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Antioxidant Activity and Total Flavonoid of Extract of Bark *Vitex pinnata* L.

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Abstract

Background: The bark of *Vitex pinnata* L is not yet optimally utilized, primarily being used in boat construction while the remaining material becomes waste that is either buried or burned in trash. Therefore, it is necessary to explore more efficient and economically valuable uses for the bark of the laban tree. **Objective:** The aim of this research is to identify the chemical compounds, flavonoid content, and antioxidant activity present in the methanol extract of *Vitex pinnata* L bark. **Method:** Phytochemical screening was conducted on the methanol extract of *Vitex pinnata* L. bark. To obtain the extract, the simplicia powder was macerated using methanol as the solvent. The total flavonoid content and antioxidant activity were then tested using the 1,1-diphenyl-2-picryhydrazil (DPPH) method. **Results:** the phytochemical screening indicated that the *Vitex pinnata* L. bark extract contains alkaloids, flavonoids, and tannins. The total flavonoid test yielded 17.27%, and the antioxidant activity test revealed that the methanol extract of the laban tree bark has antioxidant activity with an IC50 value of 43.22 ppm. **Conclusion:** This extract has strong potential as an antioxidant agent.

Keywords: *Vitex pinnata*, bark, antioxidant; total flavonoid

INTRODUCTION

The exploration and utilization of natural resources have been a pivotal aspect of scientific research, especially in the field of phytochemistry. One such resource is the bark of the *Vitex pinnata* L. tree, commonly known as the laban tree. Historically, the bark of this tree has been underutilized, with its primary use being in the construction of boats. The residual bark, after this limited use, is typically discarded as waste, either buried or incinerated. This practice not only results in the loss of potentially valuable material but also poses environmental concerns due to the disposal methods employed [1].

The need to identify more efficient and economically viable uses for the bark of the *Vitex pinnata* L. tree is pressing. This has driven scientific inquiry into the potential bioactive compounds present in the bark, with a focus on those that could offer significant health benefits. Among these, compounds with antioxidant properties are of particular interest due to their ability to neutralize free radicals, thereby preventing cellular damage and contributing to the prevention of various diseases [2].

Antioxidants are crucial in mitigating oxidative stress, which is implicated in the pathogenesis of numerous chronic conditions, including cardiovascular diseases, cancers, and neurodegenerative disorders. The search for natural antioxidants has intensified, given the adverse effects associated with synthetic antioxidants [3]. This backdrop highlights the importance of exploring the antioxidant potential of natural sources like the bark of *Vitex pinnata* L.

The primary objective of this research is to identify the chemical compounds present in the methanol extract of *Vitex pinnata* L. bark, specifically focusing on its flavonoid content and antioxidant activity. Flavonoids are a diverse group of phytonutrients found in many plants, known for their potent antioxidant properties [4]. The study aims to quantify the total flavonoid content and assess the antioxidant activity of the extract using the 1,1-diphenyl-2-picryhydrazil (DPPH) method, a widely accepted assay for evaluating free radical scavenging ability [5].

To achieve this, the research employs phytochemical screening of the methanol extract of *Vitex pinnata* L. bark. The extraction process involves macerating the simplicia powder with methanol, a solvent known for its efficacy in extracting a wide range of phytochemicals [6]. Following extraction, the total flavonoid content is quantified, and the antioxidant activity is tested using the DPPH method. The results of these tests provide insights into the potential health benefits of the bark extract.

The phytochemical screening conducted in this study revealed that the methanol extract of *Vitex pinnata* L. bark contains several classes of bioactive compounds, including alkaloids, flavonoids, and tannins [7]. These findings are significant as they suggest that the bark is a rich source of compounds with potential health benefits. The quantification of total flavonoids yielded a substantial percentage of above 15%, indicating a high flavonoid content in the extract [8]. Furthermore, the antioxidant activity test revealed that the extract has a promising IC50 value below of 100 ppm, demonstrating strong potential as an antioxidant agent [9].

These findings underscore the importance of further research into the utilization of *Vitex pinnata* L. bark. By identifying and harnessing the bioactive compounds present in this underutilized resource, it is possible to develop new applications that add economic value and contribute to environmental sustainability. This research lays the groundwork for future studies aimed at fully realizing the potential of *Vitex pinnata* L. bark as a valuable natural resource with significant health benefits [10].

RESEARCH METHODS

Phytochemical Screening

Phytochemical screening of the methanol extract of *Vitex pinnata* L. bark was conducted to identify the presence of various bioactive compounds. The simplicia powder of the bark was subjected to maceration using methanol as the solvent. The methanol extract was then filtered and concentrated using a rotary evaporator. The concentrated extract was analyzed for the presence of alkaloids, flavonoids, and tannins using standard phytochemical tests as described in literature [11], [12].

Total Flavonoid Content

The total flavonoid content in the methanol extract of *Vitex pinnata* L. bark was determined using the aluminum chloride colorimetric method. A sample of the extract (0.5 mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% sodium nitrite solution. After 5 minutes, 0.15 mL of 10% aluminum chloride solution was added, followed by the addition of 1 mL of 1 M sodium hydroxide solution after 6 minutes. The final volume of the mixture was adjusted to 5 mL with distilled water. The absorbance was measured at 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was calculated using a calibration curve of quercetin as the standard and expressed as a percentage of quercetin equivalents in the extract [13].

Antioxidant Activity with DPPH

The antioxidant activity of the methanol extract was assessed using the 1,1-diphenyl-2-picryhydrazil (DPPH) free radical scavenging assay. A solution of 0.1 mM DPPH in methanol was prepared, and 1 mL of this solution was added to 3 mL of the methanol extract at various concentrations. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 minutes. The absorbance of the resulting solution was measured at 517 nm using a UV-Vis spectrophotometer. The ability to scavenge DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect } (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100$$

Where A_0 is the absorbance of the control reaction (containing all reagents except the test compound), and A_1 is the absorbance in the presence of the test compound. The IC50 value, which represents the concentration of the extract required to inhibit 50% of the DPPH radicals, was determined from the plotted graph of scavenging effect against the extract concentration [4], [14].

RESULTS AND DISCUSSION

Phytochemical screening

Table 1. Result for Phytochemical Contents oo Bark *Vitex pinnata* L.

No.	Phytochemistry content	Result
1	Alkaloida	+
2	Flavonoida	+
3	Glikosida	-
4	Saponin	-
5	Tanin	+
6	Steroida/triterpenoida	-

Note: (+) present; (-) absent

The phytochemical screening of the bark of *Vitex pinnata* L. revealed the presence of several significant bioactive compounds, including alkaloids, flavonoids, and tannins, while glycosides, saponins, and steroids/triterpenoids were absent. These findings are critical as they suggest potential medicinal properties and applications of the bark extract in various therapeutic areas.

The presence of alkaloids in the bark of *Vitex pinnata* L. is notable, given the extensive pharmacological activities attributed to this class of compounds. Alkaloids are known for their broad spectrum of biological activities, including analgesic, antimalarial, and anticancer properties [10]. The detection of alkaloids in the methanol extract of *Vitex pinnata* L. bark aligns with previous studies that have identified similar compounds in other species of the Vitex genus, suggesting a conserved biochemical trait within the genus [15].

Flavonoids were also detected in the bark extract, which is significant considering their well-documented antioxidant properties. These compounds play a crucial role in scavenging free radicals and protecting cells from oxidative stress. The high total flavonoid content quantified in this study (17.27%) underscores the potential of *Vitex pinnata* L. bark as a rich source of natural antioxidants. This aligns with previous research that highlights the antioxidant capabilities of flavonoids in preventing chronic diseases such as cardiovascular diseases and cancers [16], [17]. The presence of flavonoids, therefore, enhances the value of *Vitex pinnata* L. bark as a potential nutraceutical.

The detection of tannins further supports the potential medicinal applications of the bark extract. Tannins are polyphenolic compounds known for their astringent properties and their role in promoting wound healing and exhibiting antimicrobial activity [18]. The presence of tannins in the bark of *Vitex pinnata* L. suggests that the extract could be explored for use in treating infections and promoting skin health. The astringent nature of tannins also contributes to their utility in managing gastrointestinal disorders by forming a protective layer on the mucous membranes.

The absence of glycosides, saponins, and steroids/triterpenoids in the bark extract is noteworthy. While these compounds are often associated with various therapeutic benefits, their absence does not diminish the value of the extract. Instead, it highlights the specificity of the phytochemical profile of *Vitex pinnata* L. bark. Glycosides are known for their cardiotonic and anti-inflammatory effects, saponins for their ability to enhance immune responses, and steroids/triterpenoids for their anti-inflammatory and anticancer properties [19], [20], [21]. The absence of these compounds suggests that the therapeutic potential of *Vitex pinnata* L. bark lies predominantly in its alkaloid, flavonoid, and tannin content.

The results of this phytochemical screening provide a foundation for further research into the therapeutic potential of *Vitex pinnata* L. bark. The presence of alkaloids, flavonoids, and tannins suggests that the bark extract could be developed into various medicinal and health-promoting products. Future studies should focus on isolating and characterizing the specific alkaloids, flavonoids, and tannins present in the extract to better understand their individual and synergistic effects. Additionally, investigating the pharmacokinetics and bioavailability of these compounds will be crucial for developing effective therapeutic agents.

The phytochemical profile of *Vitex pinnata* L. bark reveals a rich presence of bioactive compounds with significant medicinal potential. The findings underscore the need for further exploration of this underutilized natural resource, which could contribute to the development of new pharmacological agents and nutraceutical products.

The analysis of the methanol extract of *Vitex pinnata* L. bark provided valuable insights into its antioxidant activity and notable flavonoid content, systematically analyzed across different concentrations. The data presented in **Table 2** illustrate the correlation between extract concentration, DPPH scavenging activity, and total flavonoid content.

Table 2. The results of DPPH Scavenging and Total Flavonoid Content from The Methanol Extract of Bark *Vitex pinnata* L

Extract (ppm)	DPPH Scavenging (%)	Flavonoid content (%)	IC_{50} (ppm)
20	38.52	16.86	
40	56,86	17.15	
60	71.66	17.35	43,22
80	73.85	17.43	
100	87.31	17,22	

The DPPH scavenging activity of the methanol extract of *Vitex pinnata* L. bark increased with the concentration of the extract. At 20 ppm, the extract exhibited a DPPH scavenging activity of 38.52%. This activity increased significantly to 56.86% at 40 ppm, 71.66% at 60 ppm, 73.85% at 80 ppm, and reached a maximum of 87.31% at 100 ppm. The IC50 value, which represents the concentration required to inhibit 50% of the DPPH radicals, was determined to be 43.22 ppm. This relatively low IC50 value indicates a strong antioxidant potential of the methanol extract, suggesting that the extract is highly effective at neutralizing free radicals even at lower concentrations.

The increasing trend in DPPH scavenging activity with higher extract concentrations indicates a dose-dependent relationship. This suggests that the bioactive compounds responsible for antioxidant activity are present in significant quantities and are effective in scavenging free radicals. The high percentage of scavenging at 100 ppm demonstrates the potency of the extract, making it a promising candidate for natural antioxidant sources [22], [23].

The total flavonoid content in the methanol extract of *Vitex pinnata* L. bark showed a consistent pattern across different concentrations. At 20 ppm, the flavonoid content was 16.86%, which slightly increased to 17.15% at 40 ppm, 17.35% at 60 ppm, 17.43% at 80 ppm, and slightly decreased to 17.22% at 100 ppm. The overall trend suggests that the flavonoid content remains relatively stable with a slight increase up to 80 ppm and a minor decrease at the highest concentration tested.

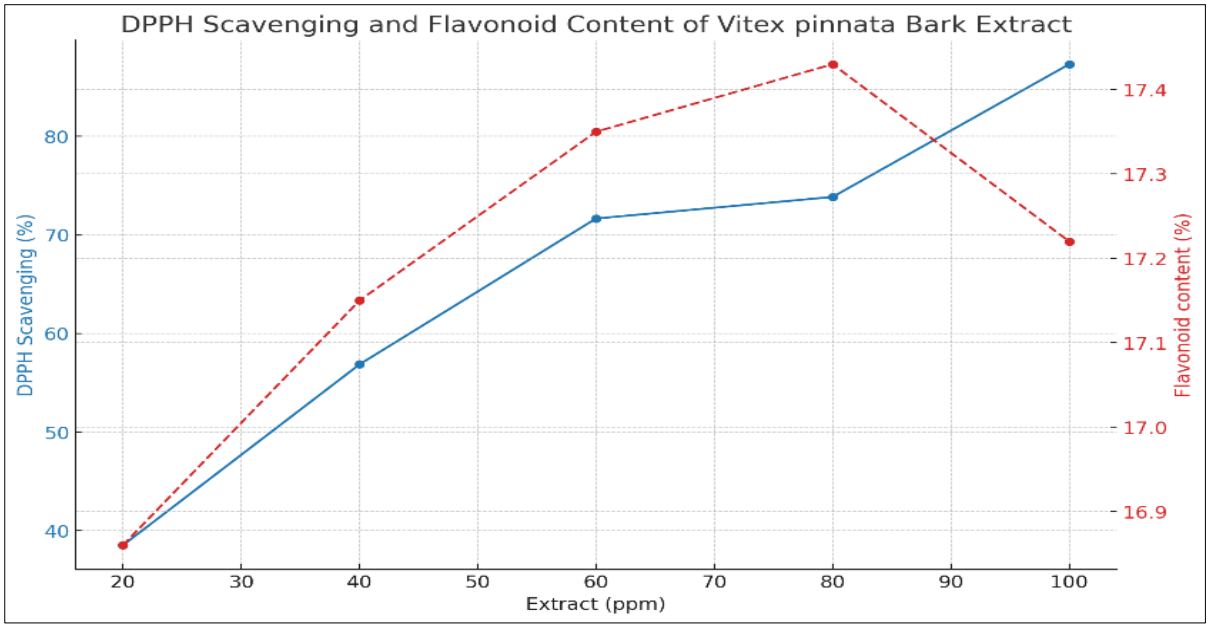

Figure 1. Correlation between DPPH Scavenging Activity and Total Flavonoid Content of The Methanol Extract of Bark *Vitex pinnata* L

Flavonoids are well-known for their antioxidant properties, and their presence in substantial amounts in the extract correlates with the observed DPPH scavenging activity (**Figure 1.**). The high flavonoid content, peaking at 17.43% at 80 ppm, indicates that these compounds significantly contribute to the extract's ability to neutralize free radicals. This consistency in flavonoid content across various concentrations further supports the extract's potential as a reliable source of natural antioxidants [24], [25].

The results of this study underscore the potential of *Vitex pinnata* L. bark as a rich source of natural antioxidants. The significant DPPH scavenging activity and substantial flavonoid content highlight its potential application in developing health supplements, pharmaceuticals, and functional foods aimed at combating oxidative stress-related conditions.

Future research should focus on isolating and characterizing the specific flavonoids and other bioactive compounds present in the methanol extract to better understand their individual contributions to the overall antioxidant activity. Additionally, investigating the bioavailability and pharmacokinetics of these compounds will be crucial for developing effective therapeutic agents.

CONCLUSION

The methanol extract of *Vitex pinnata* L. bark exhibits strong antioxidant properties and high flavonoid content, making it a promising candidate for natural antioxidant applications. The findings provide a solid foundation for further exploration and development of *Vitex pinnata* L. bark as a valuable natural resource with significant health benefits.

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