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Antioxidant and Sunscreen Activity of Bangkal Plants (Nauclea subdita (Korth) Steud)

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Abstract

Skin cancer is the one of leading causes of illness and death worldwide. The World Health Organization (WHO) state that ultraviolet (UV) radiation can cause cancer because it damages cells and triggers tumour growth. Bangkal plants (Nauclea subdita (Korth) Steud) which grows widely in South Kalimantan, contain antioxidants such as flavonoids, phenolics, saponins and tannins. These compounds, found in the plant's fruit and leaves, make bangkal extract a potential natural sunscreen. This article aims to determine the antioxidant potential of the fruit and leaf extract through scavenging hydrogen peroxide, scavenging hydroxyl radicals and chelating metals activity tests and discover the sunscreen ability based on the sun protection factor (SPF). The extracts were tested at concentrations of 2.5, 5 , 10, 25 and 50 ppm. The study results show that the fruit extract exhibited the most substantial antioxidant activity potential in metal chelating and hydroxyl radical scavenging. Conversely, the bangkal leaf extract demonstrated greater activity in hydrogen peroxide scavenging than in chelating metal and hydroxyl radical scavenging. Based on the SPF value, bangkal leaf extract has greater sunscreen activity than the fruit extract, indicating its stronger potential for development into a sunscreen formula to help prevent skin cancer.

Keywords*: scavenging, chelating_metal, SPF, Bangkal, free_radical*

Introduction

The integrity of global skin health faces a persistent and escalating threat from chronic exposure to ultraviolet (UV) radiation. Skin cancer has been identified as a primary cause of cancer-related morbidity and mortality worldwide (American Cancer Society, 2025). The World Health Organization (WHO) has formally classified UV radiation as a carcinogen due to its non-specific mutagenic and toxic effects, which possess tumor-inducing and stimulating properties (WHO, 2002). Therefore, effective and safe preventive strategies, such as the application of sunscreens, are paramount to mitigating solar-induced skin damage (D'Orazio et al., 2013). Sunscreen efficacy is primarily evaluated through the sun protection factor (SPF), a metric that quantifies the formulation's ability to delay UV-induced erythema (Darmawan et al., 2022).

The pervasive use of conventional sunscreens, particularly those employing inorganic filters like titanium dioxide (TiO_2), which has been utilized since 1952, is accompanied by significant safety controversies. The fundamental problem lies in the classification of TiO_2 by the International Agency for Research on Cancer (IARC) into the carcinogen group, raising serious concerns regarding its potential risk to consumer health and safety (Addor et al., 2022). Furthermore, scientific committees have highlighted that inhalation exposure to TiO_2 nanoparticles, especially in sprayable sunscreen formulations, may induce adverse effects, including the induction of lung tumors (Racovita, 2022)

This critical confluence of health risk (carcinogenicity of TiO_2) necessitates an urgent exploration of novel, safe, and sustainable raw materials that offer equivalent UV protection. The ideal alternative is a plant-based raw material that provides dual-action photoprotection: (1) Direct UV absorption and (2) Strong antioxidant activity (He et al., 2021). *Nauclea subdita* (Korth) Steud potential as a plant-based sunscreen raw material stems from its rich phytochemistry, specifically the presence of phenolic compounds and flavonoids, which are acknowledged for their ability to protect skin cells from oxidative mechanisms. Prior research has been done by Rahmawanty et al. (2017) and other studies, which initially focused on the bangkal stem bark. The study confirmed that the ethanol extract of bangkal bark exhibited potent UV-filtering ability, summarizing that the bangkal plant has strong sunscreen potential, evidenced by high *in vitro* SPF values, reaching 29 at a concentration of 1000 ppm.

However, a critical review of the same findings reveals a pharmacological paradox: while the bark showed high SPF, its antioxidant activity—a crucial component for holistic photoprotection—was very weak, with an IC_{50} value recorded at 84,850 ppm (Rahmawanty et al., 2017).

Furthermore, developing raw materials from tree bark presents significant sustainability challenges, as the large-scale extraction process can disrupt plant growth and reduce the longevity of natural resources. In contrast, recent data on the leaves of *N. subdita*—a more easily renewable and sustainable plant part — show significantly superior antioxidant activity. Ethanol leaf extract has been reported to possess an exceptional IC_{50} value as low as 3.424 ppm, indicating thousands of times stronger radical scavenging capacity than the bark extract (Ulfah et al., 2024)

However, this current research aims to explicitly explore the leaf part of the bangkal plant, which potentially has more comprehensive antioxidant activities and offers a sustainable resource base. Given the established superior radical scavenging potential of the leaves , the objective of this study is to move beyond simple total capacity assays and provide a robust, mechanistic understanding of its protective action.

The research therefore employs a multi-assay approach to assess distinct modes of action, which is essential for validating the leaves as a suitable active ingredient for advanced sunscreen formulations. We suggest to determine the antioxidant compounds through screening methodology that includes the metal chelating assay, which measures the extract's ability to prevent the initiation of damaging free radicals by sequestering pro-oxidant transition metal ions. The study also utilizes the hydroxyl radical scavenging assay to assess the extract's capacity to neutralize the highly reactive and destructive hydroxyl radicals generated under UV stress. Finally, the hydrogen peroxide scavenging assay is included to determine the ability to degrade non-radical oxidative species that act as precursors to further free radical formation.

By integrating these mechanistic data points, this study will scientifically validate the antioxidant superiority of *N. subdita* leaves, establishing a strong foundation for their development as a safe, effective, and sustainable raw material for the next generation of photoprotective agents.

Methods

Material

The plant materials used were the fruit and leaf of the bangkal plant (*Nauclea Subdita* (Korth) Steud). Reagents included: 96% technical grade methanol, technical grade *n*-hexane, 2N hydrochlorid acid, distilled water, 10% Sodium Chloride, 1% Iron (III) chloride, 1% gelatin, chloroform, anhydrous acetic acid, Dragendroff's reagent, Mayer's reagent, and concentrated sulfuric acid, phosphate buffer pH 7.4, phosphate buffer pH 7.8; 0.2M 1,10-phenanthroline, and Iron (II) chloride.

Equipment

The equipment utilized included a rotary evaporator (Eyela), water bath, blender, oven, and UV-VIS spectrophotometry (Shimadzu UV-1800®)

Ethical Clearance

Ethical clearance was submitted to the ethics committee of the Faculty of Medicine, Lambung Mangkurat University.

Collection of Plant Material

Leaves and fruit of the bangkal plant were collected from West Awang Bangkal, Karang Intan District, Banjar Regency, South Kalimantan,Indonesia and identified at the Basic Laboratory of the Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University.

Extract Preparation

The selected samples of *Nauclea subdita* leaves and fruits were washed under running water, cut into small pieces, and dried in an oven at 45^0C. The dried material was then pulverized into a powder (simplicia) using a blender. Simplicia was weighed 100 g and macerated using 96% methanol for 24 hours. The mixture was then filtered, and the residue was re-macerated twice more with fresh 96% methanol. The combined liquid extracts were concentrated using a rotary evaporator in a water bath until a thick, viscous extract was obtained (Putri et al., 2022)

Phytochemical Screening Test

The methanol extract was subjected to preliminary phytochemical screening to identify the presence of secondary metabolites.

Flavonoids Test (Alkaline test)

100 mg of extract was dissolved in 50 mL of 96% methanol. One mililiter (1 mL) of this solution was treated with a few drops of NaOH. A positive result was indicated if the yellow color faded upon the addition of a dilute acid mixture (Isnaini et al., 2021).

Tannin Test (Gelatin test)

100 mg extract dissolved in 50 mL of 96% methanol, then take 2 mL of solution and add 2 mL of 1% gelatin solution containing NaCl. The formation of a white precipitate indicated a positive result for tannins (Isnaini et al., 2021).

Saponin Test (Foam method)

100 mg extract dissolved in 50 mL of 96% methanol, then take 2 mL of solution and shaken with 2 mL of water. A positive result was determined if foam persisted for 10 minutes (Isnaini et al., 2021).

Polyphenol Test (Iron (III) Chloride Test)

100 mg extract dissolved in 50 mL of 96% methanol, then take 1 mL of solution and add 1 mL of 3% $FeCl_3$. The formation of a green or blackish-brown precipitate indicated a positive result for phenols. (Wardhani et al., 2018)

Steroids Test (Libermann-Burchard test)

0.05 g extract dissolved in $CHCl_3$, then filtered. Next, anhydrous acetic acid is added, then heated and cooled, and concentrated H_2SO_4 is added to the edge of the tube gradually. A brown ring indicated a positive result for steroids (Isnaini et al., 2021).

Alkaloids Test (Dragendorf test and Mayer test)

Dragendroff test : 100 mg extract dissolved with 50 mL of 96% methanol, then 1 mL is taken and 1 mL of Dragendroff reagent (potassium bismuth iodide) ; a red precipitate indicated a positive alkaloid result. A separate 1 mL aliquot was treated with 1 mL of Mayer's reagent (potassium mercury iodide); a yellow precipitate indicated a positive alkaloid result (Isnaini et al., 2021).

Terpenoids Test (Salkowski's test)

0.05 g extract dissolved with $CHCl_3$, then filtered. Two drops of concentrated H_2SO_4 are added and shaken. A color change to golden yellow indicated a positive result for triterpenes (Isnaini et al., 2021).

Antioxidants Activity Test

Metal Chelating Assay

The determination of metal chelating. using Fe^{2+} as the chelating agent, was performed based on the Carter method (Yadav, 2023). Sampel at varyng concentrations were incubated with 0.05 mL $FeCl_2.4H_2O$. The reaction was initiated by addition of 0.2 mL ferrozine solution. Methanol was then added to bring the total volume to 0.8 mL. After 10 minutes of incubation, the absorbance of the sample is measured at a wavelength 562 nm. Ethylenediamine tetraacetic acid (EDTA) was used as positive control. The metal chelation (%) was calculated using the following equation:

$$\text{Metal chelating } (\%) = \frac{(\text{A0} - \text{A1})}{\text{A0}} \text{ X } 100$$

Where A_0 was absorbance of control, and A_1 was the absorbance of the samples. The IC_{50} value, representing the concentration required to inhibit 50% of metal chelating, was determined from a plot of chelating percentage versus extract concentration.(Yadav, 2023)

Hydrogen Proxide Scavenging

Hydrogen peroxide scavenging activity was determined by mixing a 0.4 mL sample with 0.6 mL of 40 mM H_2O_2 solution. Sodium phosphate buffer solution was added to reach a total volume of 2 mL. The mixture was subsequently incubated for 40 minutes at 30°C. The absorbance was measured using a UV-VIS spectrophotometer at a wavelength of 230 nm. The inhibition percentage was calculated according to the equation below:

$$\text{Hydrogen peroxide Scavenging } (\%) = \left[\frac{(A0-A1)}{A0}\right] \text{ X } 100$$

where A_0 was the absorbance of control, and A_1 was the absorbance of the sample. The IC_{50} value, representing the concentration required to inhibit 50% of hydrogen peroxide scavenging, was determined from a plot of scavenging percentage versus extract concentration. (Ali et al., 2020)

Radical Hydroxyl Scavenging

A solution of 0.13% ferrous ammonium sulfate and 0.26% EDTA was initially prepared. Subsequently, 1 mL of this Iron-EDTA solution was combined with varying concentrations of the plant extract. To this mixture, 0.5 mL of 0.018% EDTA and 1 mL of DMSO (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added. The reaction was initiated by introducing 0.5 mL of 0.22% ascorbic acid. The entire mixture was then incubated in a water bath at 80–90°C for 15 minutes. After incubation, the reaction was halted by adding 1 mL of ice-cold trichloroacetic acid (TCA) at a concentration of 17.5% (w/v). Following this, 3 mL of Nash reagent was added, and the mixture was left to stand at room temperature for 15 minutes. The absorbance was then measured at 412 nm using a spectrophotometer, with a reagent blank as the reference. The hydroxyl radical scavenging activity was expressed as a percentage using the formula:

$$\%\ Hydroxyl\ radical\ scavenging = \left(\frac{Ac - As}{Ac}\right)\ x\ 100$$

where As represents the sample absorbance and Ac the control absorbance. The IC_{50} value, representing the concentration required to inhibit 50% of hydroxyl radical activity, was determined from a plot of scavenging percentage versus extract concentration.(Kumar, 2024)

Determination of Sun Protection Factor (SPF)

The thick extract was prepared into 5 different concentrations (2.5 ppm, 5 ppm, 10 ppm, 25 ppm and 50 ppm) using methanol 96% as solvent. The absorbance of these solutions was measured using UV-VIS spectrophotometer across the wavelength range 290 - 320 nm, with measurements taken at 5 nm intervals. The 96% methanol was used as a blank. Absorbance measurements were perfomed in triplicate. The SPF value for each concentration was calculated using the Mansur equation (Taibi et al., 2025) :

$$\text{SPF} = \text{CF x} \sum_{290}^{320} [EE\ (\lambda) x\ Abs\ (\lambda)]$$

CF = Correlation Factor (10)
EE = Efficiency Erythema
I = Solar Simulation Spectrum
Abs = Absorption

Results

The bangkal plant has been determined in the basic laboratory of Basic Laboratory of the Faculty of Mathematics and Natural Sciences Lambung Mangkurat University with no 18/LB.LABDAS/I/2022. Ethical clearance has been carried out with no.139/KEPK-FK ULM/EC/VI/2023.

The results of phytochemical screening showed that fruit and leaf extracts of the bangkal plant contained flavonoids, tannins, phenolics, saponins, steroids and terpenoids. The results of the phytochemical screening of fruit and leaf extracts of bangkal plant showed differences in the results of alkaloid compounds (Table 1).

This phytochemical screening is only qualitative , so it does not indicate the large or small content of compounds in the sample. Phytochemical screening results showed that the fruit contains flavonoids, alkaloids, tannins, phenolics, saponins, steroids, and terpenoids. Meanwhile, the leaves contain flavonoids, tannins, phenolics, saponins, steroids, and terpenoids (Table 1).

Table 1. Phytochemical Screening of Leaves and Fruit Extract of bangkal Plant

Compound	Fruit	Leaves
Flavonoids	+	+
Alkaloids	+	-
Tannin	+	+
Phenolic	+	+
Saponins	+	+
Anthraquinone	-	-
Steroids	+	+
Terpenoids	+	+

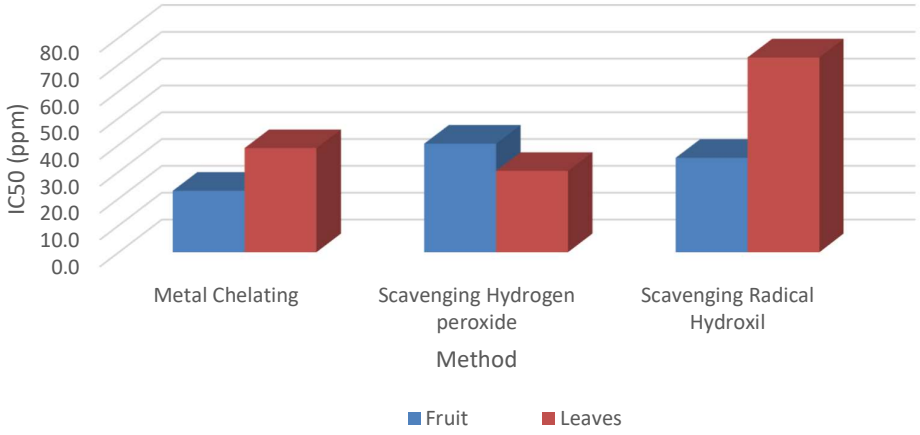

Figure 1. IC_{50} of Leaves and Fruit Extract of Bangkal Plant

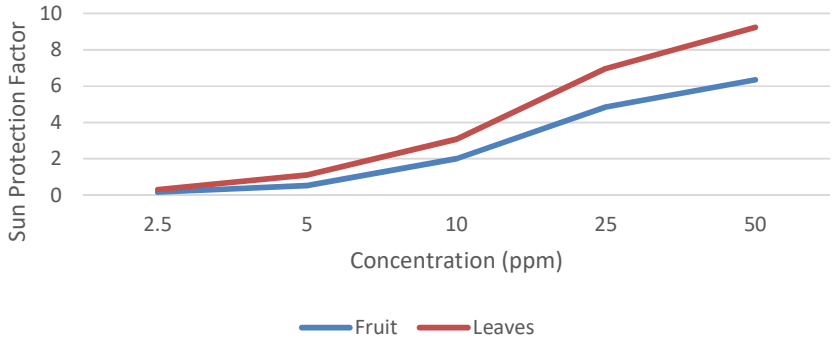

Figure 2. SPF of Leaves and Fruit Extract of Bangkal Plant

Discussion

The results of the study showed that bangkal leaf extract has a higher sunscreen ability compared to fruit extract. Based on phytochemical screening, bangkal leaf extract contains flavonoids, tannins, phenolics, saponins, steroids, and terpenoids. Research conducted by Ulfah et al. (2024) found that bangkal leaf extract contains flavonoids. (Ulfah et al., 2024). This difference is due to the different sampling locations, solvents used, and sampling times (Laily et al., 2012).

The mechanism of action of flavonoids is to act as free radical scavengers, neutralizing reactive oxygen species (ROS) by donating hydrogen atoms or electrons (Zahra et al., 2024). Flavonoids directly capture free radicals through the following reactions: (Korkina & Afanas'Ev, 1996)

Flavonoids also have the ability to act as metal chelation, which will bind metals such as Fe and Cu which act as catalysts in the formation of free radicals. Flavonoids can inhibit the activity of enzymes that play a role in producing reactive species such as xanthine oxidase and NADPH oxidase, thereby causing a decrease ROS production (Zahra et al., 2024)

Tannins have two aromatic rings which can donate hydrogen atoms to inhibit the hydrogen peroxide formation (Gülçin, et al.,

2010b). Tannins also inhibited the formation of superoxide radicals. (Chung et al., 1998).

Saponin consists of sapogenin, which is the free part of the aglycone. This compound is an antioxidant because it can reduce superoxide by forming a hydrogen-peroxide intermediate, preventing damage to biomolecules by free radicals (Pan et al., 2017).

Terpenoids are dehydrogenated and oxidized derivatives of terpene compounds. Terpenoids are antioxidants due to the terpenoid mechanism of capturing/scavenging reactive species (Jafar et al., 2020). Steroids are lipid terpenoid compounds from the triterpenoid group which have four rings and have a role as antioxidants by breaking chain reactions and converting them into more stable products which can reduce the formation of free radicals (Maulida et al., 2016).

Based on their antioxidant mechanism, leaves have a stronger hydrogen peroxide scavenging mechanism than metal chelating and hydroxyl radical scavenging. Research by Nurhidayah et al. (2022) shows that the magnitude of sunscreen activity will coincide with the magnitude of antioxidant activity. (N. Nurhidayah et al., 2022)

These results indicate that the bioactive compounds contained in the extract have a more dominant ability to neutralize H_2O_2 compared to other free radicals. Hydrogen peroxide is a relatively stable reactive oxygen species (ROS), but this compound easily penetrates cell membranes. Hydrogen peroxide compounds, through the Fenton or Haber–Weiss reaction, produce highly reactive and mutagenic hydroxyl radicals (Valko et al., 2006). The antioxidant ability to neutralize H_2O_2 plays a crucial role in preventing the formation of secondary hydroxyl radicals, which are the primary trigger of oxidative stress in skin cells.

Chronic oxidative stress is a major factor in triggering skin cancer. Exposure to ultraviolet (UV) light, especially UV-B, can increase the formation of ROS in the epidermis, including superoxide, hydroxyl radicals, and hydrogen peroxide (Pandel et al., 2013). Excessive hydrogen peroxide compounds cause oxidation of lipids, proteins, and DNA. This can trigger genetic mutations and damage cellular defense systems. The high H_2O_2 scavenging activity indicates that the plant extract has strong protective potential against oxidative damage caused by UV exposure, thus contributing to the prevention of skin carcinogenesis (Dahabra et al., 2021; Mann et al., 2020).

Furthermore, the low metal chelation activity indicates that the sample's antioxidant mechanism is not through binding transition metal ions (Fe^{2+}/Cu^{2+}), but rather through direct neutralization of non-radical oxidants such as H_2O_2. This may indicate the dominance of polar phenolic or flavonoid compounds that act as electron or hydrogen atom donors, rather than metal ligands (Hssaini et al., 2021). Meanwhile, the relatively low hydroxyl radical scavenging activity is likely due to the high reactivity of these radicals, which require electron donors with higher redox potentials than H_2O_2.

Thus, this antioxidant activity profile confirms that bangkal leaf extract has the potential to be used as a candidate active ingredient in sunscreens or skin protection agents through its mechanism of inhibiting the formation of hydroxyl radicals from H_2O_2. This protection can minimize the risk of DNA damage, p53 gene mutations, and abnormal cell proliferation that are the precursors to skin cancer (RITTIE, 2002).

The leaf extract's high hydrogen peroxide scavenging activity suggests that it has the potential to be developed as a natural active ingredient in sunscreens and anti-aging products. Its protective function stems not only from its ability to absorb UV rays but also from its ability to suppress the formation of ROS, which trigger genetic mutations in epidermal cells. This aligns with the concept of dual-action sunscreens, which combine photoprotection with antioxidant activity (Jesus et al., 2023).

Furthermore, because the primary antioxidant mechanism of bangkal leaf extract is not through metal chelation, it is relatively safe for long-term use and does not disrupt the skin's ionic homeostasis. The phenolic compounds or flavonoids responsible for this activity are also known to be highly stable in water-based formulas, making them easy to integrate into natural sunscreen cream or gel emulsion systems.

Thus, the results of this study provide a scientific basis for the development of natural ingredient-based cosmetic products that have the potential not only to protect the skin from UV radiation but also to inhibit the oxidative processes that contribute to the development of skin cancer. Further research is recommended to identify the dominant bioactive compounds through chromatographic and spectrometric analysis, as well as to test their effectiveness in vitro and in vivo on human skin cells.

Conclusion

The conclusion from the results of this study is that the extract of bangkal plant leaves has the potential to be developed into a sunscreen preparation that is useful for preventing skin cancer.

Conflict of Interest

There is no conflict of interest in this study.

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Author contribution

M. Febriansyah: Collection data, creation of extract samples, manuscript writing, Rahmaniah Ulfah: creation of extract samples, and calculation data, Isnaini: research conceptualization, analysis data, script revision, Asnawati: writing corrector.

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