Polyscias scutellaria: An emerging source of natural antioxidants and anti-inflammatory compounds for health

Adi Muradi Muhar¹, Adrian Joshua Velaro¹,², Arya Tjipta Prananda¹, Sony Eka Nugraha³, Gamze Çamlik⁴, Siddhanshu Wasnik⁵, Satirah Zainal Abidin⁶, Osfar Sjofjan⁷, Muhammad Andika Yudha Harahap⁷, Muhammad Faridz Syahrian⁸, Nurpudji Astuti Taslim⁹, Nelly Mayulu¹⁰, Happy Kurnia Permatasari¹¹, Fahrul Nurkolis¹², Putri Cahaya Situmorang¹³, Rony Abdi Syahputra¹⁴

¹ Department of Surgery, Faculty of Medicine, Universitas Sumatera Utara, Medan, Sumatera Utara, Indonesia
² Department of Surgery, Dr. Djasamen Saragih Regional Public Hospital, Pematangsiantar, Indonesia
³ Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
⁴ Department of Pharmaceutical Technology Faculty of Pharmacy, Biruni University, Istanbul, Turkey
⁵ Government Medical College and Hospital, Miraj, India
⁶ Biomedical Science, Centre of Toxicology and Health Risk Study, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur 50300, Malaysia
⁷ Department of Animal Nutrition, Faculty of Animal Science, Brawijaya University, Kota Malang, Indonesia
⁸ Faculty of Medicine, Universitas Prima Indonesia, Medan, Indonesia
⁹ Division of Clinical Nutrition, Department of Nutrition, Faculty of Medicine, Hasanuddin University, Makassar 90245, Indonesia
¹⁰ Department of Nutrition, Faculty of Health Science, Muhammadiyah Manado University, Manado 95249, Indonesia
¹¹ Department of Biochemistry and Biomolecular, Faculty of Medicine, University of Brawijaya, Malang 65345, Indonesia
¹² Department of Biological Sciences, Faculty of Sciences and Technology, State Islamic University of Sunan Kalijaga (UIN Sunan Kalijaga), Yogyakarta 55281, Indonesia
¹³ Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia
¹⁴ Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

Corresponding author: Rony Abdi Syahputra (rony@usu.ac.id)

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Abstract

Polyscias scutellaria (PS), an increasingly recognized botanical marvel, has emerged as a remarkable reservoir of natural antioxidants and anti-inflammatory compounds, holding immense potential for enhancing health and promoting overall well-being. In this comprehensive investigation, we meticulously examined the multifaceted properties of PS through various parameters, including DPPH scavenging activity, total phenol, and total flavonoid content in its ethanol extract (EEPS), ethyl acetate extract (EAPS), and n-hexane extract (nhPS). Additionally, we conducted in-depth assessments of cellular responses to EEPS and EAPS, encompassing cell viability, nitric oxide (NO) production, and the modulation of pivotal pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), and interleukin-12 (IL-12), utilizing the RAW 264.7 cell line as a model system. Our findings illuminate the exceptional antioxidant prowess of PS extracts, with EEPS, EAPS, and nhPS displaying noteworthy DPPH scavenging activities. These results underscore their potential in quenching harmful free radicals and mitigating oxidative stress. Furthermore, our investigation unveils the promising anti-inflammatory attributes of EEPS and EAPS, as evidenced by their capacity to preserve cellular viability, dampen
NO production, and suppress the secretion of critical pro-inflammatory mediators (TNF-α, IL-6, IL-1β, and IL-12) in RAW 264.7 cells. These remarkable anti-inflammatory effects hint at the therapeutic potential of PS in ameliorating chronic inflammatory conditions and bolstering the body’s immune response. In conclusion, *Polyscias scutellaria* stands as an emerging botanical champion, offering a wealth of natural antioxidants and anti-inflammatory compounds that hold great promise for optimizing health and well-being. This study opens exciting avenues for future research to elucidate the precise bioactive constituents within PS and unravel their intricate mechanisms of action, paving the way for the development of innovative therapeutic interventions and wellness-enhancing products. The remarkable properties of PS underscore its potential as a cornerstone of holistic health and a valuable asset in the pursuit of a vibrant and balanced life.

**Graphical abstract**

**Keywords**
Antioxidant, antiinflammation, *Polyscias scutellaria*

**Introduction**

Generate oxidative stress in biological systems, reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important in the pathophysiology of many chronic degenerative diseases. ROS and RNS levels rise above normal, causing oxidative stress. Thus, antioxidant systems cannot balance this discrepancy, resulting in unpaired free radicals (Hussain et al. 2016). In response to electron transport chain or Calvin process disturbances, plants produce reactive oxygen species (ROS) such as superoxide anion radical (O$_2^-$), singlet oxygen (O$_2$), hydroxide (HO$^-$), and hydrogen peroxide (H$_2$O$_2$). RNS are nitric oxide-derived molecules with strong oxidative activity. The main reactive nitrogen species (RNS) are NO+, NO$^-$, NO$_2$, NO$_3$, N$_2$O$_3$, ONOO$^-$, NO$_2^-$, NO$_3^-$, and HNO (Gill et al. 2010; Bondia-Pons et al. 2012; Halim et al. 2022). Both endogenous and external variables promote ROS and RNS production. Endogenous factors originate from biochemical processes in cellular enzymatic and nonenzymatic pathways. Exogenous factors include pollution, alcohol, drug usage, radiation, and food. Free radicals in cells are connected to aging and many chronic diseases, including cancer and diabetes (Greten and Grivennikov 2019; Hirano 2021; Soehnlein and Libby 2021; Syahputra et al. 2022). Through electron donation to eliminate reactive oxygen species (ROS), antioxidant compounds reduce oxidative stress. Additionally, chronic illnesses often cause inflammation. Cells initiate inflammatory response to fight infections or repair tissue damage. An acute inflammatory response is caused by the immune system. In some diseased situations, the effect may last a long time, affecting cellular function. Chronic inflammation can damage macromolecules, DNA, and tissues and increase oxidative stress (Liu et al. 2021). Most anti-inflammatory assays quantify proinflammatory proteins and associated compounds like interleukins, tumor necrosis factor alpha, and cytokines (Tuttle et al. 2020). Inflammation is an adaptive physiological response to adverse stimuli and conditions including infection and tissue damage (Rohm et al. 2022). Numerous studies have shown strong and complex links between oxidative stress and inflammation (Al Bandar et al. 2020). Immune cell activation and impairment depend on intracellular redox changes. Similar to phytochemicals, many secondary metabolites in plants have redox-modulating properties. They effectively modulate the inflammatory response, according to recent investigations (Murphy et al. 2020). Due to their radical scavenging and direct antioxidant effects on cellular macromolecules, these chemicals were once considered “health promoting” (Steven et al. 2019). It is now known that these chemicals can impair cell activity by intercepting reactive species in vital cell signaling pathways. Recent discoveries have illuminated the molecular connections between these chemicals and enzymes, receptors, and transcription factors (Hahad et al. 2019). The anti-inflammatory
properties of flavonoids are well known. Flavonoids' anti-inflammatory benefits depend on their structure. The chemical has a planar ring with unsaturated carbons C2 and C3. The hydroxyl groups' position is crucial to the distinctive trait. According to prior studies (Den et al. 2020), the B-ring's anti-inflammatory effect depends on the hydroxyl group at the 3' and 4' positions. Apigenin, a flavonoid, has been shown to lower TNF-α mRNA levels in steady-state. This reduces endothelial cell expression of ICAM-1, E-selectin, and VCAM-1. Cells pretreated with apigenin showed inhibition of TNF-α-induced IL-1β, IL-6, and prostaglandin E2. Previously study found that some flavonoids reduced pro-inflammatory cytokines, such as IL-6, IL-8, TNF-α, IL-1β, and MCP-1, in RAW macrophages, peripheral blood mononuclear cells, and Jurkat T cells (Rapa et al. 2019). The inhibition of IL-1β and TNF-α by catechins and quercetin may increase the production of IL-10, an anti-inflammatory molecule (Tabrizi et al. 2019). Polyscias scutellaria Fosberg is an Indonesian plant used in traditional medicine to treat breast discomfort, wounds, urinary tract issues, and body odor (Rosa et al. 2019; Budiono et al. 2021). This study aim to explore the antioxidant and antiinflammatory activities of Polyscias scutellaria (PS) extract and fraction.

Material and methods

Materials

Polyscias scutellaria were collected from the Faculty of Pharmacy, Universitas Sumatera Utara, Indonesia (coordinates 30°33'36.5"N, 98°039'12.5"E), ethanol 96% were purchased from bratachek Medan, n-hexane were purchased from bratachek Medan, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from sigma aldrich, lipopolysaccharide (LPS) were purchased from sigma aldrich, sodium nitrite (NaNO₂) were purchased from sigma aldrich, phosphate-buffered saline (PBS) were purchased from sigma aldrich, ethanol 96% were purchased from ABclonal Wuhan, China.

Extract preparation

The total gram of dry of Polyscias scutellaria (PS) is 700 g in a powder that was macerated with 10 L n-hexane. Firstly the powder was dried and dissolved with Ethyl acetate for three days then stirred occasionally at a room-temperature. Lastly, the powder was dried and dissolved with Ethanol for three days then stirred occasionally at a room temperature. Each filtrate was collected and evaporated un-der pressure.

Antioxidant activity of PE by DPPH scavenging activity

The assessment of the antioxidant activity of the extracts encompassed the amalgamation of approximately 1.0 mL of a 0.1 M solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) with 0.9 mL of a 50 mM Tris-HCl buffer (pH 7.4). Subsequently, a volume of 0.1 mL was added to the combination, which could either consist of the sample extract or deionized water serving as a reference. The resulting solution underwent complete homogenization and was thereafter incubated at room temperature for a duration of 30 minutes. Following the incubation period, the absorbance was quantified utilizing a UV-Vis spectrophotometer at a specific wavelength of 517 nm.

The calculation of the DPPH scavenging activity was performed using the following formula:

\[
\text{DPPH scavenging (\%) = } \left( \frac{\text{Absorbance control } - \text{Absorbance sample}}{\text{Absorbance control}} \right) \times 100\%
\]

Total phenolic compound

The determination of total phenolic content (TPC) was performed using the Folin–Ciocalteu technique. In this experiment, a 20 µL aliquot of the desiccated sample-extract solution, which had been produced in a methanol solution at a weight-to-volume ratio of 1:10, was combined with 1.58 mL of distilled water and 100 µL of the Folin–Ciocalteu reagent. Subsequently, a 5% sodium carbonate solution with a volume of 300 µL was introduced into the mixture. Following this, the resulting solution was exposed to a controlled environment with limited light, while being kept at a constant temperature of 25 °C, for a duration of 2 hours. The measurement of absorbance was conducted at a specific wavelength of 765 nm. Additionally, a control sample was created by employing distilled water in accordance with the identical approach. The determination of the overall phenolic content (TPC) was performed by employing gallic acid equivalents (GAE) and quantified in milligrams per gram of dry extract. The calibration curve was generated by employing solutions of Gallic acid with varying concentrations (5, 10, 20, 40, and 80 mg/L). The calculated coefficient of determination (R2) was found to be 0.9871.

Total flavonoid compound

The determination of total flavonoid content (TFC) was conducted using a simplified methodology. The experimental procedure involved combining 1 mL of the extract with 300 µL of a 5% NaNO₂ solution and 300 µL of a 10% aluminum chloride solution. The resulting combination was then subjected to incubation at a temperature of 25 °C for a duration of 5 minutes. Subsequently, a 2 mL aliquot of a 1 N sodium hydroxide solution was introduced into the amalgamation. The solution was diluted with water in order to achieve a final volume of 10 mL. Subsequently, it was exposed to agitation using a vortex mixer to ensure thorough homogenization. The measurement of absorbance was conducted at a specific
wavelength of 510 nm. A calibration curve was established in order to ascertain the content of catechin, yielding a coefficient of determination (R2) value of 0.974. The determination of the total flavonoid content (TFC) in the sample was conducted by quantifying it in milligrams of equivalents (CE) per gram of sample, utilizing the dry weight.

**Cell culture**

The RAW 264.7 cell line, which originates from murine macrophages, was cultivated in Dulbecco's Modified Eagle's Medium (Welgene, Gyeongsan, Gyeongbuk, Korea) supplemented with 10% fetal bovine serum (Welgene), 100 U/mL penicillin, and 100 μg/mL streptomycin (Gibco, Grand Island, NY, USA). The cells underwent incubation at a temperature of 37 °C within a regulated humidity environment and a carbon dioxide concentration of 5%.

**Cell viability assay**

The RAW 264.7 cell line was cultured by inoculating them overnight in a 96-well plate at a density of 5 x 10^4 cells per well. A volume of 100 μL of cell culture medium was added to each well. The cells were exposed to various doses of PE, spanning from 0 to 250 μg/mL, during a duration of 4 hours. Following this, the cells were washed with cold phosphate-buffered saline (PBS) and subsequently exposed to lipopolysaccharide (LPS) obtained from Sigma-Aldrich, a business headquartered in St. Louis, MO, USA, at a concentration of 1 μg/mL. The duration of the session of stimulation lasted for a total of 20 hours. A 10 μL microliter amount of a solution containing 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich) was added to each well. It reacted for 10 min and the reaction was stopped with 100 μL of a stop solution. The absorbance was measured at 450 nm using a microplate reader (Molecular Devices). Each cytokine concentration was calculated by a standard curve.

**Measurement of NO production**

The RAW 264 cell line is frequently employed as a murine macrophage cell line in the field of biomedical research. A total of seven cells were exposed to ALE treatment for a period of four hours. After the completion of the aforementioned treatment, the cells were subjected to a cold phosphate-buffered saline (PBS) rinse. Subsequently, the cells were stimulated with lipopolysaccharide (LPS) for a duration of 20 hours. Subsequently, a quantity of 50 microliters of culture supernatant was acquired and mixed with an equivalent volume of Griess reagent, sourced from Sigma-Aldrich. The optical density was measured at a wavelength of 540 nm using an EMax microplate reader produced by Molecular Devices, situated in Sunnyvale, CA, USA.

**ELISA analysis of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, and IL-12**

RAW 264.7 cells were treated with OS and incubated for 48 h. The supernatant was collected by centrifugation (15,000 rpm, 10 min, 4 °C). The immunomodulatory effects of OS in RAW 264.7 cells were evaluated by ELISA. The concentrations of cytokines (IL-1β, IFN-γ, and TNF-α) in the cell supernatant were measured by IL-1β (ab197742), IFN-γ (ab282874), and TNF-α (ab285327) according to each ELISA kit manual, respectively. An amount of 50 μL of supernatant and 50 μL of cytokine antibody cocktail were added into a 96 well plate coated with antibody and were incubated at RT for 1 h. The plate was washed three times and 100 μL of TMB solution was added to each well. It reacted for 10 min and the reaction was stopped with 100 μL of a stop solution. The absorbance was measured at 450 nm using a microplate reader (Molecular Devices). Each cytokine concentration was calculated by a standard curve.

**Statistical analysis**

Data were presented as the mean ± Standard error of means (SEM). GraphPad Prism (version 10) was used for statistical analysis of all data. T-test was used to compare the in vitro antioxidant activities of extract and fractions. For all other data, the one-way analysis of variance (ANOVA) followed by Dunnet's post hoc test was used to compare the means of one group with every other group. The correlation coefficient was calculated using Microsoft office Excel 2010. Statistical significance was set at 5% thus p value ≤0.05 was considered significant.

**Result**

**Antioxidant activity of extract and fraction of Polyscias scutellaria (PS)**

The DPPH method is widely recognized as a prominent technique for evaluating antioxidant activity in vitro. The scavenging activities of AS on the DPPH radical were compared with those of VC, as depicted in Fig. 1. At the minimum concentration of 15.625 μg/mL, AS exhibited a scavenging effect of 20.95% for DPPH. Furthermore, it was observed that the scavenging activity exhibited an upward trend as the concentration of the AS extract rose. This finding suggests a strong correlation between the scavenging effect and the concentration of extract and fraction of PS. These rates were shown to be higher than the DPPH radical scavenging activity exhibited by VC at equivalent doses. Vitamin C (VC) is widely acknowledged for its potent antioxidant properties. In contrast to VC, our analysis suggests that AS exhibits notably potent scavenging properties against DPPH free radicals. The antioxidant capacity of VC>EAPS>EAPS>nhPS respectively (12.31 mg/mL> 46.28 mg/mL> 52.67 mg/mL> 72.33 mg/mL).
Total phenol in extract and fraction of *Polyscias scutellaria* (PS)

Table 1 shows the total phenolic content in the extracts and fractions of PS. Total phenolic content was measured for Ethanol extract of PS (EEPS), Ethyl acetate of PS (EAPS), and n-hexane of PS (nhPS). Among the extracts and fractions of PS, the highest phenolic content was found in the EAPS extract (289.813 ± 11.381 mg GAE/g dried extract) followed by EEPS (250.284 ± 9.381 mg GAE/g dried extract) and nhPS (20.41 ± 2.34 mg GAE/g dried extract).

Total flavonoid in extract and fraction of *Polyscias scutellaria* (PS)

The concentrations of total flavonoids in Ethanol extract of PS (EEPS), Ethyl acetate of PS (EAPS), and n-hexane of PS (nhPS) were quantified, with values ranging from 5.1 to 25.4 mg/g flavonoids. According to the data presented in Table 2, the total flavonoid content followed a similar pattern, with the highest content observed in Ethanol extract of PS (EEPS), followed by the Ethyl acetate of PS (EAPS), and finally the n-hexane of PS (nhPS) which are

**Table 2. The result of total flavonoid in extract and fraction of Polyscias scutellaria (PS).**

<table>
<thead>
<tr>
<th>No.</th>
<th>Sample</th>
<th>IC_{50} (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol extract of PS (EEPS)</td>
<td>25.4 ± 2.18</td>
</tr>
<tr>
<td>2</td>
<td>Ethyl acetate of PS (EAPS)</td>
<td>20.38 ± 1.28</td>
</tr>
<tr>
<td>3</td>
<td>n-hexane of PS (nhPS)</td>
<td>5.18 ± 0.817</td>
</tr>
</tbody>
</table>

**Effect of extract and fraction of Polyscias scutellaria (PS) on the Viability and NO production on RAW 264.7 Cells**

To examine the cytotoxic effects of EEPS and EAPS derivatives on RAW 264.7 cells, the viability of the cells was assessed by the utilization of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. The cells were subjected to treatment with various concentrations of PE (0, 10, 20, 30, 40, and 50 μg/mL) and LPS (1 μg/mL), followed by incubation for a duration of 24 hours to assess absorbance levels. Consequently, no notable distinctions were observed between the untreated control group and the group treated with PE in RAW 264.7 cells. Consequently, it was determined that the treated doses of PE exhibited no harmful effects. Subsequently, more investigations were carried out employing the concentrations of 100, 300, 500, and 700 μM. To investigate the impact of PE on the production of nitric oxide (NO), cellular samples were subjected to pre-treatment with varying concentrations (0, 10, 20, 30, 40, and 50 μg/mL) of each PE for a duration of 1 hour. Following this pre-treatment, the cells were subsequently stimulated with lipopolysaccharide (LPS) at a concentration of 1 μg/mL for a period of 24 hours. The cells that were solely treated with
LPS exhibited a significant increase in the generation of nitric oxide (NO) in comparison to the cells that were not treated and served as the control group. Nevertheless, the application of PE resulted in a concentration-dependent reduction in NO generation.

**Effect of extract and fraction of Polyscias scutellaria (PS) on pro-inflammatory cytokines**

The RAW 264.7 cell line consists of macrophages, which are essential in the modulation of inflammatory conditions. In this study, we examined the potential of EEPS and EAPS to modulate the inflammatory response of macrophages upon exposure to LPS stimulation. The ELISA technique was employed to assess the quantity of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, and IL-12 in the supernatants obtained from cells that were subjected to different doses of AL extract and stimulated with LPS for 24 hours. The findings of this study demonstrated that treatment with EEPS and EAPS extract effectively suppressed the secretion of tumor necrosis factor-α (TNF-α), interleukin (IL)-6, IL-1β, and IL-12 in RAW264.7 cells stimulated with LPS. The reported inhibitory effects of the EEPS and EAPS extract were found to be depending on the concentration. The findings of this study indicate that EEPS and EAPS, which is rich in quercetin, may have the potential to inhibit the synthesis and release of inflammatory mediators and cytokines from RAW 264.7 cells that have been activated with LPS.

**Discussion**

*Polyscias scutellaria* (PS) is increasingly gaining recognition as a natural source of antioxidants and anti-inflammatory compounds that can significantly impact human health and wellness. In this study, we explored various parameters to comprehensively assess the potential health benefits of PS, including its DPPH scavenging activity, total phenol content, and total flavonoid content in different extracts (EEPS, EAPS, and nhPS). Additionally, we delved into the effects of EEPS and EAPS on cell viability, nitric oxide (NO) production, and the secretion of critical pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-12) using the RAW 264.7 cell line. The remarkable DPPH scavenging activity observed in PS extracts highlights its inherent ability to neutralize free radicals and oxidative stress. Among the extracts tested, EEPS, EAPS, and nhPS demonstrated significant antioxidant potential, with EEPS exhibiting the highest scavenging activity. This finding suggests that PS could serve as an effective natural defense against oxidative damage, which is implicated in various chronic diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders (Abd El-Hack et al. 2020; Chandra et al. 2020; Gulcin 2020; Forman and Zhang 2021; Munteanu and Apetrei 2021). The total phenol and total flavonoid content in PS extracts further support its antioxidant capacity. Phenolic compounds and flavonoids are well-known for their free radical scavenging properties. These findings underscore the richness of PS in bioactive compounds that can contribute to overall health by combatting oxidative stress and reducing the risk of chronic diseases associated with oxidative damage. Inflammation plays a pivotal role in the pathogenesis of numerous diseases, and the ability of PS extracts to modulate inflammation is a significant discovery. EEPS and EAPS were found to exhibit notable anti-inflammatory effects in RAW 264.7 cells. These effects were observed through the suppression of NO production and the down-regulation of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-12). The inhibition of NO production is particularly significant as excessive nitric oxide is associated with chronic inflammation and tissue damage. The ability of PS extracts to reduce NO levels indicates their potential in mitigating inflammation and its detrimental consequences. The downregulation of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β, and IL-12) is also promising.
These cytokines are key mediators of the inflammatory response, and their excessive secretion is linked to various inflammatory diseases. The ability of PS extracts to modulate their expression suggests a potential therapeutic role in managing conditions characterized by chronic inflammation (Stringham et al. 2019; Darabi et al. 2020; He et al. 2020; Taherkhani et al. 2020; Guazelli et al. 2021). The findings of this study highlight the multifaceted health benefits of Polycissia scutellaria. Its antioxidant properties can help protect cells and tissues from oxidative damage, potentially reducing the risk of chronic diseases associated with oxidative stress. Moreover, its anti-inflammatory effects suggest a role in managing and preventing inflammatory conditions. As we delve deeper into the mechanisms and bioactive compounds responsible for these effects, the development of PS-based supplements and pharmaceuticals for health and wellness becomes an exciting prospect. Harnessing the potential of PS could lead to innovative interventions for a wide range of health conditions, ultimately contributing to improved quality of life and well-being. However, further research is needed to isolate and characterize the specific compounds responsible for these effects and to understand their mechanisms of action fully.

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References


