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Antioxidant Potential and Phytochemical Profiles of *Scurrula ferruginea* Stem Fractions Parasitizing *Baccaurea racemosa*

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Abstract: *Scurrula ferruginea* (Roxb. ex Jack) Danser is a hemiparasitic plant of the Loranthaceae family traditionally used to treat hypertension, skin infections, digestive disorders, and cancer. Despite its ethnomedicinal importance, the antioxidant potential of the stem, particularly when growing on the host *Baccaurea racemosa*, remains largely unexplored. This study investigated the antioxidant activity and phytochemical profile of n-hexane, ethyl acetate, and water fractions derived from the methanol extract of *S. ferruginea* stem through liquid-liquid fractionation, phytochemical screening, and DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay. Absorbance was measured spectrophotometrically at 517 nm and the IC_{50} value was calculated via linear regression analysis. Phytochemical screening revealed distinct solvent-dependent metabolite distributions: the initial non-polar n-hexane fraction was dominated by terpenoids and steroids; the intermediate semi-polar ethyl acetate fraction successfully accumulated a dense combination of flavonoids and steroids; while the highly polar water fraction selectively retained glycoside-form flavonoids; no alkaloids were detected in any fraction. In strict alignment with these chemical profiles, all fractions exhibited dose-dependent radical-scavenging activity. The n-hexane fraction exhibited baseline activity with a strong antioxidant capacity (IC50 = 98.28 ± 1.15 ppm), followed by a remarkable peak in the ethyl acetate fraction, which demonstrated the most potent free radical-scavenging capacity within the very strong antioxidant category (IC50 = 45.53 ± 0.58 ppm). Lastly, the potency decreased slightly in the water fraction, which fell into the strong antioxidant tier (IC50 = 73.18 ± 0.82 ppm). The high antioxidant activity of the ethyl acetate fraction may be associated with the presence of flavonoids and steroids. provides a preliminary scientific record of the strong fractional antioxidant capacity of *S. ferruginea* stem parasitizing the *Baccaurea racemosa* host, supporting its promising potential as a natural antioxidant source while highlighting the need for future quantitative, structural, and in vivo investigations.

Keywords: Antioxidant; DPPH; Fractionation; *Scurrula ferruginea.*

Introduction

Human health is significantly influenced by the balance between free radicals and the body's antioxidant defense system [1]. Free radicals are highly reactive molecules that can induce oxidative stress, a pathological condition that damages vital biomolecules such as DNA, proteins, and lipids. The chronic impact of this condition is closely linked to the initiation of various degenerative diseases, including cancer, diabetes mellitus, cardiovascular disease, and premature aging. Therefore, antioxidant intake is essential to strengthen cellular defense mechanisms in neutralizing these reactive compounds [2].

Research on natural antioxidant sources has become a major focus in current biomedical research, as natural antioxidants remain highly sought after due to their minimal side effects, cost-effectiveness, and greater availability compared to synthetic antioxidants [3]. Indonesia, as a country with high biodiversity, has enormous potential for supplying raw materials for herbal medicines [4]. One of the plants drawing significant interest is mistletoe, a hemiparasitic plant belonging to the Loranthaceae family that is capable of inhabiting various host trees [5].

Scurrula ferruginea (Roxb. ex Jack) Danser is a type of mistletoe known to cause detrimental effects to its host trees. Nevertheless, several studies have demonstrated that *S. ferruginea* can be utilized to treat hypertension, skin infections, gastrointestinal disorders, and cancer [6]. Furthermore, numerous scientific investigations have validated its pharmacological potential, which includes significant anti-inflammatory, antimicrobial, and antioxidant activities [7].

However, the vast majority of scientific literature on *S. ferruginea* to date has focused almost exclusively on its foliar portions. Although the leaves have been extensively documented to possess elevated phenolic and flavonoid profiles, comprehensive data concerning the bioactive properties of its stem fractions remain notably sparse. From a biological standpoint, the stem acts as the primary vascular conduit for nutrient translocation and structural metabolite accumulation, which inherently yields a qualitatively and quantitatively distinct distribution of secondary metabolites compared to the leaves [8].

Furthermore, as a hemiparasitic organism, the secondary metabolic profile of *S. ferruginea* is highly dynamic and heavily modulated by its host species, as the parasite actively sequesters water, essential minerals, and localized host-derived secondary metabolites. Consequently, the identical mistletoe species parasitizing divergent host plants will exhibit profound variations in its secondary metabolite composition and subsequent biological activities. Previous investigations have reported the antioxidant

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potential of *S. ferruginea* hosted on alternative plant species, such as *Syzygium aquaeum*, thereby confirming robust radical-scavenging capabilities. The established fact that distinct host plants exhibit variable bioactivity profiles and secondary metabolite yields provides a compelling rationale for systematically exploring the influence of other specific host species [9].

Despite these intricate host-parasite chemical interactions, a critical knowledge gap persists regarding how specific host trees modulate the therapeutic architecture of *S. ferruginea* stems. In particular, the antioxidant capacity and secondary metabolite profile of *S. ferruginea* stems specifically parasitizing *Baccaurea racemosa* (locally known as kepundung) have yet to be empirically evaluated. The distinct chemical characteristics of the *Baccaurea racemosa* host, which is rich in specific organic compounds, are hypothesized to significantly influence and modulate the type and efficacy of the secondary metabolites accumulated within the parasitic stem. Consequently, investigating this specific host-parasite relationship adds valuable insights into how these dynamics shape the distribution of its active fractions [10].

To address this empirical gap, this study investigates the fractional secondary metabolite profiles and antioxidant capacities of *S. ferruginea* stems parasitizing *Baccaurea racemosa*. The investigation initiated with a solid extraction protocol to secure the crude extract [11]. Typically, a crude plant extract represents a complex matrix comprising various classes of secondary metabolites with diverse polarities. Conducting bioassays solely on a crude extract limits the ability to discern which specific metabolite class drives the observed biological response. Therefore, a sequential multi-stage liquid-liquid fractionation process is executed using solvents of increasing polarity: n-hexane (non-polar), ethyl acetate (semi-polar), and water (polar). This principle facilitates the separation of active principles based on their solubility affinities, thereby pinpointing the fraction primarily responsible for the overall antioxidant performance [12].

Phytochemical screening serves as the foundational step for qualitatively mapping the classes of secondary metabolites present in the matrix. Secondary metabolites are specialized organic compounds that, while not directly involved in the primary growth, development, or reproduction of the plant, play deterministic roles in ecological interactions and adaptive defense against environmental stressors. Classes of compounds routinely identified through phytochemical screening include flavonoids, alkaloids, terpenoids, steroids, tannins, and saponins, each presenting a distinct biological activity profile[13]. The quantitative assessment of antioxidant capacity was conducted using the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, a rapid, stable, and highly reproducible standard protocol grounded in the mechanism of hydrogen atom donation [14].

Conclusively, this study aims to elucidate the secondary metabolite constituents and determine the IC_{50} (Inhibitory Concentration) values of the n-hexane, ethyl acetate, and water fractions derived from the methanolic extract of *S. ferruginea* stems. The explicit novelty of this research lies in establishing the provides a preliminary scientific record of the phytochemical mapping and fractional antioxidant capacity of *S. ferruginea* stems uniquely derived from the *Baccaurea racemosa* host. The findings of this study are expected to provide a valuable scientific baseline regarding natural secondary metabolites in *S. ferruginea* stems, offering critical insights to support further exploration of this species as a source of natural antioxidants.

Research Methods

Materials and instrumentation

The laboratory experimental approach in this study involved phytochemical screening and antioxidant activity testing of fractions from the methanol extract of *S. ferruginea*. The stems of the mistletoe parasitizing *Baccaurea racemosa* were collected from the Lubuk Minturun area, Koto Tangah subdistrict, Padang, West Sumatra, Indonesia, and identified by the Herbarium of Andalas University (ANDA). The materials used in this study are methanol (Merck), n-hexane (Merck), ethyl acetate (Merck), HCl p.a, magnesium powder (Mg), ammonia, H_2SO_4 p.a, Wagner reagent, Mayer reagent, acetic anhydride, chloroform, DPPH (1,1-diphenyl-2-picrylhydrazyl), Ascorbic Acid and distilled water. Instruments used include a UV-Vis spectrophotometer (Genesys 50), analytical balance (Ohaus), rotary evaporator (Buchii), and standard laboratory glassware.

Sample Preparation

The stems of mistletoe kepundung (*S. ferruginea* (Roxb. ex Jack) Danser) were collected, washed, and air-dried. The dried stems were then sliced and ground into simplicia powder.

Extraction and Fractionation

The simplicia powder was macerated with methanol for 3 × 72 hours at room temperature with occasional stirring, then filtered [15]. The filtrate was concentrated using a rotary evaporator to obtain a thick methanol extract [16]. The methanol extract was then subjected to liquid-liquid partitioning using n-hexane, water, and ethyl acetate solvents sequentially, producing three fractions with different polarities [17].

Phytochemical Tests

Phytochemical tests were performed on each fraction (n-hexane, ethyl acetate, and water) to detect the presence of secondary metabolite compound classes. Alkaloid identification was conducted using Wagner and Mayer reagents; a positive result is indicated by the formation of brown (Wagner) and white (Mayer) precipitates [18]. Steroid detection was performed using the Liebermann-Burchard test by reacting each fraction with chloroform, acetic anhydride, and concentrated H_2SO_4, with a positive result shown by a green color change [19]. Terpenoid screening was performed through the Salkowski test using chloroform and concentrated H_2SO_4. The appearance of a reddish-brown color indicates a positive result [20]. Flavonoid detection was conducted using the Shinoda test with HCl p.a and Mg

powder, a positive result being marked by a color change to red [21].

Antioxidant Activity Test (DPPH Method)

The DPPH free radical scavenging activity was determined using a modified spectrophotometric method. Stock solutions of each fraction (n-hexane, ethyl acetate, and water) were diluted into a series of concentrations (20, 40, 60, 80, and 100 ppm). Purified ascorbic acid (Vitamin C) was prepared across the same concentration range to serve as the standard positive control.

A 2 mL aliquot of each sample concentration or positive control was homogeneously mixed with 2 mL of 0.25 mM DPPH solution (prepared by dissolving 5 mg of DPPH powder in 50 mL of methanol). The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance of the solution was then measured using a UV-Vis spectrophotometer at the maximum wavelength of 517 nm against a methanol blank [12].

Every concentration level for all fractions and the positive control was tested in triplicate (n=3) to ensure the reproducibility and accuracy of the experimental data. The percentage of free radical inhibition was calculated using the following equation:

$$\%\ \text{Inhibisi} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}}\ \text{X}\ 100\%$$

Description:
$\text{Abs}_{\text{control}}$: DPPH absorbance 50 ppm sol
$\text{Abs}_{\text{sample}}$: Sample absorbance

The IC_{50} (Inhibitory Concentration 50%) value was determined via the linear regression equation (y = ax + b) correlating the sample concentration (X) with the percentage of inhibition (Y), where the value of Y was set at 50 [22]. The IC_{50} values obtained were subsequently used to classify the strength of antioxidant activity according to the criteria presented in Table 1.

Table 1. Classification of antioxidant activity strength [23]

IC_{50} value (ppm)	Strength Category
<50	Very strong
50-100	Strong
101-150	Quite powerful
151-200	Weak
>200	Inactive

Results and Discussion

Extraction and Partitioning

The dried stems of *S. ferruginea* were extracted with methanol by maceration for 3 × 72 hours. Maceration was selected as it is a straightforward cold-extraction technique that prevents the degradation of thermolabile secondary metabolites [24]. Methanol was selected because its relatively high polarity enables efficient extraction of a broad range of secondary metabolites from plant material [25]. The methanol extract is then concentrated using a rotary evaporator, yielding 254 grams of concentrated methanol extract. Furthermore, the concentrated extract is fractionated using n-hexane, water and ethyl acetate solvents in order to produce three fractions [17].

Phytochemical Tests

Phytochemical testing was performed as a preliminary screening to characterize the secondary metabolite profiles of the sample. The identified secondary metabolites for each fraction are summarized in Table 2.

Table 2. Results of phytochemical screening of *S. ferruginea* stem fractions

No	Compound Groups	Fraction		
		n-hexane	Ethyl acetate	Water
1	Flavonoids	-	+	+
2	Alkaloids	-	-	-
3	Terpenoids	+	-	-
4	Steroids	+	+	-

Table 2 presents the phytochemical screening results of the three fractions from the *S. ferruginea* stem. The ethyl acetate fraction contains flavonoids and steroids, the n-hexane fraction contains terpenoids, whereas the water fraction contains flavonoids. No alkaloids were detected in any of the three fractions. These phytochemical screening results indicate that the distribution of secondary metabolites is highly influenced by the polarity of the solvents used. Flavonoid compounds were detected in both the ethyl acetate and water fractions, which aligns with the semi-polar to polar nature of flavonoids. Terpenoids, which are non-polar, were only detected in the n-hexane fraction, while steroids were identified in the ethyl acetate fraction [26].

Test of Antioxidant Activity of All Fractions

The free radical scavenging activity of each fraction obtained from the stems of *Scurrula ferruginea* was quantitatively evaluated using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The assays were conducted in independent triplicates (n=3) to minimize experimental errors and closely monitor data variability. Absorbance values were measured using a UV-Vis spectrophotometer at the maximum wavelength of 517 nm, with pure ascorbic acid (Vitamin C) utilizing as the standard positive control [27]. The antioxidant potency was determined based on the IC_{50} (Inhibitory Concentration 50%) value, which represents the sample concentration required to reduce 50% of the initial DPPH free radical activity. The IC_{50} values were mathematically calculated via interpolation from linear regression equations correlating the variations in sample concentration (20, 40, 60, 80, and 100 ppm) with their respective percentage inhibition values [28]. To ensure scientifically valid data and accurately reflect data variability, the deviation metrics are reported as the mean ± standard deviation (Mean ± SD) in Table 3.

Figure 1 presents the relationship between concentration and DPPH radical inhibition, while the IC_{50} values obtained for each fraction are presented in Table 3. Furthermore, the antioxidant activity of each fraction was classified according to the criteria presented in Table 1. For comparison, the IC_{50} value of ascorbic acid, the positive control, is presented in Table 4.

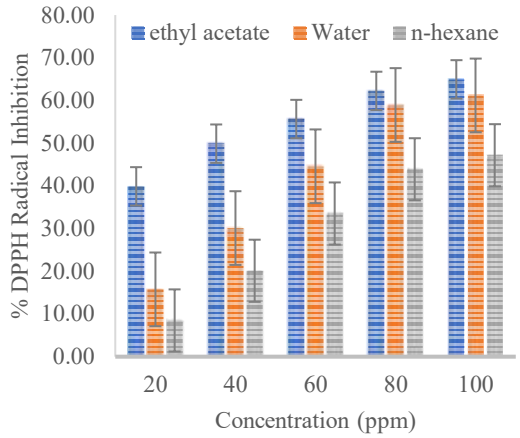


Figure 1. Graph Showing the Relationship Between Concentration and % DPPH Inhibition of the Stem Fraction of the *S. ferruginea* (Roxb. ex Jack) Danser.

Based on the experimental data presented in Table 3, all tested fractions exhibited dose-dependent radical-scavenging activity, with higher sample concentration directly corresponding to greater DPPH radical inhibition. This linear relationship is strongly supported by the high coefficients of determination ($R^2 > 0.96$) observed across all regression lines, indicating a robust correlation between concentration and antioxidant capacity.

The antioxidant intensity classification of the samples was determined using the reference criteria in Table 1. According to these parameters, the ethyl acetate fraction (IC_{50}= 45.53±0.58 ppm) officially falls into the Very Strong antioxidant category. Meanwhile, both the water fraction (IC_{50}= 73.18±0.82 ppm) and the n-hexane fraction (IC50= 98.28±1.15 ppm) are successfully designated within the Strong antioxidant tier, as their values fall firmly within the specified range for that class.

Table 3. Comparison of IC_{50} values for each fraction of the *S. ferruginea* stem

No	Fraction	Linear Regression Equation	R^2	Average ± SD(ppm)	Category
1	n-hexane	y = 10.115x + 0.2918	0.9663	98.28 ± 1.15	Strong
2	ethyl acetate	y = 6.2471x + 35.776	0.9632	45.53 ± 0.58	Very strong
3	Water	y = 11.965x + 6.2235	0.9602	73.18 ± 0.82	Strong

Table 4. IC_{50} Value of Ascorbic Acid Antioxidant Activity Test Results

No	Testing	IC_{50} (ppm)	Average ± SD (ppm)	Category
1	Replicate 1	11.85	12.11 ± 0.24	Very strong
2	Replicate 2	12.33		
3	Replicate 3	12.15		

The n-hexane fraction (non-polar) exhibited an IC_{50} value of 98.28 ± 1.15 ppm, which is classified as a Strong antioxidant [23]. The antioxidant activity in this initial non-polar phase is entirely driven by the simultaneous action of terpenoids and steroids, which were detected positively during the qualitative screening. The absolute absence of hydrophilic flavonoids in this fraction forces the radical scavenging mechanism to rely heavily on the lipophilic nature of terpenoids and steroids. These specific metabolites stabilize free radicals via addition reactions across their double bonds or through hydrogen abstraction from their structural rings. Spectrophotometrically, this lipophilic kinetic pathway progresses at a significantly slower rate compared to phenolic donor compounds, thus positioning its IC_{50} value close to the upper threshold of the strong category [29].

In contrast, the ethyl acetate fraction (semi-polar) demonstrated a remarkable increase in potency, yielding the lowest IC_{50} value of (IC50= 45.53 ± 0.58 ppm), elevating it into the Very Strong antioxidant category [23]. However, since this study did not conduct quantitative total flavonoid profiling or compound isolation, attributing this high antioxidant capacity exclusively to flavonoids would be scientifically premature. Instead, this potent radical-scavenging activity is highly likely the result of a complex, synergistic interplay among multiple co-extracted secondary metabolites.

Qualitative screening revealed that the ethyl acetate fraction successfully accumulated a dense combination of both flavonoids and steroids. While flavonoids provide a rapid kinetic pathway via hydrogen atom donation from their aromatic hydroxyl (–OH) groups, the co-existing steroidal frameworks act as vital chain-breaking antioxidants [24]. Steroids participate in mitigating lipid radical proliferation and stabilizing the reaction medium through alternative spatial orientations. Therefore, the observed "Very Strong" status is a collective bioactivity of these multi-target metabolite groups working in tandem, rather than the singular action of an isolated compound class.

Lastly, the water fraction (highly polar) yielded an IC_{50} value of (IC50= 73.18 ± 0.82 ppm), falling back into the Strong category [23]. Although the water fraction tested positive for flavonoids, its scavenging capacity was notably lower than that of the semi-polar ethyl acetate fraction. Biochemically, highly polar solvents tend to selectively extract flavonoids bound to sugar moieties, known as glycoside forms. The physical presence of these glycone (sugar) chains creates significant steric hindrance, which drastically reduces the physical flexibility of hydrogen transfer from the flavonoid's remaining hydroxyl groups to the DPPH radical [30]. This directly explains the lower performance compared to the ethyl acetate fraction, which is predominantly composed of flavonoid aglycones (free from sugar groups) that possess much faster kinetic reactivity [31].

This multi-component synergy is also heavily modulated by a unique ecological variable in this research: the specific host tree, *Baccaurea racemosa* (locally known as kepundung). As an obligate hemiparasite, *S. ferruginea* lacks a fully independent root system and relies fundamentally on its haustorium to absorb water, mineral nutrients, and various metabolic precursors directly from the xylem sap of *Baccaurea racemosa* [10]. Previous eco-phytochemical studies indicate that host-mistletoe

interactions induce a chemical defense response in the host, altering the secondary metabolite flux. Baccaurea species are well-documented to be rich in organic acids, phenolic derivatives, and alkaloids. The absorption of these diverse host precursors may trigger upregulated biosynthetic pathways within the stem tissues of S. ferruginea, leading to high-yield accumulation of highly active aglycone flavonoids and lipophilic steroids that are optimally extracted by the semi-polar ethyl acetate solvent.

To critically contextualize these findings within the wider field of mistletoe research, a comparative analysis with previous literature reveals significant variations that highlight the impact of host-dependent and tissue-specific factors. For instance, an ethyl acetate fraction derived from the leaves of *Scurrula ferruginea* hosted by the coffee plant (Coffea canephora) was reported to exhibit a slightly lower IC_{50} value of 35.20 ppm. Conversely, investigations of related species within the same genus, such as the stems of Scurrula parasitica hosted by Mangifera indica (IC_{50} = 88.40 ppm) and the leaves of Scurrula atropurpurea hosted by Camellia sinensis (IC_{50} = 62.14 ppm), demonstrated significantly weaker antioxidant activities.

This quantitative disparity provides critical insight: S. ferruginea in this study exhibits a superior antioxidant profile compared to other Scurrula species, even when comparing stem tissues. The fact that the stem extract from a tea host (Scurrula atropurpurea) demonstrates a significantly lower antioxidant capacity with an IC_{50} value of 142.15 ppm, as the *S. ferruginea* stem from the kepundung host (45.53 ppm) strongly validates the hypothesis that the chemical profile of *Baccaurea racemosa* plays a pivotal role in boosting the bioactivity of its parasite. The host-dependent nutrient transfer not only alters the concentration of the metabolites but also shapes the specific chemical architectures synthesized by the mistletoe, creating a distinct phytocomplex that optimizes free radical reduction.

Although the ethyl acetate fraction exhibited remarkably potent activity, its activity remained statistically below that of pure ascorbic acid (Vitamin C), which served as the positive control (IC_{50} = 12.11 ± 0.24 ppm). This significant difference is highly rational, given that ascorbic acid is a high-density, single, pure molecule, whereas the tested fractions are complex crude extract mixtures containing a multi-target blend of active and inactive compounds. Connection to this, the very narrow standard deviation intervals across all data replications reaffirm that the DPPH radical scavenging data in this study possess high experimental accuracy, low data variability, and solid reproducibility [32].

Conclusion

The phytochemical evaluation and antioxidant activity of *Scurrula ferruginea* stems across three fractions sequentially align with their polarity levels, where the non-polar n-hexane fraction is dominated by terpenoids and steroids (IC_{50}= 98.28 ±1.15 ppm, Strong), the semi-polar ethyl acetate fraction accumulates dense flavonoid and steroid complexes (IC_{50} = 45.53 ± 0.58 ppm, Very Strong), and the polar water fraction retains glycoside-form flavonoids (IC_{50}= 73.18 ± 0.82 ppm, Strong). These outcomes confirm that *S. ferruginea* stems, particularly the ethyl acetate fraction, hold great potential as a natural source of free-radical scavengers, a potential that is strongly modulated by its unique host-tree interaction with *Baccaurea racemosa*. However, this study is strictly limited to preliminary qualitative screening and in vitro chemical assays (DPPH), meaning that single-compound structures remain unverified and these crude results cannot be extrapolated for direct pharmaceutical development without comprehensive biological evaluations. To address these limitations, future research should move beyond these baseline findings toward quantitative phytochemical analysis (TPC/TFC), bioassay-guided chromatographic isolation (HPLC/NMR), and advanced toxicity and in vivo antioxidant testing to safely evaluate their complex mechanisms in living systems.

Author's Contribution

E.H.Br. Siadari: Compiling and conducting research as well as writing the paper. Suryelita: supervising lecturer and determining the type of analysis and data analysis techniques.

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