



Phytochemical Screening and Antioxidant Activity of *Piper Nigrum* and *Aframomum Melegueta* Seeds Extracts

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ABSTRACT

Natural products remain a vital source of bioactive compounds with therapeutic potential, particularly in the management of oxidative stress-related diseases. This study evaluated the phytochemical composition and antioxidant activity of seed extracts of *Piper nigrum* and *Aframomum melegueta*. Dried and pulverized seeds were subjected to successive solvent extraction using *n*-hexane, ethyl acetate, and methanol. Qualitative phytochemical screening, such as tests for flavonoids, tannins, saponins, reducing sugars, alkaloids, anthraquinones, and steroids on the extracts, was conducted using standard methods, and antioxidant activity was assessed by the DPPH free radical scavenging assay, with ascorbic acid as the reference standard. Both plant extracts exhibited appreciable yields: *P. nigrum* (4.26 %) and *A. melegueta* (4.57 %), and contained diverse phytochemicals, including flavonoids, alkaloids, tannins, steroids, and anthraquinones. Antioxidant analysis revealed concentration-dependent radical scavenging activity across all fractions. *A. melegueta* demonstrated superior antioxidant activity, with IC₅₀ values (0.4224-0.4398 mg/mL) comparable to ascorbic acid (0.3656 mg/mL), whereas *P. nigrum* showed moderate activity (IC₅₀: 0.5766 - 1.0276 mg/mL). These findings indicate that both plants are rich in bioactive compounds with significant antioxidant potential, with *A. melegueta* exhibiting greater efficacy. The study supports their traditional medicinal use in treating oxidative diseases and highlights their potential as natural antioxidant sources for pharmaceutical and nutraceutical applications.

Keywords: *Piper Nigrum*; *Aframomum melegueta*; Qualitative phytochemical screening; DPPH free radical scavenging assay

INTRODUCTION

Natural products remain an important source of bioactive compounds for pharmaceutical drug discovery and development (Chaachouay and Zidane, 2024). Although considerable progress has been made in phytochemical research, many medicinal plants remain insufficiently investigated, highlighting the need to explore their chemical constituents and biological activities (Nwozo et al., 2023; El-Saadony, et al., 2025). The increasing burden of antimicrobial resistance and the high cost of conventional therapies have further intensified the search for safe and effective plant-derived alternatives (Halder and Jha, 2023; Chapman, 1997). Phytochemical screening plays a vital role in identifying therapeutically valuable secondary metabolites, including alkaloids, flavonoids, phenolics, tannins, saponins, steroids, and terpenoids, many of which exhibit diverse pharmacological activities (Agidew, 2022; Shahrajabian et al., 2023).

Piper nigrum (black pepper) and *Aframomum melegueta* (alligator pepper) are widely used medicinal spices with reported antimicrobial, anti-inflammatory, digestive, and antioxidant properties (Bennett & Inengite, 2022; Parthasarathy et al., 2024). Their phytochemical constituents have attracted growing interest because of their potential therapeutic applications.

Natural antioxidants have received increasing attention due to their ability to neutralize reactive oxygen species and reduce oxidative stress, thereby lowering the risk of chronic diseases such as cardiovascular disorders and cancer (Herberg et al., 1998; Goyal et al., 2025). The antioxidant potential of plant extracts is commonly evaluated using assays such as DPPH

and ORAC (Cao et al., 1993; Gulcin & Alwasel, 2023). Despite the widespread traditional use of *Piper nigrum* and *Aframomum melegueta* seeds, scientific evidence supporting their antioxidant potential remains limited. Therefore, this study aimed to evaluate the phytochemical composition and antioxidant activity of these seeds.

MATERIALS AND METHODS

Plant Material Collection and Identification

Seeds of *Piper nigrum* and *Aframomum melegueta* were collected from Lokoja, Kogi State, Nigeria, in August 2022. The plant materials were identified at the Department of Biological Sciences, Federal University Lokoja.

Extraction Procedure

The collected seeds were air-dried and pulverized into a coarse powder. A total of 400 g of *Piper nigrum* and 500 g of *A. melegueta* seeds were powdered and subjected differently to successive cold maceration at room temperature using *n*-hexane, ethyl acetate, and methanol (0.5 L each) for 24 h per solvent. The resulting extracts were filtered and concentrated under reduced pressure using a rotary evaporator. The concentrates were subsequently combined and dried to obtain the crude extracts.

Phytochemical Screening

Qualitative phytochemical analyses of the extracts were carried out using standard procedures:

Saponins: Approximately 0.5 g of the extracts was vigorously shaken with distilled water. Persistent frothing indicated the presence of saponins (Halilu et al., 2024).

Alkaloids: The extracts (0.5 g) were treated with 5 mL of 1% aqueous hydrochloric acid and heated on a steam bath. The filtrate (1 mL) was then treated with Dragendorff's reagent. Formation of a precipitate indicated the presence of alkaloids (Sheel *et al.*, 2014).

Steroids: The extracts (0.5 g) were dissolved in 2 mL of chloroform, followed by careful addition of concentrated sulfuric acid. A reddish-brown coloration at the interface indicated the presence of steroids (De *et al.*, 2010).

Tannins: The extracts (0.5 g) were stirred with 10 mL of distilled water, warmed, and filtered. The filtrate was treated with ferric chloride solution. Formation of a blue-black precipitate confirmed the presence of tannins (Usman *et al.*, 2008).

Reducing Sugars: A portion of the extracts was dissolved in distilled water and heated with Fehling's solutions A and B. A brick-red precipitate indicated the presence of reducing sugars (Karu *et al.*, 2019).

Anthraquinones: The extracts (2 g) were shaken with 5 mL of benzene and filtered. To the filtrate, 10 mL of aqueous sulfuric acid was added, and the mixture was shaken. The appearance of pink, red, or violet coloration indicated the presence of anthraquinones (Manonmani *et al.*, 2026).

Flavonoids: A portion of the aqueous filtrate was treated with 5 mL of dilute ammonia solution, followed by concentrated sulfuric acid. The development of a yellow coloration, which may fade upon standing, indicated the presence of flavonoids (Shah *et al.*, 2015).

Determination of Antioxidant Activity

The antioxidant activity of the extracts was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay. A freshly prepared DPPH solution (100 ppm) was obtained by dissolving 3.94 mg of DPPH in 100 mL of methanol. A stock solution of the extract (1 mg/mL) was prepared by dissolving 3.0 mg of crude extract in 3 mL of methanol. From this stock, serial dilutions were prepared to obtain concentrations of 1.0, 0.5, 0.25, 0.125, and 0.0625 mg/mL. For the assay, 3.0 mL of DPPH solution was added

to 0.5 mL of each extract concentration. The mixtures were shaken and allowed to stand in the dark for 10 min. Absorbance was measured at 517 nm using a spectrophotometer. The percentage inhibition of the DPPH radical was calculated by comparing the sample absorbance with that of the control. Ascorbic acid was used as the reference standard as the same concentration (Okpala *et al.*, 2022).

Statistical Analysis

The findings of the antioxidant experiments were analyzed in Microsoft Excel. By comparing % inhibition to concentration, nonlinear regression was used to determine IC₅₀ values.

RESULTS AND DISCUSSION

Extraction Yield

The extraction yields of the crude extracts and solvent-partitioned fractions obtained from the seeds of *Piper nigrum* and *Aframomum melegueta* are presented in Table 1. From 400 g of *P. nigrum* seed powder, 17.03 g of crude extract was obtained, corresponding to a percentage yield of 4.26%. Similarly, 500 g of *A. melegueta* seeds yielded 22.87 g of crude extract, corresponding to a 4.57% yield. Subsequent solvent fractionation revealed distinct distribution patterns of phytoconstituents across solvents of varying polarity. In *P. nigrum*, the methanol fraction accounted for the highest proportion (10.52 g), followed by ethyl acetate (3.92 g) and hexane (2.59 g), suggesting a predominance of polar constituents. Conversely, *A. melegueta* exhibited a relatively higher yield in the hexane fraction (8.46 g), indicating a substantial presence of non-polar compounds, alongside appreciable quantities in the ethyl acetate (5.96 g) and methanol (8.45 g) fractions. These variations in yield and fraction distribution reflect differences in the chemical composition of the two plant species and highlight the influence of solvent polarity on extraction efficiency. The relatively comparable overall yields suggest that both plants are viable sources of bioactive compounds.

Table 1: The Extraction Results from the Seed Extracts of *P. Nigrum* and *A. Melegueta*

Plant Sample and parts	Sample Weight (g)	Extract Weight (g)	% yield	Hexane Fraction (g)	EtOAc Fraction (g)	MeOH Fraction (g)
<i>P. nigrum</i> seed.	400	17.03	4.26	2.59	3.92	10.52
<i>A. melegueta</i> seed	500	22.87	4.57	8.46	5.96	8.45

Phytochemical Composition

The qualitative phytochemical screening results for the different solvent fractions of *P. nigrum* and *A. melegueta* seeds are summarized in Tables 2 and 3. The analysis revealed a diverse array of secondary metabolites, including flavonoids, tannins, saponins, alkaloids, steroids, anthraquinones, and reducing sugars. In *P. nigrum*, flavonoids and alkaloids were consistently present across all fractions, indicating their broad solubility and abundance. Steroids were prominent in the ethyl acetate and hexane fractions, while tannins and anthraquinones were primarily detected in the methanol fraction, suggesting their polar nature. Saponins were detected only in the hexane fraction, albeit at low intensity.

For *A. melegueta*, a broader spectrum of phytochemicals was observed across all fractions. Alkaloids, steroids, and

anthraquinones were consistently present, while tannins and reducing sugars were detected in all solvent fractions. Notably, flavonoids were predominantly present in the methanol fraction, indicating their affinity for polar solvents. The hexane fraction was unique in containing saponins, reflecting the plant's complex phytochemical profile. The presence of flavonoids in both plant species is particularly significant, given their well-documented antioxidant and anti-inflammatory properties. Previous studies have demonstrated that flavonoids can mitigate oxidative stress and associated tissue damage. Their occurrence in these extracts may therefore contribute to the observed biological activities and support the traditional medicinal use of these plants. Additionally, the coexistence of multiple bioactive compounds suggests possible synergistic interactions that enhance therapeutic efficacy.

Table 2: Phytochemical Screening of *P. Nigrum* Seed Extract

Phytochemicals	MeOH	EtOAc	Hexane
Flavonoids	+++	+++	+++
Tannins	+	-	-
Saponin	-	-	+
Reducing sugar	-	-	-
Alkaloids	+++	+++	+++
Anthraquinones	+	-	-
Steroids	-	+++	+++

+ = present; +++ = strongly present; - = absent; MeOH = methanol; EtOAc = ethyl acetate

Table 3: Phytochemical Screening on *A. Melegueta* Seeds' Extract

Phytochemicals	MeOH	EtOAc	Hexane
Flavonoids	+	-	-
Tannins	+	+	+
Saponin	-	-	+
Reducing sugar	+	+	+
Alkaloids	+++	+++	+++
Anthraquinones	+++	+	+
Steroids	+++	+++	+++

+ = present; +++ = strongly present; - = absent; MeOH = methanol; EtOAc = ethyl acetate

Antioxidant Activity

The antioxidant activities of the solvent fractions of *P. nigrum* and *A. melegueta* seeds were evaluated using a free radical scavenging assay, with ascorbic acid serving as the reference standard. The percentage inhibition values at varying concentrations and the corresponding IC₅₀ values are presented in Tables 4 and 5. For *P. nigrum*, all fractions exhibited concentration-dependent antioxidant activity. The hexane fraction demonstrated the strongest activity (IC₅₀ = 0.5766 mg/mL), followed closely by the methanol fraction (IC₅₀ = 0.6489 mg/mL), while the ethyl acetate fraction showed comparatively weaker activity (IC₅₀ = 1.0276 mg/mL). Despite this, none of the fractions matched the potency of ascorbic acid (IC₅₀ = 0.3656 mg/mL). The relatively higher activity of the methanol and hexane fractions may be attributed to the presence of flavonoids and alkaloids, which are known to contribute significantly to radical

scavenging mechanisms. In contrast, *A. melegueta* fractions exhibited notably strong antioxidant activity across all solvent systems. The IC₅₀ values for methanol, ethyl acetate, and hexane fractions were 0.4330, 0.4398, and 0.4224 mg/mL, respectively, indicating comparable efficacy among the fractions and close proximity to the standard ascorbic acid. This suggests that antioxidant compounds in *A. melegueta* are distributed across both polar and non-polar fractions. The superior antioxidant performance of *A. melegueta* may be linked to its richer and more diverse phytochemical composition, particularly the presence of flavonoids, tannins, and phenolic compounds, which are known to act as hydrogen donors and free radical scavengers. The consistent activity across fractions also implies that multiple classes of compounds contribute synergistically to its antioxidant potential.

Table 4: Antioxidant Activity of Fractions of *P. Nigrum*

Concentration (mg/mL)	% Inhibition Methanol	Ethyl Acetate	n-Hexane	Ascorbic Acid
1.0	62.45	37.63	66.54	98.98
0.5	50.77	34.85	59.48	97.26
0.25	41.25	26.77	51.24	86.71
0.125	33.54	19.08	38.32	75.84
0.0625	21.07	10.87	25.99	54.42
IC ₅₀	0.6489	1.0276	0.5766	0.3656

Table 5: Antioxidant Activity of Fractions of *A. Melegueta*

Concentration (mg/mL)	% InhibitionMethanol	Ethyl acetate	n-Hexane	Ascorbic Acid
1.0	86.38	89.77	91.77	98.98
0.5	78.53	72.07	79.07	97.26
0.25	69.75	60.64	62.64	86.71
0.125	61.51	57.82	59.02	75.84
0.0625	48.71	52.11	53.11	54.42
IC ₅₀	0.4330	0.4398	0.4224	0.3656

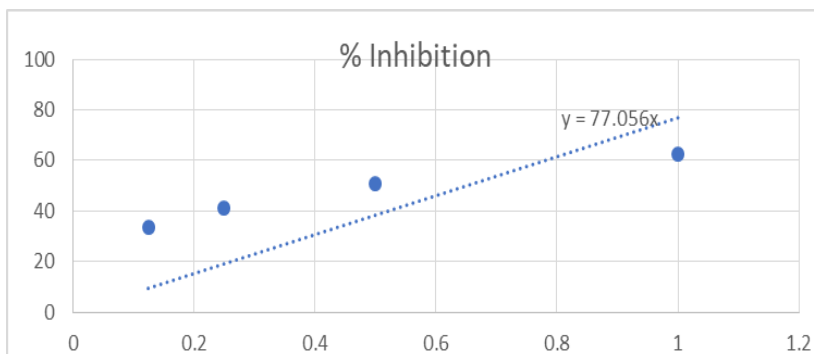


Figure 1: Graph of the IC₅₀ of the methanol fraction of *Piper nigrum* seed

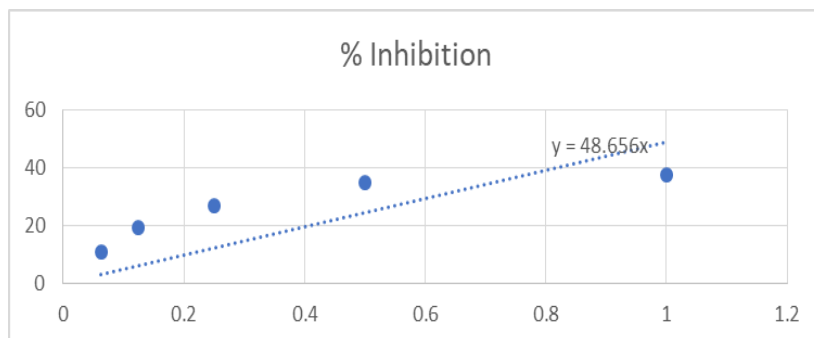


Figure 2: Graph of the IC₅₀ of the ethylacetate fraction of *Piper nigrum* seed

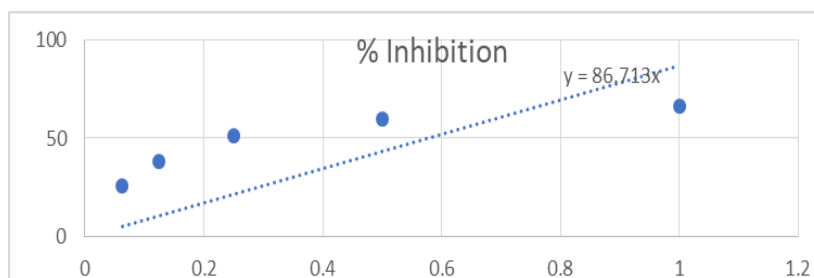


Figure 3: Graph of the IC₅₀ of the hexane fraction of *Piper nigrum* seed

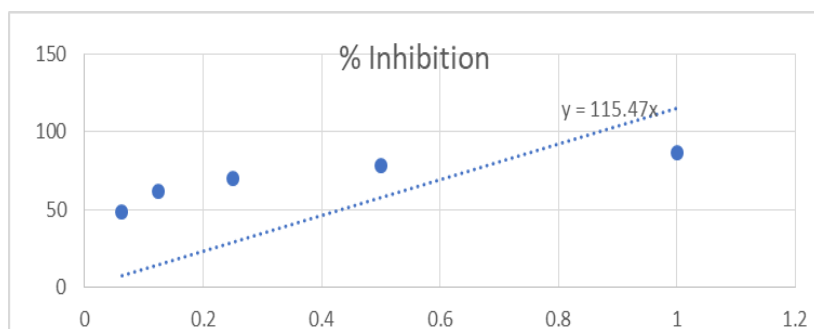


Figure 4: Graph of the IC₅₀ of the methanol fraction of *A. melegueta*

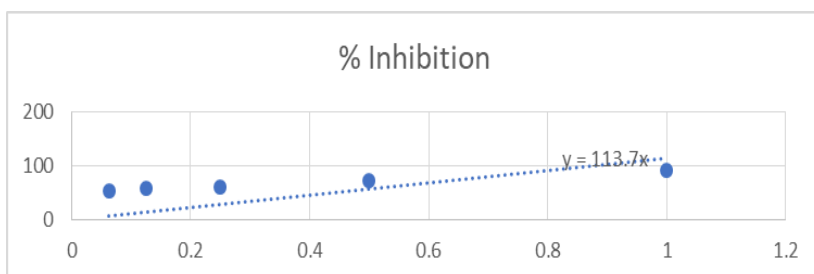
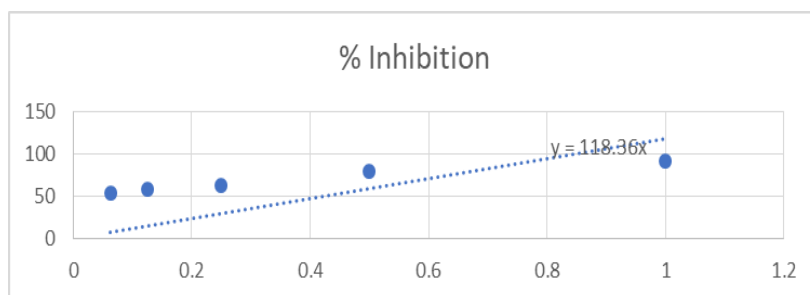
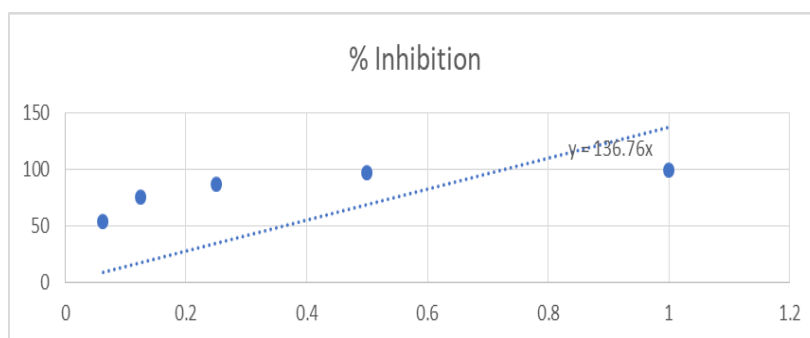


Figure 5: Graph of the IC₅₀ of the ethylacetate fraction of *A. melegueta*

Figure 6: Graph of the IC₅₀ of the Hexane fraction of *A. melegueta*Figure 7: Graph of the IC₅₀ of Ascorbic acid

Overall, the results demonstrate that both *P. nigrum* and *A. melegueta* seeds are rich sources of bioactive phytochemicals with significant antioxidant properties. However, *A. melegueta* exhibited comparatively higher and more consistent antioxidant activity across all fractions, suggesting a broader distribution of active compounds. The observed antioxidant activities correlate well with the phytochemical profiles, particularly the presence of flavonoids, tannins, and alkaloids. These compounds are known to neutralize free radicals, chelate metal ions, and inhibit oxidative processes, thereby contributing to their therapeutic potential. The findings support the potential application of these plant extracts as natural antioxidants in pharmaceutical and nutraceutical formulations. Furthermore, the results provide a scientific basis for their traditional use in managing oxidative stress-related conditions. Future studies focusing on isolation, characterization, and mechanistic evaluation of the active constituents are recommended to further validate their pharmacological relevance.

CONCLUSION

This study confirms that *P. nigrum* and *A. melegueta* seeds are valuable sources of bioactive compounds with notable antioxidant properties. While both plants produced comparable extraction yields, *A. melegueta* showed a slightly higher yield and a wider distribution of phytochemicals across solvent fractions. Phytochemical analysis revealed the presence of important secondary metabolites, including flavonoids, alkaloids, tannins, and steroids, which are known to contribute to antioxidant activity. The antioxidant assay demonstrated that all extracts exhibited dose-dependent radical-scavenging activity, with *A. melegueta* fractions showing stronger and more consistent activity, approaching that of ascorbic acid. Overall, the findings highlight the greater antioxidant potential of *A. melegueta* and support the medicinal relevance of both plants. These results suggest their suitability as natural antioxidant sources, with potential applications in pharmaceutical and nutraceutical development. This study is limited by the absence of quantitative estimation of total phenolics and flavonoids, and

isolation of active compounds is required to confirm these findings.

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