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Research Article

Phytochemical profiling and comparative antioxidant activity of n-hexane and ethyl acetate leaf extracts of *Solanum nigrum*

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ABSTRACT

This study evaluated the phytochemical constituents and antioxidant potential of *Solanum nigrum* leaf extracts using n-hexane and ethyl acetate solvents. Preliminary phytochemical screening revealed the presence of key secondary metabolites. Ethyl acetate extract showed the presence of alkaloids (+), flavonoids (++), carbohydrates (++), and terpenoids (+++), while the n-hexane extract predominantly contained terpenoids (+++). Other constituents such as saponins, tannins, and anthraquinones were absent in both extracts. Antioxidant activity was assessed using three in vitro assays: DPPH assay, H_2O_2 assay, and Ferric reducing antioxidant power. Results demonstrated a concentration-dependent increase in antioxidant activity (250–1000 µg/mL) across all assays. Ethyl acetate extract exhibited significantly higher scavenging activity, with maximum values of 82.58 ± 0.04% (DPPH), 64.16 ± 0.20% (H_2O_2), and 58.19 ± 0.01% (FRAP) at 1000 µg/mL, compared to n-hexane extract. The calculated IC_{50} values were recorded as thus for DPPH, H_2O_2, and FRAP as 549, 617 and 878 µg/mL respectively. Statistical analysis indicated significant differences ($P \leq 0.05$) among treatments. Among the assays, DPPH showed the highest sensitivity, followed by hydrogen peroxide and FRAP. The enhanced antioxidant activity of the ethyl acetate extract is attributed to its richer phytochemical profile, particularly flavonoids and phenolic compounds known for free radical scavenging. These findings support the potential application of *Solanum nigrum* as a natural antioxidant source for pharmaceutical and nutraceutical development. Further studies on bioavailability and isolation of active compounds are recommended.

Keywords: Antioxidant activity, Assay, Ethyl acetate extract, Phytochemical screening, *Solanum nigrum*, Terpenoids

Introduction

Medicinal plants continue to play a vital role in global healthcare systems, particularly in developing countries where access to conventional medicine is limited [1]. According to [2], more than 80% of the world's population depends on traditional medicine for primary healthcare. The therapeutic properties of these plants are largely attributed to the presence of phytochemicals, which are bioactive compounds produced through primary and secondary metabolic processes. Alkaloids, flavonoids, tannins, saponins, and phenolics, are known to contribute significantly to plant defense mechanisms and exhibit various pharmacological activities such as antimicrobial, anti-inflammatory, and antioxidant effects [3], [4].

Phytochemical screening is an essential preliminary step in identifying the chemical constituents of plant extracts and evaluating their biological activities. To date, over 4,000 phytochemicals have been identified, many of which possess protective roles against oxidative stress-related diseases [5]. Oxidative stress, caused by an imbalance between free radical production and antioxidant defenses, contributes to the development of several chronic diseases [6], [7]. Due to safety concerns associated with synthetic antioxidants, natural antioxidant sources

have gained increasing attention [8]. n-Hexane and ethyl acetate were selected as extraction solvents because their different polarities enable the extraction of distinct classes of phytochemicals that may contribute to antioxidant activity.

Solanum nigrum Linn (family Solanaceae), commonly known as black nightshade is widely distributed across Africa and Asia. The plant has been traditionally used for the treatment of various ailments, including inflammation, liver disorders, skin diseases, and respiratory infections [9], [10]. Previous phytochemical studies have reported that *S. nigrum* contains a wide range of bioactive compounds such as steroidal alkaloids, flavonoids, glycoproteins, and phenolic acids, which are associated with antioxidant, anticancer, and hepatoprotective activities [11-13]. Additionally, experimental studies have demonstrated its cytoprotective and neuropharmacological properties, largely attributed to its ability to scavenge reactive oxygen species and reduce oxidative stress [14].

Despite these findings, there remains limited comprehensive information on the influence of extraction solvents on the phytochemical composition and antioxidant potential of *S. nigrum*, especially the one that is cultivated in Jos. The choice of solvent plays a crucial role in determining the type and quantity of phytochemicals extracted, thereby influencing the biological activity of plant extracts. Furthermore, many traditional uses of this plant lack sufficient scientific validation, highlighting the need for systematic investigation.

Therefore, the present study aims to investigate the phytochemical constituents and evaluate the antioxidant activities of n-hexane and ethyl acetate extracts of *Solanum nigrum* leaves. The specific objectives are to (i) obtain the crude extracts using different solvents, (ii) determine the phytochemical components present, and (iii) comparatively assess the antioxidant activity using DPPH, hydrogen peroxide (H_2O_2) scavenging, and ferric reducing antioxidant power (FRAP) assays. This study contributes to existing knowledge by providing comparative data on solvent extraction efficiency and comparative antioxidant potential, thereby supporting the scientific validation of the traditional use of *S. nigrum* and its potential application in pharmaceutical and nutraceutical development. Further studies will be required to isolate the specific bioactive components and also quantify their effectivity.

Material and Methods

Sample Collection and Preparation

The plant was harvested and packaged in a polyethylene bag from old legislative Quarters Jos, Plateau State. The leaves were thoroughly washed under running water and shade dried for 14 days at ambient temperature in the laboratory. The dried sample was pulverized into fine powder and stored in an airtight container for further use. The plant leaf sample was identified and authenticated with a voucher number ABUH0917 at Ahmadu Bello University Zaria.

Crude Extraction

The dried powdered leaf (120 g each) was exhaustively extracted with n-hexane and ethyl acetate for 72 hrs using a Soxhlet extraction method. All the extracts were concentrated to dryness at a reduced pressure using rotary evaporator.

Percentage Yield

The percentage yield of the extracts was calculated using the formula below:

$$\% \text{ yield} = \frac{Weight\ of\ the\ crude\ extract\ (g)}{Weight\ of\ the\ pulverized\ powerder\ (g)}\ x\ 100$$

Qualitative Phytochemical Screening of *Solanum nigrum* leaf extract

All qualitative phytochemical screening (Carbohydrates Terpenoids Alkaloids Flavonoids Saponin Tannins) were determined using the methods adopted by [1], [15].

Hydrogen Peroxide (H_2O_2) Scavenging Activity of *Solanum nigrum* leaf extract

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Different concentrations (250, 500, 750 and 1000 µg/mL) of the crude extract and ascorbic acid as the control were added to a hydrogen peroxide solution (0.6 mL, 40 mM). The absorbance of hydrogen peroxide at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The hydrogen peroxide percentage scavenging activity was then calculated.

$$\text{Anitioxidant capacity} = \left(\frac{1-\text{sample absorbance}}{Blank\ \text{absorbance}}\right)\ x\ Dilution\ Factor \text{ x Volume of sample}$$

Ferric Reducing Antioxidant Power (FRAP) Assay of *Solanum nigrum* Leaf extract

The FRAP assay measures the reducing ability of antioxidants through a redox-linked colorimetric reaction, using an easily reduced oxidant system present in stoichiometric excess. Sample extract (100 µL) was mixed with 3 mL of freshly prepared FRAP reagent, and the absorbance was measured at 593 nm immediately after vortexing (0 min). The reaction mixture was then incubated at 37 °C for 4 min, after which the absorbance was measured again. Ascorbic acid standard solutions (100–1000 µM) were treated under the same conditions to generate a calibration curve by plotting absorbance against concentration. The antioxidant capacity of the extracts was determined from the standard curve and expressed relative to ascorbic acid equivalents.

$$\text{Antioxidant capacity} = (\text{absorbance of sample} - \text{absorbance of blank})x\ \frac{Dilution\ factor\ \text{x } 100}{\text{absorbance of standard}}$$

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay of *Solanum nigrum* Leaf extract

Sample stock solutions (1.0 mg/mL) were diluted to final concentrations of 250, 500, 750, and 1000 µg/mL, in ethanol. One mL of a 0.3 mM DPPH ethanol solution was added to 2.5 mL

of sample solutions of different concentrations, and allowed to react at room temperature. After 30 min, the absorbance values were measured at 518 nm and converted into the percentage antioxidant activity (AA) using the following formula:

$$\text{DPPH (\%)} = \frac{(Ao - A1)}{Ao} x\ 100$$

Where A_o is the absorbance of the control
A_1 = is the absorbance of the sample

Ethanol (1.0 mL) plus plant extract solution (2.5 mL) was used as a blank. DPPH solution (1.0 mL; 0.3 mM) plus ethanol (2.5 mL) was used as a negative control. Ascorbic acid was used as the positive control at concentrations of 250, 500, 750, and 1000 µg/mL and was treated under the same experimental conditions as the extracts.

All values and measurements of the antioxidant analysis were done in triplicates.

Results and Discussion

Percentage Yield of the Extracts

a. % yield for ethyl acetate extract $\frac{83}{120} x\ 100 = 69\%$
b. % yield for n-hexane extract $\frac{68}{120} x\ 100 = 56.67\%$

The leave sample was dried for 14 days under ambient temperature in the laboratory during raining season.

Qualitative Phytochemical Screening

Table 1. Phytochemical screening of *Solanum nigrum* leaf extract

S/N	Test	n-hexane extract	Ethyl acetate extract
1	Carbohydrates	-	++
2	Terpenoids	+++	+++
3	Alkaloids	-	+
4	Flavonoids	-	++
5	Saponins	-	-
6	Tannins	-	-
7	Cardiac glycosides	-	+
8	Anthraquinone	-	-

Key: + present, ++ moderately present, +++ highly present, - absent

The phytochemical profile of *Solanum nigrum* leaf extracts reveals a clear solvent-dependent pattern, where the non-polar n-hexane fraction is dominated by terpenoids (+++), while the moderately polar ethyl acetate extract shows a broader spectrum of bioactive constituents, including carbohydrates (++), flavonoids (++), alkaloids (+), and cardiac glycosides (+). This suggests that ethyl acetate is more efficient in extracting polar to semi-polar phytochemicals, some of which are known contributors to antioxidant activity, particularly flavonoids and alkaloids, due to their redox properties and ability to scavenge free radicals [16]. The absence of saponins, tannins, and anthraquinones in both extracts may indicate either low abundance in the plant material or poor solubility in the selected solvents. The high terpenoid content in both extracts aligns with previous reports that *S. nigrum* is rich in terpenoid compounds with anti-inflammatory and antioxidant potentials [17]. Similarly, the presence of flavonoids and alkaloids in ethyl acetate extract supports findings by [18], who linked these compounds to enhanced antioxidant capacity in plant extracts.

Antioxidant Study

Table 2. The percentage scavenging activity of the different extracts using DPPH radicals.

Extracts	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
n-hexane	11.25± 0.03^c	17.28± 0.02^c	26.47± 0.03^c	38.88± 0.20^c
Ethyl acetate	31.81± 0.30^b	45.53± 0.01^b	68.46± 0.33^b	82.58± 0.04^b
Standard (AA)	52.94± 0.35^a	65.90± 0.02^a	79.26± 0.02^a	93.80± 0.03^a
L.S.D	0.30			
P-value	<0.0001 ****			

At P≤0.05 there was a significant difference in the antioxidant activity of the different extracts using the DPPH substrate. Values are presented as mean±standard error of means, n = 3. Ranking was done across the plants species and values with the same super script are not significant.

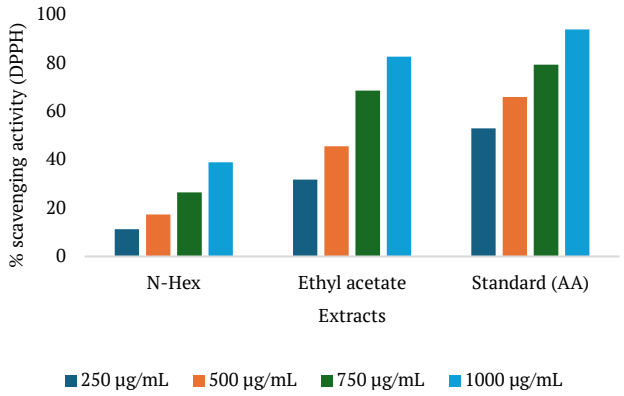


Figure 1. Percentage DPPH radical scavenging activity of n-hexane and ethyl acetate leaf extracts of *Solanum nigrum* compared with ascorbic acid (AA) at concentrations of 250, 500, 750, and 1000 µg/mL. n = 3 with values expressed as mean ± SEM

The results show a clear concentration-dependent increase in DPPH radical scavenging activity for both extracts of *Solanum nigrum*, with the ethyl acetate extract consistently exhibiting significantly higher antioxidant activity than the n-hexane extract (P≤0.05). This suggests that moderately polar solvents like ethyl acetate are more effective at extracting bioactive antioxidant compounds, likely phenolics and flavonoids, compared to non-polar n-hexane. Despite this, both extracts demonstrated lower activity than the standard ascorbic acid, indicating comparatively weaker but still notable antioxidant potential. The significant differences observed (P<0.0001) highlight the influence of solvent polarity on phytochemical yield and bioactivity. These findings align with previous reports that solvent polarity enhances the extraction of antioxidant constituents in medicinal plants [19], [20], and support studies indicating that *Solanum nigrum* possesses appreciable antioxidant properties due to its rich phytochemical composition.

Table 3. The percentage scavenging activity of the different extracts using H_2O_2 radicals.

Extracts	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
n-hexane	8.48± 0.03^c	15.05 ± 0.02^c	27.55 ± 0.30^c	40.18 ± 0.01^c
Ethyl acetate	28.35± 0.30^b	41.58 ± 0.33^b	59.56 ± 0.03^b	64.16 ± 0.20^b
Standard (AA)	46.27± 0.02^a	56.21 ± 0.03^a	75.24 ± 0.40^a	94.81 ± 0.48^a
L.S.D	0.08			
P-value	<0.0001 ****			

At P≤0.05 there was a significant difference in the antioxidant activity of the different extracts using the H2O2 substrate. Values are presented as mean ± standard error of means, n = 3. Ranking was done across the plants species and values with the same super script are not significant.

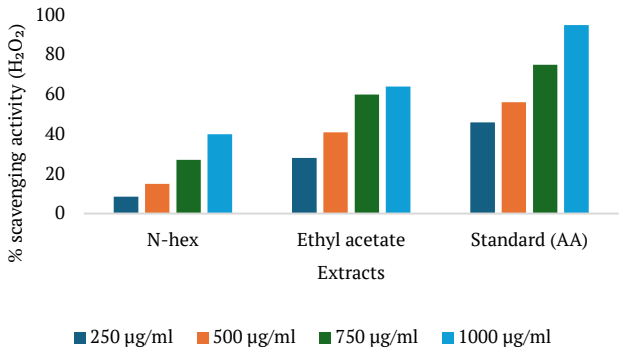


Figure 2. Percentage hydrogen peroxide (H_2O_2) scavenging activity of n-hexane and ethyl acetate leaf extracts of *Solanum nigrum* compared with ascorbic acid (AA) at concentrations of 250, 500, 750, and 1000 µg/mL. n = 3 with values expressed as mean ± SEM

From figure 2 above, the pattern of results shows that hydrogen peroxide (H_2O_2) scavenging activity increased steadily with concentration in both the n-hexane and ethyl acetate leaf extracts of *Solanum nigrum*. Notably, the ethyl acetate extract exhibited consistently stronger antioxidant activity across all tested concentrations, with the difference being statistically significant (P ≤ 0.05). This trend points to the effectiveness of moderately polar solvents in isolating antioxidant constituents, particularly phenolic and flavonoid compounds that are widely recognized for their free radical neutralizing properties.

The highly significant variation observed (P < 0.0001) reinforces the robustness of the data and highlights the critical role of solvent polarity in determining phytochemical extraction efficiency.

Table 4. The percentage scavenging activity of the different extracts using FRAP radicals.

Extracts	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
n-hexane	6.05 ± 0.02^b	12.51 ± 0.01^b	19.64 ± 0.04^c	25.28 ± 0.02^b
Ethyl acetate	23.53 ± 0.06^a	33.37 ± 0.03^b	41.36 ± 0.05^a	58.19 ± 0.01^a
tandard (AA)	31.42 ± 0.03^a	46.78 ± 0.02^a	61.55 ± 0.04^a	79.86 ± 0.03^a
L.S.D	0.10			
P-value	< 0.0001 ****			

At P≤0.05 there was a significant difference in the antioxidant activity of the different extracts using the FRAP substrate. Values are presented as mean±standard error of means. Ranking was done across the plants species and values with the same super script are not significant.

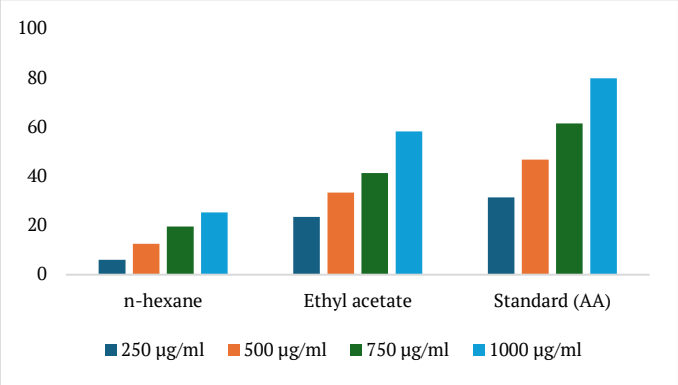


Figure 3. Ferric reducing antioxidant power (FRAP) activity of n-hexane and ethyl acetate leaf extracts of *Solanum nigrum* at different concentrations (250–1000 µg/mL). n = 3 with values are expressed as mean ± SEM.

The results in figure 3 above depicts the statistically significant difference (P ≤ 0.05) confirms that solvent polarity strongly influences antioxidant yield and efficacy. The higher FRAP radical scavenging activity observed in the ethyl acetate extract aligns with previous reports that link increased antioxidant potential to the presence of bioactive compounds capable of electron donation and free radical neutralization [21], [22].

Table 5. The comparative percentage scavenging activity (SADPPH) of the different extracts using DPPH, H2O2, and FRAP radicals

Extracts	Substrate	250 µg/ml	500 µg/ml	750 µg/ml	1000 µg/ml
n-hexane	DPPH	11.25 ± 0.03^a	17.28 ± 0.02^a	26.47 ± 0.03^b	38.88 ± 0.20^b
	H_2O_2	8.48 ± 0.03^b	15.05 ± 0.02^b	27.55 ± 0.30^a	40.18 ± 0.01^a
	FRAP	6.05 ± 0.02^c	12.51 ± 0.01^c	19.64 ± 0.04^c	25.28 ± 0.02^c
Ethyl acetate	DPPH	31.81 ± 0.30^a	45.53 ± 0.01^a	68.46 ± 0.33^a	82.58 ± 0.04^a
	H_2O_2	28.35 ± 0.30^b	41.58 ± 0.33^b	59.56 ± 0.03^b	64.16 ± 0.20^b
	FRAP	23.53 ± 0.06^c	33.37 ± 0.03^c	41.36 ± 0.05^c	58.19 ± 0.01^c
Standard (AA)	DPPH	52.94 ± 0.35^a	65.90 ± 0.02^a	79.26 ± 0.02^a	93.80 ± 0.03^b
	H_2O_2	46.27 ± 0.02^b	56.21 ± 0.03^b	75.24 ± 0.40^b	94.81 ± 0.48^a
L.S.D		0.20			
P-value		<0.0001 ****			

At P≤0.05 there was a significant difference in the antioxidant activity of the different extracts using the DPPH, H_20_2, and FRAP substrate. Values are presented as mean ± standard error of means. Ranking was done across the plants species and values with the same super script are not significant.

The results showed a clear concentration-dependent increase in antioxidant activity for both extracts of *Solanum nigrum*, with the ethyl acetate extract consistently exhibiting significantly higher scavenging activity across DPPH, H_2O_2, and FRAP assays ($P \leq 0.05$), indicating its superior capacity to donate electrons and neutralize free radicals. However, both extracts showed lower activity than the standard ascorbic acid, implying that although the plant possesses notable antioxidant potential, its efficacy is comparatively moderate. The observed trend aligns with previous studies [23], which reported higher antioxidant activity in polar or semi-polar extracts of *Solanum nigrum* due to higher phytochemical content (phenolics and tannins).

The IC_{50} values obtained in this study for the ethyl acetate extract were 549 µg/mL (DPPH), 617 µg/mL (H_2O_2), and 878 µg/mL (FRAP), whereas the n-hexane extract exhibited IC_{50} values greater than 1000 µg/mL in all assays, indicating weaker antioxidant activity. These values are higher than those reported for other *Solanum nigrum* extracts, including a methanolic fruit extract with IC_{50} values of 70.73 µg/mL (DPPH) and 59.72 µg/mL (H_2O_2), and an aqueous leaf extract with IC_{50} values of 165 µg/mL (DPPH) and 417 µg/mL (H_2O_2) [24], [25], suggesting comparatively lower antioxidant potency in the present extracts. The differences may be attributed to variations in plant part, extraction solvent, geographical origin, and phytochemical composition.

Conclusions

The study evaluated the phytochemical composition and comparative antioxidant potential of n-hexane and ethyl acetate leaf extracts of *Solanum nigrum*. The findings confirm that solvent polarity significantly influences both the type of phytochemicals extracted and the antioxidant effectiveness of the plant. Ethyl acetate proved to be a more suitable solvent for extracting bioactive compounds associated with antioxidant activity, thereby demonstrating greater potential compared to n-hexane extract. The study supports the traditional use of *Solanum nigrum* and highlights its relevance as a promising natural source of antioxidants for pharmaceutical and nutraceutical applications. However, further research is recommended to isolate and characterize the individual flavonoids from the ethyl acetate extract and testing their antioxidant activity in cell-based models.

Author Contributions

ST: Conceptualisation, Methodology, Investigation, Writing. APA: Methodology, Review and Editing. DIG: Data Curation, Formal Analysis, Review and Editing. ABF: Statistical analysis, review and editing. JJA: Plant identification, review and editing. All authors have read and approved the final version of the paper and its submission and have consented to publication.

Declaration of Generative AI and AI-Assisted Technologies in Scientific Writing

ChatGPT was used for language editing and improvement of grammatical clarity. The authors reviewed and verified all content and remain responsible for the accuracy and integrity of the manuscript

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Data Availability Statement

All relevant data are available within the manuscript

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None

Conflict of Interest

The authors declare that they have no conflicts of interest.

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