



Comparative Antioxidant Properties of Crude Leaf and Stalk Extracts of *Ocimum Gratissimum* L. (Scent Leaf) Cultivated in Jos, Nigeria

*¹Chukuka Achuenu, ¹Simon Tanko, ²Susona Joshua Ngullah,
³Silas Johnson Ngandi and ¹Rowland Tuamyen Hoomkwap



¹Department of Chemistry, University of Jos, PMB 2084, Jos, Nigeria.

²Department of Chemistry, College of Education, Gindiri, Plateau State, Nigeria.

³Department of Chemical Sciences, Taraba State University, Jalingo, Nigeria.

*Corresponding Author's email: achuenuc@unijos.edu.ng

ORCID iD: <https://orcid.org/0000-0002-5686-679X>

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ABSTRACT

Ocimum gratissimum is commonly found in different communities across Africa and Asia. In Nigeria, the plant is usually incorporated into menus in different cultures. Studies have shown that the plant possesses high antioxidant properties. However, both researchers and consumers have paid little attention to the stalk, with more focuses targeting the leaf. This study investigated the comparative antioxidant properties of crude extracts of the leaf and stalk of *O. gratissimum* cultivated in Jos, Nigeria. The leaves and stalks were separately extracted with various solvents using maceration. The antioxidant activities of the extracts were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging and ferric reducing antioxidant power (FRAP) assays in different concentrations (6.25–100 µg/mL). The results showed that antioxidant activities were concentration-dependent with various extracts of both plant parts. The DPPH activity demonstrated significantly high percentage inhibitions with the various extracts explored. The ethanol extract (up to 97.16 %) and ethanol extract (95.58 %) of the leaf provided remarkable antioxidant properties in analogy to the ascorbic acid(standard). Similarly, the stalk ethanol extract (up to 97.02 %) and methanol extract (96.91 %) exhibited very significant inhibition. The results of the FRAP assay for the leaf and stalk extracts are consistent with those of the DPPH assay as evidenced by the high percentage reducing power obtained from the various extracts. The FRAP results of leaf showed that the ethanol extract gave the highest reducing activity across all concentrations (up to 92.30 %). From the stalk's FRAP results, ethanol extract (up to 92.17 %) and methanol extract (up to 92.07 %), exhibited the highest reducing powers. Although leaf extracts demonstrated the highest antioxidant properties in all cases, the study suggests that the stalk possesses remarkable antioxidant properties as well. Further study is required to comparatively explore the toxicology of both plant's parts.

CITATION

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INTRODUCTION

Antioxidants are an important group of bioactive compounds that protect biological systems against the harmful effects of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are continuously generated during normal cellular metabolism and in response to environmental stressors (Kiran *et al.*, 2023). Although these reactive species play important physiological roles in cellular signaling and host defense, their excessive production can overwhelm the body's antioxidant defense mechanisms and result in oxidative damage to cellular components such as lipids, proteins and nucleic acids (Jomova *et al.*, 2023). Oxidative stress, which arises from an imbalance between the production of reactive species and the capacity of antioxidant defense systems to neutralize them, has been associated with cellular dysfunction, tissue damage and the development or progression of several chronic diseases, including cardiovascular disorders, neurodegenerative diseases, diabetes and certain forms of cancer (Sharifi-Rad *et al.*, 2020; Kiran *et al.*, 2023). Consequently, increasing attention has been focused on the identification and utilization of naturally occurring antioxidants, particularly those obtained from medicinal and edible plants. Plant-derived antioxidants are of considerable interest because they contain diverse phytochemicals capable of donating electrons or hydrogen atoms, chelating metal ions and neutralizing free radicals. This has encouraged continued research into medicinal plants as potential sources of safer and more accessible antioxidant agents for nutritional, pharmaceutical and other health-related applications.

O. gratissimum L., commonly known as scent leaf (Olaniyi-Olushola & Udourioh, 2026), is an aromatic medicinal plant that is widely distributed and traditionally utilized in Nigeria and other parts of Africa for the management of various health conditions or used for foods. The plant is valued for its culinary, medicinal and ethnobotanical importance and has been reported to contain a wide range of biologically active constituents, including phenolics, flavonoids, tannins, terpenoids and other secondary metabolites that may contribute to its pharmacological

properties and antioxidant activity (Maphetu *et al.*, 2026; Olaniyi-Olushola & Udourioh, 2026). Phenolic compounds and flavonoids are particularly important because of their ability to scavenge free radicals and reduce oxidative reactions, while other phytochemical constituents may contribute synergistically to the overall biological activity of the plant. Several investigations have demonstrated that extracts of *O. gratissimum* possess appreciable free-radical-scavenging and reducing activities against DPPH and other reactive species, suggesting that the plant may serve as a valuable source of natural antioxidant compounds (Akintunde *et al.*, 2025). The antioxidant potential of the plant is also of interest because its bioactive constituents may provide protection against oxidative processes implicated in various physiological and pathological conditions. Furthermore, a recent study has reported the analgesic activity and phytochemical properties of *O. gratissimum* leaves cultivated in Jos (Achuenu *et al.*, 2026), providing additional evidence of the biological activity of the locally cultivated plant and suggesting that environmental conditions in Jos may influence the accumulation of its bioactive constituents positively.

However, most previous investigations have focused mainly on the leaves, with limited comparative information on the antioxidant potential of the stalk to our knowledge, despite the possibility that different plant parts may contain varying concentrations and compositions of bioactive compounds. This knowledge gap limits effective utilization of the plant and leads to underutilization of the stalk as a source of natural antioxidants. Therefore, comparative investigation of the crude extracts of the leaf and stalk of *O. gratissimum* cultivated in Jos is necessary to possibly establish their antioxidant properties and determine the plant part with greater potential. The findings may provide scientific data on the full utilization of the locally cultivated *O. gratissimum* and encourage researchers to explore every part of the plant for pharmacological applications, and food-related developments. Plate 1 demonstrates the fresh leaves and stalks of *O. gratissimum* grown in Jos.



Plate 1: *Ocimum gratissimum* leaf and stalk

MATERIALS AND METHODS

Sample Collection and Preparation

Plant Material Collection and Preparation

Fresh leaves and stalks of *Ocimum gratissimum* were obtained from local farms in Jos, Plateau State, Nigeria. The plant was authenticated by a taxonomist at the Herbarium unit, Federal College of Forestry Jos, with a voucher number of FHJ-847. Fresh leaves and stalks of *Ocimum gratissimum* were carefully washed with clean water to remove dirt and air-dried at room temperature for two weeks. The dried samples were ground into coarse powder using a mortar and pestle, then stored in airtight containers until extraction.

Samples Extraction

The extraction of the powdered leaf and stalk samples was carried out using maceration method. A 100 g of each powdered sample was separately immersed in 500 mL of ethanol, methanol, chloroform, and ethyl acetate. The mixtures were allowed to stand for 72 hours with intermittent shaking. The extracts were filtered with Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at 40 °C. The crude extracts obtained were preserved in amber bottles at 4 °C until employed for analysis.

Antioxidant Assay

Invitro Antioxidant Activity Assay using DPPH as substrate

The effect of fractions on DPPH radical was estimated using the method of Liyana-Pathiranan & Shahidi, (2005). A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of this was mixed with 1.0 mL of different concentrations (100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL and 6.25mg/mL) of the different fractions in methanol in the ratio of 1:1(v/v). The reaction mixture was vortexed thoroughly and left in the dark at room temperature 27° C for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Ascorbic acid was used as reference. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{Scavenging activity (\%)} = \frac{\text{Absorbance of blank} - \text{Absorbance of samples}}{\text{Absorbance of blank}} \times 100$$

Invitro Antioxidant Activity Assay using FRAP as substrate

The reducing power of different plant extracts was assessed using the Ferric Reducing Antioxidant Power (FRAP) assay as outlined by Benzie & Strain (1996) with slight modifications. The FRAP reagent was freshly

prepared by mixing acetate buffer (300 mM, pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl, and 20 mM FeCl₃•6H₂O in the ratio of 10:1:1 (v/v/v), followed by pre-warming to 37 °C. For the assay, 100 µL of each plant extract at different concentrations was added to 2.9 mL of FRAP reagent, incubated at 37 °C for 30 minutes, and the absorbance was recorded at 593 nm using a UV-Vis spectrophotometer against a reagent blank. The percentage reducing power (%RP) of each extract was calculated relative to a standard (FeSO₄•7H₂O calibration curve, 100–1000 µM) using the following formula:

$$\%RP = \frac{A_{\text{sample}}}{A_{\text{standard}}} \times 100$$

where A_{sample} is the absorbance of the plant extract and A_{standard} is the absorbance corresponding to an equivalent concentration of the Fe²⁺ standard. Results were expressed as percentage reducing power of the extracts, which reflects their electron-donating ability and overall antioxidant capacity.

Data Analysis

All experiments were performed in triplicate, and the data were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) on a Microsoft excel, followed by Duncan's multiple range test. A probability level of $p < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidant Activity with DPPH Scavenging Radicals on the Leaf Extracts

Table 1 and figure 1 demonstrate that the leaf various extracts of the plant exhibited significant DPPH scavenging percentage inhibition (up to 97 %) in analogy to the standard (98 %). The DPPH radical scavenging activity showed concentration-dependent as evidenced by the highest inhibition observed with high concentrations (Table 1, Figure 1). Ethanol extract showed the highest activity (97.16% at 100 µg/mL), followed by methanol (95.58%), ethyl acetate (86.98%) and chloroform (86.11%). The high percentage activity of the ethanol and methanol extracts may be attributed to their efficiency in the extraction of polar antioxidant compounds such as phenolics, flavonoids and others due to their relatively high polarity. The result is consistent with recent reports on concentration-dependent antioxidant activity in the leaf extracts of the plant (Ike *et al.*, 2024; Akintunde *et al.*, 2025). In a similar study, Kabubii *et al.* (2023) demonstrated an interesting scavenging activity in methanol extracts. Whereby the team observed increased DPPH percentage inhibitions as the concentrations of the extracts increased.

Table 1: The percentage scavenging activity of different solvents of the leaf extracts on DPPH radicals

Extract	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Chloroform	54.07±0.13 ^e	61.51±0.09 ^d	70.53±0.11 ^d	81.75±0.13 ^c	86.11±0.12 ^c
Ethyl acetate	57.65±0.13 ^d	58.88±0.15 ^e	60.04±0.09 ^e	79.75±0.13 ^d	86.98±0.15 ^b
Ethanol	81.47±0.11 ^b	85.30±0.20 ^b	91.54±0.13 ^c	95.40±0.13 ^b	97.16±0.11 ^d
Methanol	63.96±0.19 ^c	77.40±0.09 ^c	94.53±0.11 ^b	95.12±0.13 ^b	95.58±0.11 ^e
Ascorbic Acid	98.03±0.05 ^a	98.58±0.08 ^a	98.43±0.08 ^a	98.47±0.08 ^a	98.66±0.03 ^a
P-Value	<0.0001				

At $P \leq 0.05$, there was a significant difference in the percentage scavenging activity (SA_{DPPH}) of different concentrations of leaf extracts on DPPH radicals. Values are presented as mean±standard error of means. Ranking was done across the extracts, and values with the same superscript are not significant.

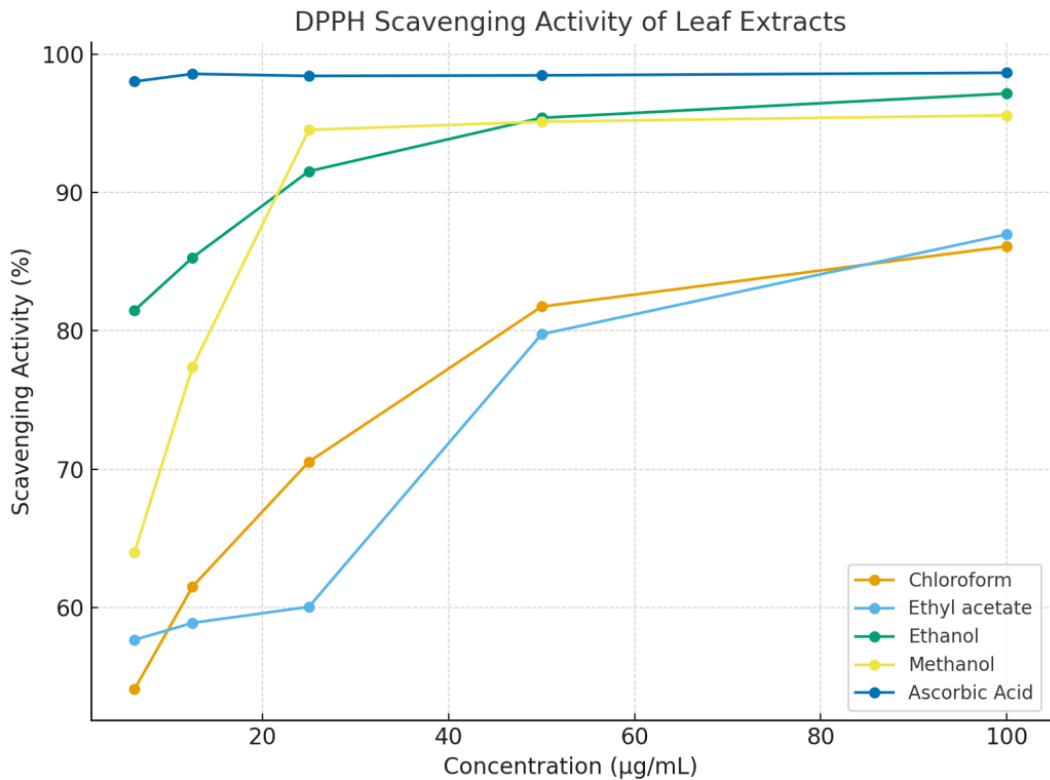


Figure 1: DPPH % scavenging activity of leaf extract of different solvents

Antioxidant Activity with DPPH Scavenging Radicals on the Stalk Extracts

The DPPH radical-scavenging assay (Table 2, Figure 2) revealed the highest percentage inhibitions in all cases at 100 µg/mL. Remarkably, there was a concentration-dependent increase in the antioxidant activity of the stalk extracts similar to the results obtained with the leaf extracts. And the extracts also exhibited differences in antioxidant activity (Figure 2). Chloroform extract exhibited the lowest percentage inhibitions ($51.40 \pm 0.15\%$ at 6.25 µg/mL to $61.54 \pm 0.13\%$ at 100 µg/mL) in all concentrations, which could be attributed to the chloroform’s relatively lowest polarity than the other three solvents. The extracts of ethyl acetate, ethanol and methanol demonstrated the highest percentage inhibitions providing $83.44 \pm 0.13\%$, $97.02 \pm 0.09\%$ and

$96.91 \pm 0.09\%$, respectively, at 100 µg/mL (Table 2). The significantly high activities obtained with the more polar solvent’s extracts could be associated to their efficiency to rip-off phenolic compounds and other antioxidant phytochemicals from plant materials. The obtained result is in agreement with a recent report on *O. gratissimum* of possessing a wide range of secondary metabolites capable of donating hydrogen or electrons to stabilize free radicals (Maphetu *et al.*, 2026). It is noteworthy that the percentage inhibitions of both ethanol extract (97.02% and methanol extract (96.91 %) are only slightly lower than that of the ascorbic acid (98.66 %) at high concentrations (100 µg/mL). The results therefore demonstrate that the stalks of *O. gratissimum* cultivated in Jos possess considerable antioxidant potentials as much as that of the leaves and all parts of the plant contain very valuable bioactive compounds.

Table 2: The Percentage Scavenging activity (SA_{DPPH}) of different Concentrations of Stalk Extracts on DPPH radicals

Extract	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Chloroform	51.40±0.15 ^c	51.51±0.18 ^d	58.25±0.44 ^a	59.23±0.41 ^e	61.54±0.13 ^d
Ethyl acetate	60.07±0.13 ^b	65.72±0.15 ^c	71.05±0.11 ^d	76.63±0.12 ^d	83.44±0.13 ^c
Ethanol	61.65±0.53 ^b	72.81±0.07 ^b	88.88±0.15 ^b	93.19±0.09 ^c	97.02±0.09 ^b
Methanol	60.21±0.12 ^b	72.18±0.13 ^b	82.25±0.09 ^c	94.49±0.13 ^b	96.91±0.09 ^b
Ascorbic Acid	98.03±0.05 ^a	98.58±0.08 ^a	98.43±0.08 ^a	98.47±0.08 ^a	98.66±0.03 ^a
P-Value	<0.0001				

At P≤0.05 there was a significant difference in the percentage scavenging activity (SA_{DPPH}) of different concentrations of stalk extracts on DPPH radicals. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

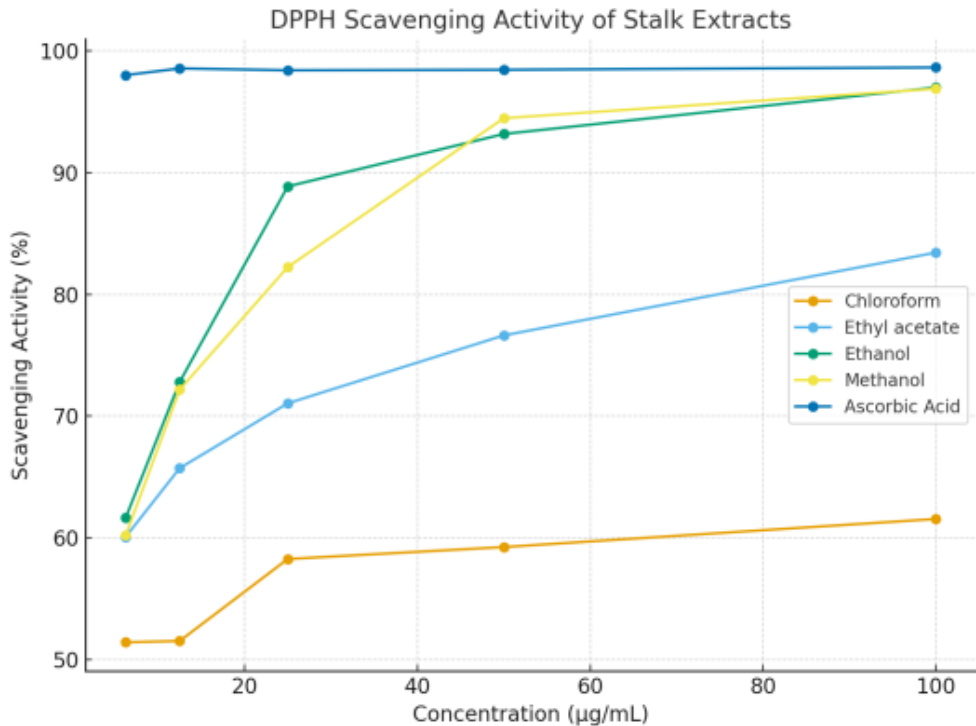


Figure 2: DPPH scavenging activity of stalk extract

Antioxidant Activity with FRAP Percentage Reducing Power Model of the Leaf Extracts

The FRAP assay results (Table 3, Figure 3) demonstrate that the leaf extracts of the plant exhibited appreciable and concentration-dependent ferric reducing power. The result shows a progressive increase in reducing power with increasing concentration. Ethanol extract provided the highest reducing activity across all concentrations (up to 92.30 %) followed by methanol extract (up to 90.80%), while chloroform extract (up to 81.80 %) and ethyl acetate (82.62 %) generally exhibited lower activities; their percentage reducing powers are, however, significantly high. The strong electron-donating compounds in the extracts act by effectively donating electrons to reduce Fe³⁺ to Fe²⁺, thereby demonstrating antioxidant properties. The superior activity of the ethanol extract and methanol

extract may be associated with the solvents' ability to efficiently extract polar antioxidant compounds, particularly phenolic compounds and flavonoids. The obtained result is consistent with prior literature that demonstrated a great exhibition of reducing power with methanol extract (Ikokide *et al.*, 2025). In another report, Ibeabuchi *et al.* (2023) reported that the reducing power of *O. gratissimum* leaf extracts increased with increasing concentration, supporting the concentration-dependent pattern observed in the present study. This study is also consistent with the report of Chatepa *et al.* (2024), which demonstrated concentration-dependent ferric reducing power activity of *O. gratissimum* leaf extract. Interestingly, the FRAP result of the leaf extract is in agreement with that of the DPPH, confirming the strong antioxidant property of the plant.

Table 3: The Percentage Reducing Power (RP_{FRAP}) of different Concentrations of Leaf Extracts on FRAP radicals

Extract	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Chloroform	51.37±0.12 ^d	58.43±0.09 ^c	67.00±0.10 ^c	77.67±0.09 ^b	81.80±0.12 ^d
Ethyl acetate	54.77±0.12 ^c	55.93±0.15 ^d	57.03±0.09 ^d	75.77±0.12 ^c	82.63±0.15 ^c
Ethanol	77.40±0.10 ^a	81.10±0.15 ^a	86.97±0.12 ^a	90.63±0.15 ^a	92.30±0.10 ^a
Methanol	60.77±0.18 ^b	73.53±0.09 ^b	89.80±0.10 ^b	90.37±0.12 ^a	90.80±0.10 ^b
P-Value	<0.0001				

At P≤0.05 there was a significant difference in the percentage reducing power (RP_{FRAP}) of different concentrations of leaf extracts on FRAP radicals. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

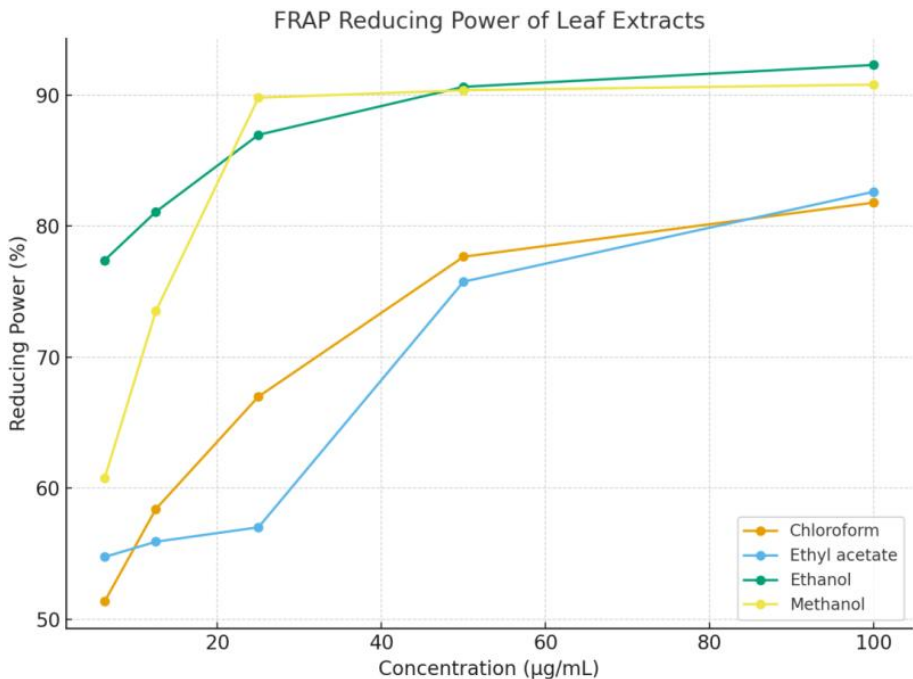


Figure 3: FRAP reducing power of leaf extracts

Antioxidant Activity with FRAP Percentage Reducing Power Model of the Stalk Extracts

The results in Table 4 and Figure 4 demonstrate that the stalk extracts of the plant exhibited appreciable ferric reducing antioxidant power, with reducing activity increasing progressively as extract concentration increased (from 6.25 to 100 µg/mL). At 6.25 µg/mL, the percentage reducing power ranged from 48.83 ± 0.15% for chloroform extract to 58.57 ± 0.50% for ethanol extract, while at 100 µg/mL the values increased to 57.80 ± 0.71, 79.27 ± 0.12, 92.17 ± 0.09 and 92.07 ± 0.09% for chloroform extract, ethyl acetate extract, ethanol extract and methanol extract respectively. The significantly higher activity of the ethanol and methanol extracts, particularly at higher concentrations, suggests that these solvents

extracted greater amounts of electron-donating antioxidant constituents from the stalk. The overall concentration-dependent increase (Figure 4) agrees with the findings of Poullos *et al.* (2025), in their report, the group stated that Basil (*Ocimum basilicum*) extracts exhibited concentration-dependent reducing activity and that the methanol extract showed stronger FRAP activity than other examined extracts. Nganteng *et al.* (2022) also observed that the reducing power of *O. gratissimum* increased with increasing extract concentration, supporting the result of this study. The statistically significant difference among the extracts at P≤ 0.05 further confirms that solvent type and concentration significantly influenced the antioxidant response. The strong reducing activity of the extracts of the stalk suggests that it is not inferior in antioxidant property to the leaf.

Table 4: The Percentage Reducing Power (RP_{FRAP}) of different Concentrations of Stalk Extracts on FRAP radicals

Extract	6.25 µg/mL	12.5 µg/mL	25 µg/mL	50 µg/mL	100 µg/mL
Chloroform	48.83±0.1 ^c	48.93±0.1 ^d	53.67±0.1 ^d	56.27±0.3 ^d	57.80±0.7 ^c
Ethyl acetate	57.07±0.1 ^b	62.43±0.1 ^c	67.50±0.1 ^c	72.80±0.1 ^c	79.27±0.1 ^b
Ethanol	58.57±0.5 ^a	69.17±0.0 ^a	84.43±0.1 ^a	88.53±0.0 ^b	92.17±0.0 ^a
Methanol	57.20±0.1 ^b	68.57±0.1 ^b	78.13±0.0 ^b	89.77±0.1 ^a	92.07±0.0 ^a
P-Value	<0.0001				

At P≤0.05 there was a significant difference in the percentage reducing power (RP_{FRAP}) of different concentrations of stalk extracts on FRAP radicals. Values are presented as mean±standard error of means. Ranking was done across the extracts and values with the same super script are not significant.

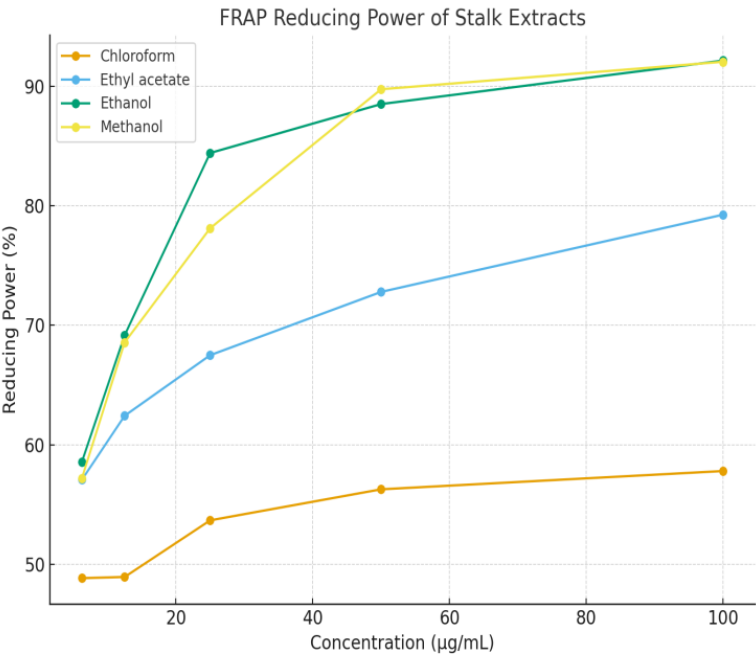


Figure 4: FRAP reducing power of stalk extract

CONCLUSION

The study established that the crude extracts of the leaf and stalk of *O. gratissimum* cultivated in Jos possess considerable antioxidant properties, as demonstrated by their DPPH radical-scavenging and FRAP reducing activities. Both plant parts exhibited concentration-dependent antioxidant activity. Although ethanol and methanol extracts generally showed superior activities compared with chloroform and ethyl acetate extracts, with ethanol leaf extract producing particularly strong responses, the stalk is not inferior to the leaf in antioxidant properties. The finding addresses the identified research gap concerning the limited information on the antioxidant potential of the stalk compared with the commonly used and explored leaf extract. The study therefore provides scientific empirical evidence contributing to the potential therapeutic importance of both leaves and stalks of the plant, as a natural antioxidant alternative for pharmaceuticals, nutraceuticals, and food-related applications. The utility of the stalk could also reduce the wastage of this part of the plant and contribute to the farmer’s fortune. Further studies are recommended to

evaluate the comparative anti-inflammatory properties, analgesic properties, and toxicology of the leaf and stalk of the plant.

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