids and flavonoids are known to have anti-inflammatory abilities by interfering with several stages of its development, including key enzyme inhibition, reactive oxygen and nitrogen species scavenging, and pro-inflammatory gene suppression [79].

Fig. 8 Relation of sungkai leaf development stage to its anti-oxidant activity: the younger, the higher the activity

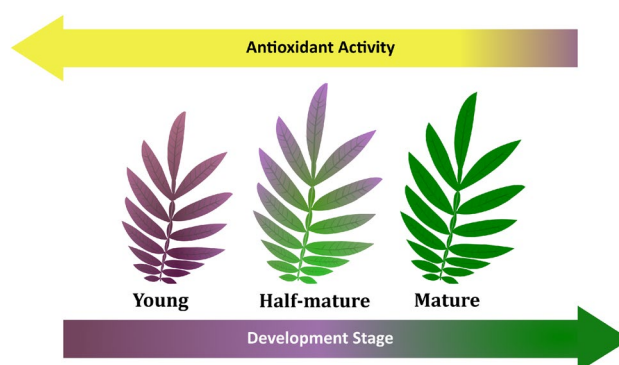


Table 3 Anti-inflammatory studies of sungkai

Sample	Method	Result	Reference
Leaf ethanol extract 15%	Carrageenan induction to mice: volume of exudate measurement	Inhibition percentage: 87,78%	[77]
Leaf ethanol extract in various concentrations	Cell Counting of Lymphocytes, Monocytes, and Neutrophils	Significant decrease	[77]
Leaf n-hexane extract 15%	Carrageenan induction to mice: volume of exudate measurement	Inhibition percentage: 54.87%	[83]
Ethanolic-fraction-derived isolate of the leaf: apigenin	Carrageenan induction to mice: volume of exudate measurement	Inhibition percentage: 58.12%	[30]
n-haxane-fraction-derived isolate of the leaf: squalene	Carrageenan induction to mice: volume of exudate measurement	Inhibition percentage: 56.59%	[30]
Leaf extract	Mice lung histopathological appearance assessing, Polymorphonuclear cell number counting, Macrophages number counting	Positive	[82]
Leaf ethyl acetate extract 10%	Carrageenan induction to mice: volume of exudate measurement	Inhibition percentage: 83,40%	[78]
Sirup formulation of leaf ethanol extract	C-reactive protein test	Negative	[84]

In 2022, two isolates from sungkai leaves were successfully isolated in our laboratory, a flavonoid and a terpenoid [30]. The exact structures of the compounds were not elucidated. However, according to FTIR characterization, the isolated compound from the ethanolic fraction was similar to apigenin and the one from the n-hexane fraction had similar bands with squalene. Both compounds showed strong anti-inflammatory activity (> 40%). Apigenin is known to modulate several important cell signaling pathways, including MAPK, protein kinase C, phosphatidylinositol-3 kinase, nuclear factor- κ B, and cyclooxygenase (COX), which collectively give anti-inflammatory effects [80]. On the other hand, squalene has anti-inflammatory activity on the LPS-mediated inflammatory response and targets pro-inflammatory mediators such as TLR4, iNOS, COX-2, or MPO (Fig. 9). In addition, squalene was also found to enhance the expression of anti-inflammatory enzymes (HO-1) [81].

The ability of sungkai extract to affect macrophage numbers has also been proven. Mice that were induced with the COVID-19 vaccine showed improvement in lung histopathological appearance, polymorphonuclear cell numbers, and macrophage numbers after ethanol sungkai leaf extract intake [82]. This is in line with the finding that sungkai leaves have a squalene-type compound [30], which has been demonstrated to manage inflammatory conditions characterized by the excessive activation of neutrophils, monocytes, and macrophages [81].

The anti-inflammatory activity of sungkai has also been investigated by using a C-reactive protein test. The test used a syrup formulation of ethanol extract from sungkai leaves. However, the results did not show any detectable C-reactive protein, even for the negative control [84]. The authors gave commentary about the result stating that the findings might be caused by the method used, which used rat paw injection instead of the intraperitoneal injection like in previous successful research.

Scientific research on the anti-inflammatory properties of sungkai is relatively new and is still limited to a few studies. Nonetheless, the available reports already give insight that sungkai has high potential for providing anti-inflammatory compound(s). Further studies are needed to understand the mechanisms and compounds responsible for its

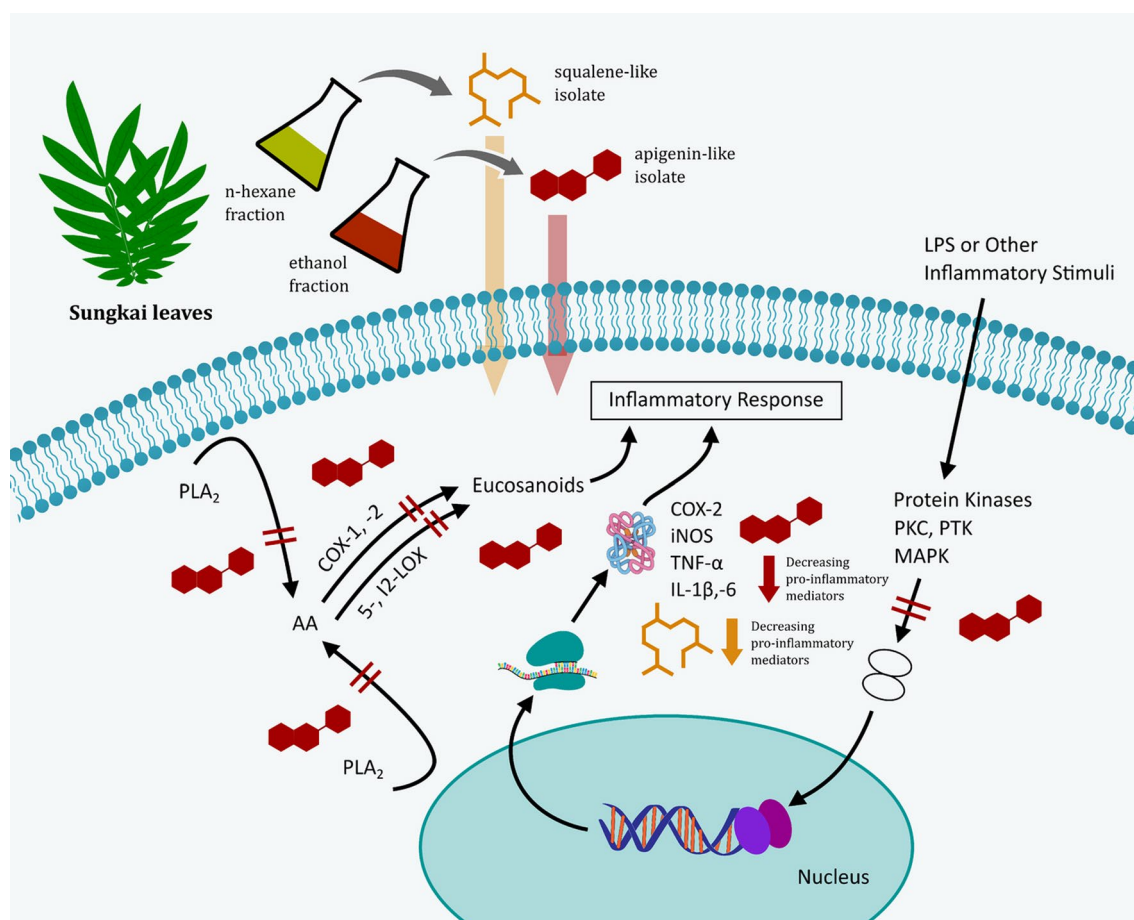


Fig. 9 Mechanism of sungkai isolates in inhibiting inflammation

anti-inflammatory effects. The scientific report on this bioactivity can support the validity of its traditional uses and can be used as the basis to determine its various related potential applications in modern medicine, including as an immunomodulator.

4.6 Immunomodulator

The consumption of brewed sungkai water has long been believed to boost the immune system, raising the notion of its potential as an immunomodulator. The presence of terpenoids, flavonoids, alkaloids, and tannins in the extract also gives preliminary support for its immunomodulatory property potential. These secondary metabolites are known to play roles in modulating immune responses. Current research on the immunomodulatory activity of sungkai was driven by the belief that it could heal COVID-19 infection among local people in Jambi in 2020. The belief might derive from the centuries-old practice of sungkai utilization to cure colds and fever in the local tradition. Even though it seems to be unlikely for a plant extract to have the ability to cure COVID-19 entirely, and it was indeed classified as a hoax by the government, plants are well known to improve the immune system. Thus, it is still worth pursuing to seek scientific evidence on sungkai's potential as an immunomodulator.

Numerous approaches have been employed in attempts to obtain evidence of sungkai's immunomodulatory activity. The works were mostly reported in the last three years and are summarized in Table 4. A preliminary study found that young leaf ethanol extract of sungkai intake at a dose of 0.567 mg/kg BW increased leukocyte numbers by 36% in mice [85]. A later study agreeingly reported that daily oral administration of sungkai leaf ethanol extract to male mice at doses of 25, 50, and 100 mg/kg BW for 7 days increased their phagocytic index, total leukocyte count, and segmented neutrophil percentage as well [86]. Leukocytes are responsible for defending the body against infections, pathogens, and foreign substances. Segmented neutrophils are among leukocyte types that are critical components of the immune system and play a central role in the body's defense against bacterial and fungal infections through phagocytosis, a non-specific mechanism that destroys pathogens and foreign particles in the body that involves digestion of the particles by phagocytic cells [87]. Increasing the phagocytic index means improvement of the process and thus assists in improving the immune system. Hence, the ethanol extract of sungkai leaves has been scientifically proven to have potential as an immunomodulator in animals. A significant increase in leukocyte number was also observed in mice that were orally administered with sungkai leaf infusion daily for 7 days at a dose of 20%/30 g BW [16]. Furthermore, immunomodulatory activity was also observed from methanol and ethyl acetate extract of sungkai leaves in our laboratory [31]. An *in silico* approach was conducted by Rahardhian et al. [88] to test the docking interactions of the seven molecules isolated by Kitagawa et al. [11] with interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α). It was found that peronemin C₁ has the best molecular docking ability compared to the other six molecules towards IL-6 and TNF- α based on its binding affinity, inhibition constants, and interactions with amino acids in the proteins which implies its potential as their inhibitors [88]. IL-6 and TNF- α are cytokines, a group of small proteins that play a crucial role in cell signaling and communication within the immune system. Increased levels of IL-6 and TNF- α are known to be related to illness severity [89]. Thus, their inhibition in severe conditions can modulate the immune system which may help to cope with unwanted inflammatory responses. However, it is also important to bring to attention that excessive inhibition of these cytokines could interfere with the overall body's immune system and more studies are needed to confirm how far compounds from sungkai can inhibit these cytokines.

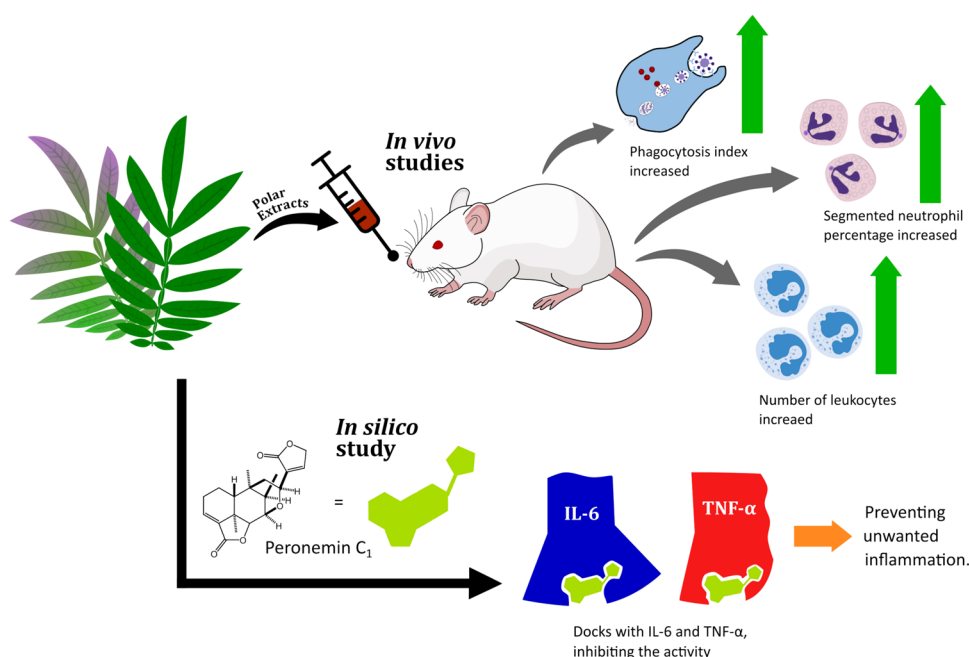
A closer look into sungkai's immunomodulatory role shows remarkable details with respect to leukocyte action and immune response (Fig. 10). Several studies have shown that an increased leukocyte count indicates the activation of the innate immune system by sungkai leaf extracts. The increase in the percentage of segmented neutrophils and the acceleration of the phagocytosis index highlight its role in enhancing the body's primary defense against bacterial and fungal infections. Besides the evidence from animal models, the *in silico* molecular docking study by Rahardhian et al. [11] offers clues to the molecular mechanisms underlying this immunomodulatory effect. Peronemin C₁, a compound extracted from sungkai, demonstrated the strongest binding affinity for IL-6 and TNF- α , two essential cytokines that play significant roles in inflammatory responses.

This finding is particularly significant, as elevated IL-6 and TNF- α are markers of severe inflammatory diseases (autoimmunity, sepsis). Sungkai's compounds may have the potential to inhibit these cytokines and be used as natural immunomodulatory agents, particularly for managing inflammation-driven diseases. This information is an important consideration for future research. However, too much suppression of these cytokines can inhibit normal immune function, so a better understanding of dosing and modes of action is critical. Sungkai has become a desirable candidate for immunotherapy, as it shows potential in targeting both innate immune responses (by leukocyte, phagocytosis) and specific

Table 4 Immunomodulatory studies of sungkai

Sample	Method	Subject	Result	Reference
Leaf ethanol extract	Carbon clearance	Male mice	Phagocytosis index; 1.00 (Na CMC 0.5%, the negative control) 1.269 (the dose of 25 mg/kg BW) 1.548 (the dose of 50 mg/kg BW) 1.737 (the dose of 100 mg/kg BW) 1.548 (Stimuno® 50 mg/kg BW, the positive control)	[86]
Leaf ethanol extract	Total leukocyte counting	Male mice	Increase in total leukocytes	[86]
Leaf ethanol extract	Calculation of segmented neutrophil cell type percentage	Male mice	Increase in the percentage of segmented neutrophil cell types with increasing doses	[86]
Leaf methanol and ethyl acetate extract	Leukocyte cell calculation	Male mice	Activity of leukocytes increased proportionally with the increase of the dose of the sample	[31]
Leaf methanol and ethyl acetate extract	Calculating the value of the phagocytic activity of peritoneal macrophages of mice	Male mice	Phagocytic activity of macrophages is proportionally increase with the dosage of the sample administered	[31]
Sungkai leaf infusion (brewed at 90 °C)	Leukocyte counting	Male mice	At the extract daily dose 5%; 15%; and 20%/30 gBW, higher leukocyte number than the negative control was observed. However, only at a dose of 20%/30 g BW that increased leukocyte number above the positive control	[16]
Sungkai active compounds	In silico docking of sungkai active compounds with IL-6 and TNF- α	Peronemin A ₂ Peronemin A ₃ Peronemin B ₁ Peronemin B ₂ Peronemin B ₃ Peronemin C ₁ Peronemin D ₁	Peronemin C ₁ has the best docking interaction to IL-6 and TNF- α based on binding energy, inhibition constant, and interactions with amino acids of the proteins	[88]

Fig. 10 Immunomodulatory studies on sungkai metabolites, in vivo and in vitro



cytokine pathways, including IL-6 and TNF- α . All these studies reiterate the urgent requirement to conduct detailed investigations to establish sungkai as an efficacious and safe therapeutic tool that can modulate the immune response.

4.7 Antidiabetic

The scientific exploration of antidiabetic-related activity of sungkai was first recorded in 2013. The study was conducted in vitro and found there was no enhancement of glucose uptake in 3T3-L1 preadipocyte cells shown by sungkai leaf extract [90]. However, recent in vivo studies have found that sungkai leaf extracts exhibit antidiabetic activity. The intake of sungkai leaf infusion by diabetic rats has been shown to affect the decrease of blood sugar levels [91]. An earlier study conducted in our laboratory also found that ethanol extract of sungkai with the dose of 350 mg/kg BW reduced blood glucose levels by up to 71.98% in mice which is comparable to the positive control, glibenclamide with the dose of 3 mg/kg BW, that can reduce the blood glucose by up to 72.69%, as well as urine volume, daily drinking, and body weight [24]. Separate research additionally found that ethyl acetate extract of sungkai leaf at a dose of 300 mg/kg BW can reduce blood glucose of male mice after alloxan induction in 14 days by up to 71.7%, which is comparable with the positive control, metformin 65 mg/kg BW, 71.4% under the same condition [92]. Studies from our laboratory further found that antidiabetic activity was shown by an isolate from the methanol fraction of sungkai leaf at a dose of 700 mg/kg BW, reducing blood glucose levels by 42.20%, and at a dose of 350 mg/kg BW by 42.00% in mice [93]. The ethyl acetate fraction of sungkai leaf extract was also found to bear antidiabetic activity at a dose of 600 mg/kg BW to reduce 50.16% of blood glucose levels [94]. In contrast, in vitro antidiabetic testing using the α -glucosidase enzyme inhibition mechanism in the latter two studies did not show any antidiabetic activity. In vitro studies seem to not give positive results for sungkai antidiabetic activity as also reported by Khattak et al. [90] that sungkai leaf extract showed no effectiveness in promoting glucose uptake in 3T3-L1 preadipocyte cells. Kanedi et al. [95] in their brief review commented that the report from Khattak et al. [90] makes the result from our laboratory [24] less convincing. However, these findings in turn suggest that the mechanism of anti-diabetic activity of sungkai may not mainly lie in the enhancement of glucose uptake by adipose and muscle cells. Instead, it could proceed via different mechanisms. In a very recent study, ethyl acetate extract of sungkai leaf has been found to inhibit α -glucosidase. LC-MS/MS analysis showed the extract contains some antidiabetic compounds such as pheophorbide A, luteolin-7-O-glucuronide, calceolarioside, peronemin D₁, and coprostanone [96]. The results note that sungkai extract can act as an antidiabetic by inhibiting α -glucosidase, an enzyme that catalyzes the digestion of carbohydrates into simpler sugars. Inhibition of α -glucosidase can delay the glucose uptake of the body and help diabetes sufferers in controlling their blood sugar [97]. The potential for sungkai utilization needs to be further investigated to provide scientific evidence to the community, which may help them in achieving more affordable treatment for this medical condition.

In vitro data suggest that the antidiabetic actions of sungkai are not likely due to glucose uptake regulation alone and support the possible involvement of additional mechanisms. In vivo, antidiabetic studies conducted in our laboratory [24, 93, 94] and others have previously established sungkai's ability to decrease blood glucose levels, significantly comparable to glibenclamide or metformin, prompting an inquiry into the specific biological pathways involved. As illustrated in Fig. 11, although when tested in vitro on 3T3-L1 preadipocytes, sungkai leaf extract had no significant effect on glucose uptake [90], inhibition of α -glucosidase in vivo can also be a possible pharmacological mechanism for the hypoglycemic action of sungkai leaf extract [96]. Sungkai has the ability to slow down carbohydrate digestion and hence reduce postprandial blood glucose spikes, which would consequently be helpful for people with diabetes. Furthermore, a study using LC–MS/MS analysis found three bioactive compounds that have an inhibitory effect on α -glucosidase in sungkai: luteolin 7-O-glucuronide, pheophorbides A, and peronemin D1 [96]. This observed inhibition, combined with the evidenced hypoglycemic activity of sungkai in vivo, confirms specific effects that may significantly reduce glucose levels and underscores its therapeutic potential as an antidiabetic drug for people seeking natural alternatives to pharmacologically synthetic drugs.

In addition, although in vivo studies consistently produce favorable results, the differences identified when comparing in vitro and in vivo data reflect the intricate interactions between sungkai's compounds and the biological system. One possible reason might lie in the pharmacodynamics and bioavailability of active compounds in sungkai when metabolized, as opposed to isolated cell experiments. This difference indicates the need for additional clinical studies to understand these mechanisms better and identify the best dosing strategies for successful diabetic treatment.

4.8 Other bioactivities

Research on sungkai bioactivity can be traced back as far as 1990. However, research about the plant has only just boomed since 2020, as discussed earlier. Research on sungkai bioactivities has been extended to more diverse and specific activities, such as analgesic, antihyperlipidemic, antityrosinase, and more, just within the last four years. The conduct of the studies is driven by the need to provide safety information and explore more potential pharmacological uses of sungkai. Several studies confirm that sungkai extract has analgesic activity in mice and rats [98, 99]. Sungkai has also been found to have antihyperlipidemic activity in vitro [100]. Additionally, it was claimed from a preliminary qualitative study using thin-layer chromatography that the n-butanol fraction of sungkai leaf ethanol extract showed tyrosinase

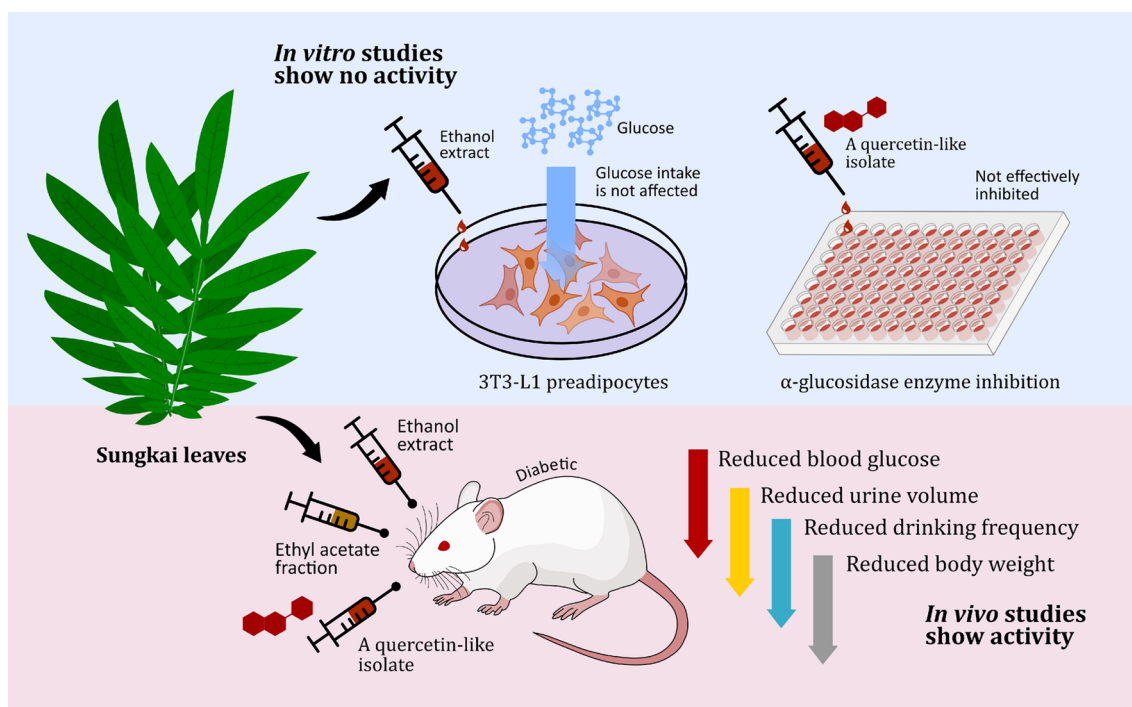


Fig. 11 Antidiabetic studies of sungkai metabolites in vitro and in vivo

inhibition besides its antioxidant capacity [17]. The diverse sungkai bioactivity studies indicate that it is a progressive area of research, with countless bioactivities are waiting to be explored and lined up for publication in the coming years.

5 Potential for future development

Sungkai, which is easily available to people in Southeast Asia, could provide an alternative medicine source for local communities in the region. The practice of healing with sungkai begun thousands of years ago certainly needs to be supported by scientific evidence, which is already provided by many studies, mostly since 2020. The pharmacological development of sungkai through time is summarized in Fig. 12. The plenty of scientific evidence for sungkai bioactivity has led to the development of sungkai into various practical products, such as tea [101], leaf powder [25], emulsion [102], micro-encapsulants [103], sunscreen [104], and even the whole dried leaves, some of which are already commercially available. Simple products based on sungkai parts might have less effective pharmacological effects, such as found by Fendri et al. [25] that sungkai leaf instant powder has nine times weaker antioxidant activity than its ethanol extract. However, such simple products may be easier to be accessed by the community and preferred, which gives them affordable economic value. The phenomenon of sungkai leaves as a commercial commodity, which has developed recently, needs to be studied separately.

Even though sungkai gained its vogue only since 2020 because of the hoax that sungkai can treat COVID-19 infection, it turns out that plenty of scientific evidence suddenly appeared afterward confirming its various bioactivities. This phenomenon implies that sungkai indeed has pharmacological properties. However, even though sungkai has bioactivities, the use of the plant should not be exaggerated to the extent that it leads to misinformation in the community. Thorough scientific studies must be conducted before it is widely used, moreover, to address the concern about its toxicity and other safety issues.

One of the safety concerns that has been brought to scientific attention is its teratogenic effect. A study on mice found that administration of ethyl acetate fraction of sungkai leaf during pregnancy affected the body weight of the animals. However, no significant effects were found on the body weight and number of the fetus. A closer inspection of the fetuses showed that there were no abnormalities in the gaps of the palate, while bone abnormalities in the caudal and phalanx were observed [105]. This study is important in providing information about sungkai use safety during pregnancy and prompting other studies to address safety concerns of sungkai use. Judging from the current trend in sungkai studies, publications addressing such concerns could be expected to appear in the years to come.

Several researchers in addition claimed to succeed in isolating compounds from sungkai, yet there are still plenty of compounds waiting to be identified from the plant. Compounds bearing more potent bioactivity can still be discovered. Meanwhile, the identified isolated compounds having several bioactivities may be used as the foundation for the development of more potent compounds by modifying their chemical structure. Reported isolated compounds with elucidated structures from sungkai have also sparked in silico studies on them, such as those conducted by Rahardhian et al. to study their immunomodulatory potential [88], Nurjannah et al. to predict their anti-inflammatory activity [106], and Fikriansyah et al. to study their anticancer activity [58]. All studies use the seven compounds identified by Kitagawa et al. [11], which were isolated from sungkai leaf acetone extract.

With the diversity of its secondary metabolites, sungkai has the potential to have various bioactivities. Some bioactivities of sungkai are currently under extensive research, while others have only recently been brought to the scientific community's attention. Thus, there are still many bioactivities of sungkai that need to be explored. The exploration is

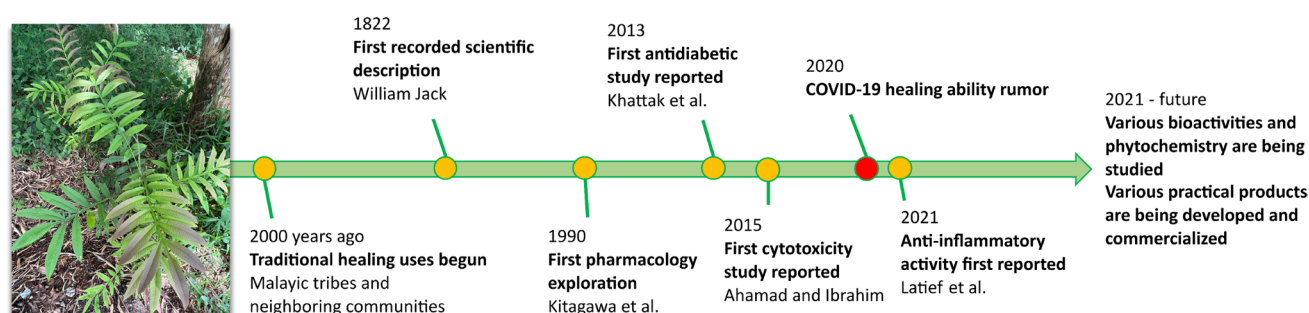


Fig. 12 Sungkai pharmacology development timeline (plant image is the authors' own documentation)

not limited to sungkai extracts but is also currently expanded to the bioactivity of its endophytic fungi's secondary metabolites [107] and the bitter honey from its flowers [108]. Many aspects of sungkai still need to be studied, such as safety concerns, more active metabolite discovery, and more bioactivities, all of which are fruitful areas to be developed in the future.

Acknowledgements The authors would like to thank *Lembaga Penelitian dan Pengabdian kepada Masyarakat* (LPPM) of Universitas Jambi for the support in the development of the research and the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, International Islamic University Malaysia for the collaboration permission.

Author contributions All authors made substantial contributions to the writing of this review article. Muhammad Irhash Shalihin lead the design of concept, drafted initial manuscript, and revised the final manuscript. Alfi Khatib performed data analysis and contributed to critical revisions. Yusnadar gave a valuable input in convenient aspects and scrutinized the review process. Indra Lasmana Tarigan contributed to bioactivity discussion and final edits. Madyawati Latief organized contributions and assisted in refining the structure of the manuscript. All the authors have read and approved the final manuscript and accept its submission for publication.

Funding The review article is a part of the ongoing further experimental study to investigate metabolomics profile of sungkai and their α -glucosidase inhibitors and antioxidant activity relationship that is funded by LPPM of Universitas Jambi with contract agreement letter number: 2210/UN21.11/PT.01.05/SPK/2023 under the International Collaboration Scheme.

Data availability No new data was generated for the article.

Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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