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Evaluation of Antioxidant Activity in Solanum Ferox (Through DPPH and FRAP Assays)

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Abstract
The antioxidant properties of natural compounds have garnered significant attention for their potential therapeutic applications. Solanum ferox, a plant traditionally used in several countries for medicinal purposes, has been identified as possessing potential antioxidant properties. However, the extent and efficacy of its antioxidant capacity have not been thoroughly examined, creating a gap in understanding its effectiveness compared to established antioxidants like ascorbic acid. This study aims to evaluate the antioxidant capacity of Solanum ferox using DPPH and FRAP assays across concentrations ranging from 20 to 100 µg/µl, with ascorbic acid serving as a standard reference. The DPPH assay measured the scavenging activity of both samples to assess their radical-quenching properties, while the FRAP assay evaluated their reducing power. Findings indicate that ascorbic acid exhibits significantly higher scavenging activity than Solanum ferox across all concentrations, with consistent activity ranging between 88.4% and 90.88% in the DPPH assay and from 45.16% to 83.89% in the FRAP assay. In contrast, Solanum ferox shows a gradual but much lower increase in activity, from 6.8% to 35.5% in the DPPH assay and from 10.59% to 33.82% in the FRAP assay, suggesting limited antioxidant potential. The study contributes to the understanding of the antioxidant properties of Solanum ferox, highlighting its limited efficacy compared to ascorbic acid and suggesting that further research is needed to explore its potential at higher concentrations or through alternative methods. These insights provide a foundational basis for future studies investigating Solanum ferox as a potential natural antioxidant source.

Keywords: Solanum Ferox, Antioxidant, DPPH, FRAP Assays. .

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1. INTRODUCTION

Solanum ferox L., commonly referred to as "Terung Asam," is a flowering plant that belongs to the Solanum genus within the Solanaceae family (Raduan et al., 2019). The Solanaceae family encompasses thousands of species with both commercial and medicinal significance, including "Terung Dayak," which is highly regarded in Central Kalimantan (Fadhli & Fitriyah, 2023). This plant is widely distributed across most regions of Central Kalimantan, where its fruit is traditionally utilized by local communities in sour vegetable dishes and fish sambals. In addition to Indonesia, Solanum ferox L. is also found in countries such as Bangladesh, Sri Lanka, and the Philippines (Meyer et al., 2014; Ranil et al., 2017; Aubriot & Knapp., 2022).

Previous research has showcased the remarkable medicinal potential of Solanum ferox L., a plant long valued in traditional medicine for its diverse therapeutic applications. In many ethnicities, the root decoction is used to treat a variety of conditions, including syphilis (Hazimah et al.,2023; Ugoeze., 2022), body pain (Abdullah et al., 2012), and even loss of appetite (Kalalinggi, 2024; Rahardjo et al., 2022). Additionally, it has been applied as a medication for common issues such as fever, itching, wounds, bruises, and as a natural antipyretic (Abdullah et al., 2012). In Bangladesh, the plant holds a reputation for improving respiratory problems like coughs, asthma, and sore throats. Meanwhile, in India, it has been recognized for its anti-rheumatic effects (Putri et al., 2023) and has also been utilized for its anti-asthmatic and antiviral properties (Arthan & Nanakorn, 2002). Beyond these benefits, Solanum ferox L. has shown promise as an anticancer agent, further highlighting its potential in modern medicine (Dailah, 2022; Raduan et al., 2019). The breadth of these traditional applications strongly suggests that Solanum ferox L. contains bioactive compounds capable of providing significant therapeutic effects. These findings make a compelling case for further scientific investigation to better understand the plant's chemical composition and validate its historical uses.

In addition to its medicinal benefits, studies have demonstrated that Solanum ferox L. possesses remarkable antioxidant properties, as evidenced by multiple investigations (Hamzah et al., 2022; Rosmainar et al., 2023). Antioxidants are essential compounds that help prevent or slow cellular damage caused by free radicals—unstable and highly reactive molecules capable of harming cells and contributing to chronic diseases such as cancer, cardiovascular disorders, and premature aging (Pisoschi & Pop, 2015; Poljsak et al., 2013; Pham et al., 2008). The bioactive compounds present in Solanum ferox L., particularly phenolics and flavonoids, have shown significant promise in combating oxidative stress, making the plant a potential candidate for disease prevention and treatment (Ranil et al., 2017; Jan et al., 2024; Hamzah et al., 2022). Considering its extensive medicinal applications and antioxidant potential, further research on Solanum ferox L. is imperative to identify and develop its bioactive constituents for pharmaceutical purposes. This study focuses on evaluating the antioxidant properties of the plant, aiming to uncover new compounds that could significantly enhance disease treatment and drug development. The findings are anticipated to provide a robust scientific basis for the medicinal use of Solanum ferox L. and establish its potential as a natural antioxidant source, contributing to advancements in both traditional medicine and modern pharmacology (Meyer et al., 2014; Isla et al., 2022).

2. RESEARCH METHOD

The research used an experimental method to evaluate the antioxidant activity of *Solanum ferox. L* extracts through DPPH and FRAP assays. The fruit were collected and cleaned then dried, ground into powder and extracted with a 75% ethanol-water solution. The DPPH assay measured radical scavenging activity by incubating varying extract concentrations with DPPH solution and measuring absorbance. The FRAP assay assessed the extract's ability

to reduce ferric ions by mixing the extract with a FRAP solution and measuring absorbance. Both assays provided data on the antioxidant capacity of the extracts by comparing results to standard references.

Preparation of *Solanum Ferox L*. the samples of *Solanum ferox. L* were collected from the forests of Palangkaraya, Central Kalimantan, and underwent a cleaning process before being air-dried in a shaded area to preserve their natural compounds. These dried fruit were then ground into a fine powder using a powder grinder machine. For extraction, 10 grams of the powdered leaves were steeped in 250 ml of a 75% ethanol-water solution at a 50:50 ratio for five days. The re sulting mixture was filtered through a Buchner funnel with Whatman No-1 filter paper and concentrated using a vacuum concentrator (BUCHI R210). The final product was obtained by lyophilizing the concentrated extract into a powder using a freeze dryer machine, ensuring the preservation of essential plant properties for subsequent nanoparticle synthesis (Al-Garadi et al., 2022; Raspe et al., 2021)

1,1,-Diphenyl-2-picrylhydrazyl (DPPH) Assay. The antioxidant capacity of *Solanum ferox. L* extracts was assessed using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay, a method widely recognized for its efficacy in measuring radical scavenging activity. In this procedure, 500 µl of *Solanum ferox. L* extracts at different concentrations (20-100 µg/µl) were combined with an equal volume of 0.3 mM ethanolic DPPH solution and incubated for 30 minutes in darkness. The absorbance was then measured at 517 nm, with a water: ethanol (1:1) mixture as the blank and the DPPH solution as the control. The antioxidant activity was quantified by calculating the inhibition percentage using the formula:

Inhibition % = (1- (A Sample)/Ao) x 100 %

In this formula, A(sample) represents the absorbance of the *Solanum ferox. L* extract sample, while A0 denotes the absorbance of the DPPH solution.

Ferric Ion Reducing Antioxidant Power (FRAP) Assay. FRAP assay was utilized to measure the antioxidant capacity of *Solanum ferox. L* extracts, following the methodology by (Sivapalan et al., 2024). In this assay, varying concentrations of the extracts (20-100 µg/µl) were tested against a FRAP working solution, made by combining acetate buffer, TPTZ, and $FeCl3.6H_2O$ in a 10:1:1 ratio. For each test, 60 µL of the extract was mixed with 1800 µL of the FRAP solution and incubated for 5 minutes at room temperature in the dark. The absorbance was then measured at 593 nm, with water as the background control. The antioxidant power was quantified using a formula comparing the absorbance values to those of ascorbic acid, serving as the standard, providing a relative measure of the extracts' ability to reduce ferric ions.

FA = ((A Sample- A Blank)/A Sample) x 100 %

In this formula, A represents the absorbance of the sample at 593 nm.

3. RESULTS AND DISCUSSION

DPPH Scavenging Activity of *Solanum Ferox*. Table 1 illustrates the DPPH scavenging activity of ascorbic acid and *Solanum ferox* across concentrations ranging from 20 to 100 µg/µl. Ascorbic acid exhibits consistently high scavenging activity, starting at 88.4% and slightly increasing to 90.88%, with minimal variation (STD ranging from 0.05 to 0). This consistency indicates stable antioxidant effectiveness across all tested concentrations. In contrast, Solanum ferox shows a progressive increase in DPPH scavenging activity from 6.8% to 35.5% as the concentration rises. However, its activity remains considerably lower than that of ascorbic acid. The standard deviations for *Solanum ferox* (0.03 to 0.04) demonstrate reliable results but reflect less potent antioxidant capacity. Overall, while *Solanum ferox* displays concentration-dependent scavenging activity, its effectiveness is significantly lower than that of ascorbic acid, suggesting limited antioxidant potential within the tested concentration range.

Table 1. DPPH scavenging activity of ascorbic acid and *Solanum Ferox* in consentration 20-100 µg/µl

Sample Concentration (µg/µl)	**Ascorbic acid DPPH scavenging activity (%)**	**STD**	***Solanum Ferox* DPPH scavenging activity (%)**	**STD**
20	88.4	0.05	6.8	0.03
40	89.3	0.5	15.1	0.04
60	90	0	21.2	0.03
80	90.4	0	27.9	0.03
100	90.88	0	35.5	0.04

Figure 2 illustrates that ascorbic acid consistently exhibits high DPPH scavenging activity, around 90% across all concentrations with minimal variability, while *Solanum ferox* displays significantly lower yet progressively increasing activity from 7% to 36%, accompanied by greater variability at lower concentrations.

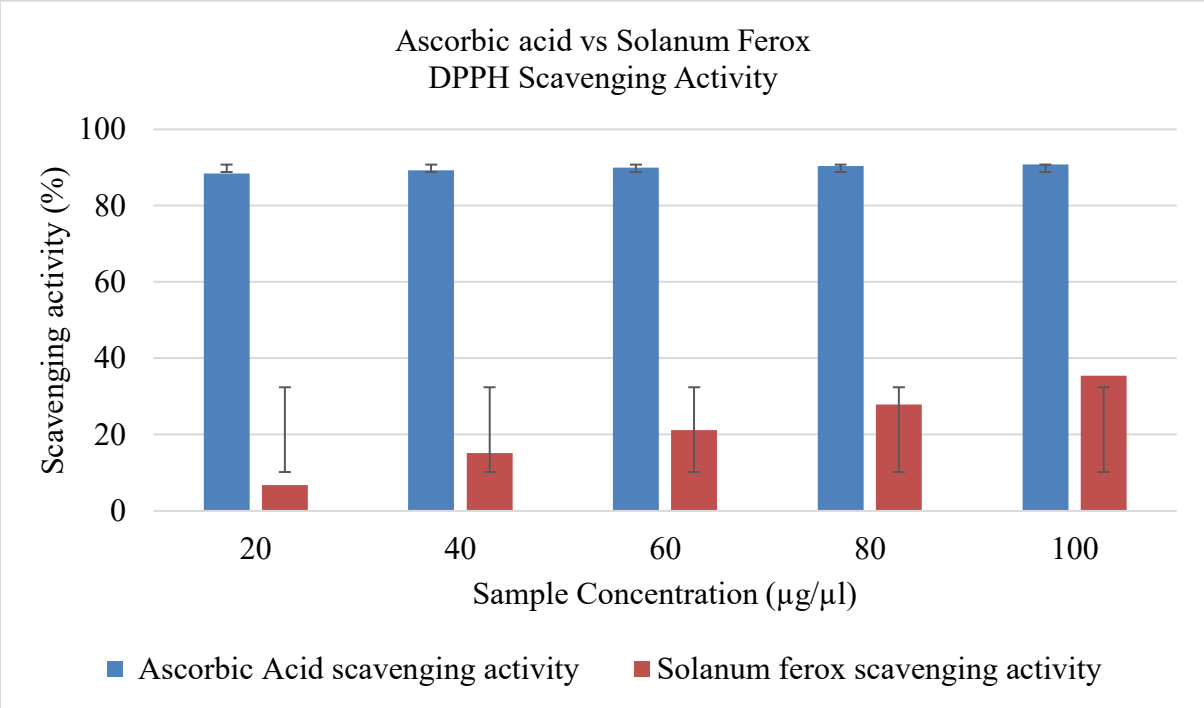

Figure 2. DPPH scavenging activity of ascorbic acid and *Solanum Ferox* in consentration 20-100 µg/µl

FRAP Scavenging Activity of *Solanum Ferox*. Table 2 displays the FRAP scavenging activity of ascorbic acid and *Solanum ferox* across concentrations ranging from 20 to 100 µg/µl. Ascorbic acid exhibits a strong, concentration-dependent increase in activity, rising from 45.16% to 83.89%, with only a slight increase in variability (STD: 0.08 to 0.14). In contrast, Solanum ferox shows a lower scavenging activity, gradually increasing from 10.59% to 33.82% as concentration increases. The standard deviation for *Solanum ferox* remains low (0 to 0.01), indicating consistent results. Despite this increase, Solanum ferox demonstrates substantially lower scavenging effectiveness compared to ascorbic acid across all concentrations tested.

Table 2. FRAP scavenging activity of ascorbic acid and *Solanum Ferox* in concentration 20-100 µg/µl

Sample Concentration (µg/µl)	Ascorbic Acid FRAP scavenging activity (%)	STD	Solanum Ferox FRAP scavenging activity (%)	STD
20	45.16	0.08	10.59	0.01
40	62.86	0.09	14.56	0
60	73.29	0.11	19.64	0
80	80.2	0.14	29.32	0.01
100	83.89	0.12	33.82	0

Figure 3 illustrates the FRAP scavenging activity of ascorbic acid and *Solanum ferox* across concentrations of 20 to 100 µg/µl. Ascorbic acid displays consistently higher scavenging activity, increasing from approximately 45% to over 80%, with moderate variability as shown by the error bars. In contrast, *Solanum ferox* exhibits significantly lower scavenging activity, ranging from around 10% to 34%, with minimal variability across concentrations. This comparison clearly demonstrates that ascorbic acid possesses a substantially greater antioxidant capacity than Solanum ferox at all tested concentrations.

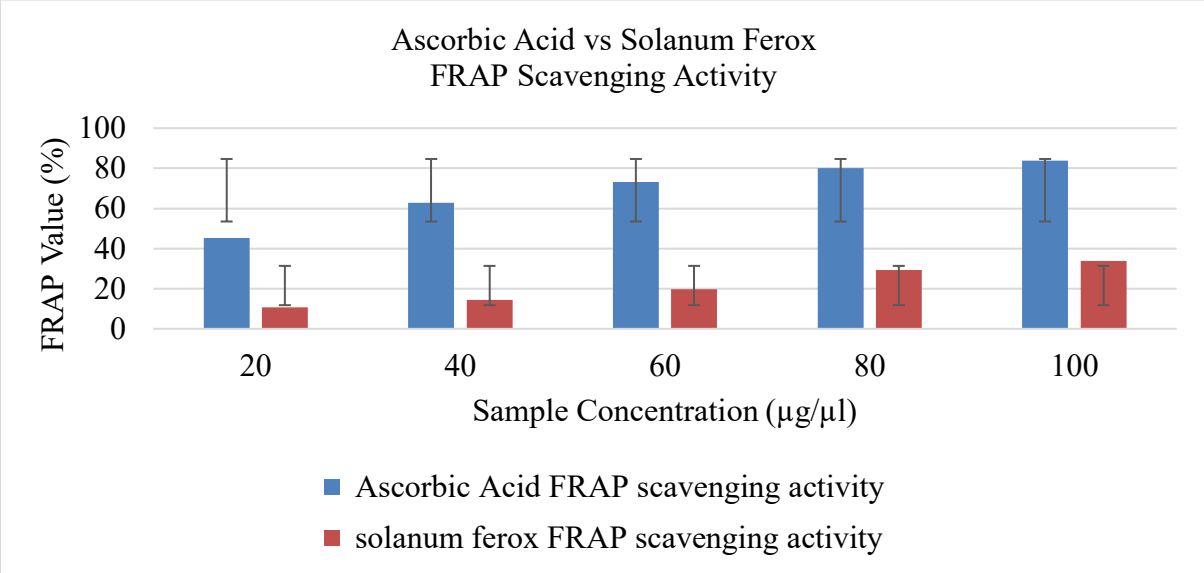

Figure 3. FRAP scavenging activity of ascorbic acid and *Solanum Ferox* in Concentration 20-100 µg/µl

DISCUSSION

The DPPH scavenging assay results demonstrate a clear contrast in antioxidant activity between ascorbic acid and *Solanum ferox*. Ascorbic acid, a well-known standard antioxidant (Osjecki et al.,2010 ; Bajić et al., 2017), exhibited consistently high scavenging activity across all concentrations, ranging from 88.4% to 90.88%, with minimal variability (STD: 0.05 to 0). This stability underscores its strong radical scavenging ability, attributed to its efficient hydrogen-donating properties (Pisoschi & Pop, 2015; Qi et al., 2021; Jing et al., 2019). In comparison, Solanum ferox displayed a concentration-dependent increase in scavenging activity, rising from 6.8% at 20 µg/µl to 35.5% at 100 µg/µl. Although this trend indicates that higher concentrations of *Solanum ferox* extracts enhance radical scavenging, its maximum activity remains significantly lower than that of ascorbic acid, suggesting limited antioxidant capacity within the tested range.

The observed difference in antioxidant activity may be attributed to variations in the bioactive compounds present in ascorbic acid and *Solanum ferox*. Phenolic compounds and flavonoids, commonly found in Solanum species, are known contributors to antioxidant activity (Rosmainar et al., 2023; Hamzah et al., 2022; Medina-Medrano et al., 2015; Nandi et al., 2021). However, their concentration and composition in *Solanum ferox* might be insufficient to match the potent radical scavenging effects of ascorbic acid. Moreover, the structural complexity of these compounds could influence their interaction with DPPH radicals, leading to the observed variability in activity. These findings highlight the potential of *Solanum ferox* as a moderate natural antioxidant source, while emphasizing the need for further investigation into its specific phytochemical constituents.

The FRAP assay results further differentiate the antioxidant capacities of ascorbic acid and *Solanum ferox*. Ascorbic acid exhibited a strong, concentration-dependent increase in reducing power, with activity ranging from 45.16% at 20 µg/µl to 83.89% at 100 µg/µl. The relatively low variability (STD: 0.08 to 0.14) across all concentrations confirms its consistent ability to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), which is indicative of its high reducing potential (Yin et al., 2024; Bai et al., 2024). On the other hand, *Solanum ferox* showed a gradual increase in FRAP activity, starting at 10.59% and reaching 33.82% at the highest tested concentration. While the results suggest that *Solanum ferox* has a measurable reducing capacity, its performance is considerably lower than that of ascorbic acid, underscoring its limited efficacy as a reducing agent.

The discrepancy between the FRAP activities of ascorbic acid and *Solanum ferox* can be explained by differences in the abundance and nature of their antioxidant compounds. While ascorbic acid directly participates in electron transfer reactions, the reducing power of S*olanum ferox* is likely influenced by its phenolic content, which may not be as effective at converting ferric ions under the assay conditions (Benzie & Devaki., 2018; Granoto et al., 2016). Additionally, the lower variability observed in *Solanum ferox's* results (STD: 0 to 0.01) reflects consistency in its reducing ability, albeit at a much lower level than ascorbic acid. These findings indicate that, while *Solanum ferox* exhibits antioxidant potential, its overall capacity to neutralize oxidative stress through electron transfer mechanisms is limited compared to a standard like ascorbic acid. Future research should focus on optimizing extraction methods to enhance the yield and efficacy of its bioactive compounds.

4. CONCLUSION

This study compared the antioxidant capacity of Solanum ferox and ascorbic acid using DPPH and FRAP assays across multiple concentrations. The results clearly demonstrate that ascorbic acid exhibits significantly higher and more consistent scavenging activity in both assays, confirming its strong antioxidant potential. While Solanum ferox displays a concentration-dependent increase in activity, its overall antioxidant effectiveness remains substantially lower, indicating limited utility at the tested concentrations. These findings emphasize the superior antioxidant capacity of ascorbic acid, suggesting that Solanum ferox may require further investigation at higher concentrations or with alternative methods to fully assess its potential as a natural antioxidant source.

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