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THE ANTIOXIDANT PROPERTIES AND PHYTOCHEMICAL SCREENING OF THE LEAF OF *OCIMUM GRATISSIMUM* (SCENT LEAF) GROWN IN JOS, NIGERIA

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Abstract

Ocimum gratissimum (commonly known as Scent leaf) is widely recognized for its medicinal and culinary applications. This study evaluates the antioxidant potential of *O. gratissimum* leaves using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and Thin Layer Chromatography (TLC)-DPPH bioautography analysis. Ethanol and ethyl acetate extracts were tested at various concentrations (20–100 mg/mL). Results show that the ethanol extract exhibited the highest percentage inhibition at 96%, surpassing the activity of the standard reference, ascorbic acid (93%). Notably, significant antioxidant activity was observed even at the lowest tested concentration (20 mg/mL). The TLC-DPPH bioautography analysis further confirmed the presence of antioxidant compounds, evidenced by a distinct yellow spot. These findings suggest that *O. gratissimum* grown in Jos could serve as a valuable natural source of antioxidants, with potential applications in the management of oxidative stress-related diseases.

Keywords: Antioxidant property, *O. gratissimun*, TLC-DPPH assay, Ethanolic extract, Ethylacetate extract

Introduction

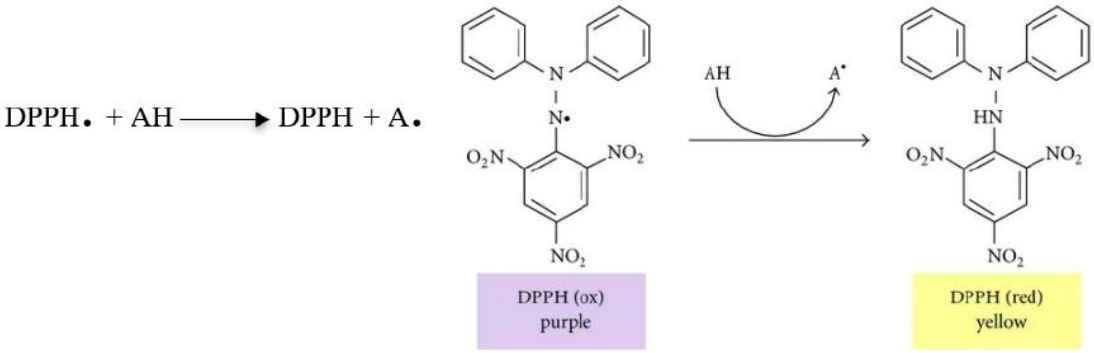
Ocimum gratissimum, a member of the *Lamiaceae* family, is widely cultivated for both culinary and medicinal purposes in West Africa. It is known by various names in different Nigerian languages, including “Nchuanwu,” “Ahinji,” and “Ahigbu” (Igbo), “Efirin” (Yoruba), “Ihirieziza” (Bini), and “Dai doyatagida” (Hausa).(Okoli *et al.*, 2010) The plant is indigenous to Southern Asia, the Bismarck Archipelago, Africa, and Madagascar, and has naturalized in regions such as Polynesia, Hawaii, Mexico, Panama, the West Indies, Brazil, and Bolivia (Edo *et al.,* 2023). Traditionally, it has been used to treat infections, digestive disorders, and inflammatory conditions. Plants are not only sources of food but also contain medicinal compounds that contribute to health and disease management(Mann, 2011). Approximately 80% of individuals in developing countries rely on traditional medicine for primary healthcare needs.

Oxidative stress is a key factor in aging and the progression of neurodegenerative diseases. It results from an imbalance between the production of reactive oxygen species (ROS) and the body’s ability to neutralize them. A disturbance in the normal redox state of the body could lead to the production of peroxides and free radicals that

damage all compounds of the cell, including protein, lipids, and DNA, contributing to conditions such as Antioxidants are categorized into endogenous and exogenous types based on their origin. These compounds mitigate oxidative damage to proteins, lipids, and DNA, thereby reducing the risk of chronic diseases (Neha *et al.,* 2019). Given that food items containing antioxidant compounds can help slow the aging process and prevent disease progression(Sherma, 2018), it is essential to further explore the antioxidant properties of *O. gratissimum*, which is widely consumed as a dietary component in Nigeria and strategically in Jos Farin Gada market where most vegetables in Jos are sold.

cancer, diabetes, and cardiovascular diseases (Ojo *et al.*, 2014).

Despite extensive research on the antioxidant properties of *O. gratissimum*, further investigation is necessary to assess its bioactivity in different environmental conditions, such as those found in Jos, Nigeria. Given the increasing interest in natural antioxidant agents, this study aims to evaluate the antioxidant potential of *O. gratissimum* using DPPH radical scavenging and TLC bioautography techniques.



Scheme 1: The mechanistic steps of the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radicals scavenging property(Nagarajan *et al*., 2020)

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and solvents used were of analytical grade. Pre-coated silica gel 60F254 TLC plates were obtained from Merck.

Collection and Preparation of Plant Material

Fresh *O. gratissimum* leaves were collected in February 2024 from a farm in Farin Gada, Jos North Local Government Area, Plateau State, Nigeria. The leaves were washed with running tap water and distilled water, and then air-dried in the shade for two weeks. The dried samples were coarsely pound into powder using mortar and pestle. The plant was identified and authenticated by Forest Herbarium in the College of Forestry, Jos. The voucher number is FHJ847

Extraction Process

Two 300 g portions of coarsely powdered *O. gratissimum* were macerated separately with ethanol (500 mL) and ethyl acetate (500 mL). The crude mixtures were filtered with Whatman 1 filter paper and then evaporated in vacuo. The obtained extracts were stored at ambient temperature for further analysis(Obasi, 2020).

DETERMINATION OF ANTIOXIDANT ACTIVITY

The antioxidant activity was determined using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) as the substrate. DPPH is a commercially available stable free radical that can be reduced by antioxidants. In its oxidized

form, DPPH appears purple, but upon reduction by an antioxidant, it changes to yellow**.**

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of the extract of O. gratissimum was measured by 1,1-dihenyl-2-picryl-hydrazil (DPPH).The antioxidant activity was assessed using the standard DPPH assay with a little modification (El-Euch et.al., 2014). Different concentrations of ethanol and ethyl acetate extracts (0.02, 0.04, 0.06, 0.08, 0.1 mg/mL) were prepared in methanol. A 10 mL freshly prepared 1 mM DPPH solution was mixed with 20 mL of each extract concentration. Ascorbic acid (standard antioxidant) was used as a reference. The mixtures were incubated in the dark for 15 minutes, and absorbance was measured at 517 nm using a spectrophotometer. Radical scavenging activity (%) was calculated using equation 1 (El-Euch *et al*., 2014).

$$\% \text{ Free radical scavenging} = \frac{\text{Abs}_{\text{standard}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{standard}}} \times 100 \quad (1)$$

where:
Abs standard = Absorbance of ascorbic acid solution
Abs sample = Absorbance of extract solution

Thin-Layer Chromatography bioautography using DPPH

To visualize antioxidant compounds, 5 µL of each extract was applied to a silica gel TLC plate. The plate was developed using toluene as a mobile phase in a saturated chamber to an 80 mm distance. After drying, the plates were immersed in 0.05% (w/v) DPPH methanol solution and incubated in the dark for 30 minutes. Antioxidant compounds appeared as yellow spots on a purple background (Odumosu et al., 2019)

RESULTS AND DISCUSSION

ANTIOXIDANT ACTIVTY BY SPECTROPHOTOMETRIC DPPH ASSAY

The scavenging activities of DPPH exerted by each extract (Ethanol and Ethyl acetate) against ascorbic acid as a standard were summarized in Table 1 indicating the various inhibition percentage.

At 20 mg/mL, both extracts exhibited high inhibition (ethanol: 96%, ethyl acetate: 95%) comparable to ascorbic acid (93 % as shown in Fig 1 and Table 1).At 40 mg/mL, the ethanol extract showed a decline (78 %), while ethyl acetate extract remained relatively high (83 %).At 60 mg/mL, the ethanol extract maintained moderate activity (70 %), while ethyl acetate extract showed a sharp drop (36 %). At 80 mg / mL, both extracts had similar inhibition (47 % for ethanol, and 47 % for ethyl acetate). At 100 mg/mL, the ethyl acetate extract exhibited significantly higher DPPH radical scavenging activity (92 %) compared to the ethanol extract, which showed a lower inhibition of 49 %, suggesting possible concentration-dependent variations of the phytochemicals in the extracts. The bar chart (Fig. 1) representation highlights how the antioxidant potential of *O. gratissimum* varies with concentration and solvent type.

Table 1: DPPH percentage inhibition of the different extracts and ascorbic acid

SN	Concentration (mg/ml)	Ascorbic acid inhibition (%)	Ethanol inhibition (%)	Ethyl acetate inhibition (%)
1	20	93	96	95
2	40	96	78	83
3	60	65	70	36
4	80	41	47	47
5	100	96	49	92

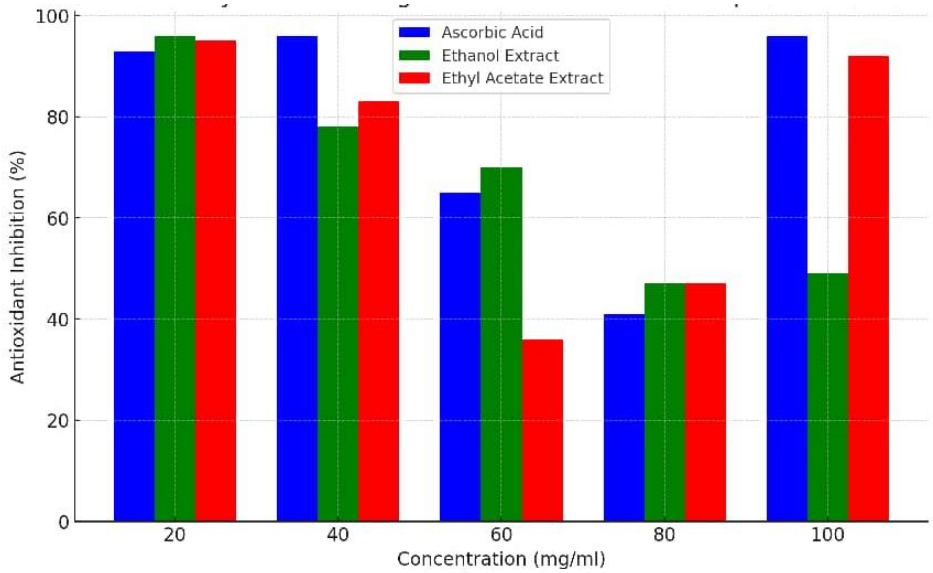


Fig 1. Antioxidant percentage inhibition of ethanol and ethyl acetate extracts of O. gratissimum compared to ascorbic acid.

In Fig 2, the Ascorbic Acid (Blue Line) shows consistently high inhibition, with a slight drop at 60 mg/mL (65 %) but recovering at 100 mg/mL (96 %).This suggests its strong and stable antioxidant capacity across all concentrations.

Ethanol Extract (Green Line). Exhibits high inhibition at 20 mg/mL (96 %) but gradually declines with increasing concentration, reaching 49 % at 100 mg/mL. This indicates that its effectiveness is concentration-dependent, with maximum activity at lower concentrations.

Ethyl Acetate Extract (Red Line)Shows a trend like ethanol extract but with a sharper decline at 60 mg/mL (36 %).However, it recovers at 100 mg/ml (92 %), indicating possible delayed release or solvent-specific interactions.

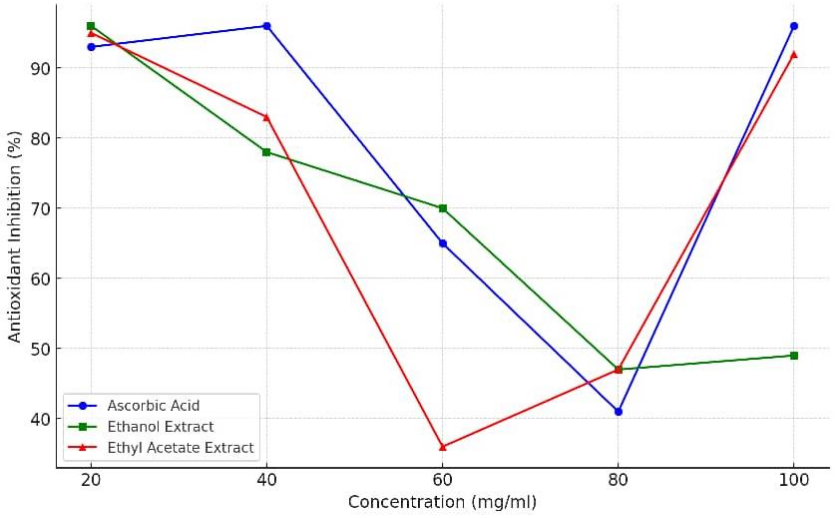


Fig 2. Trend of antioxidant inhibition (%) of *O. gratissimum* extracts compared to ascorbic acid at different concentrations.

TLC-DPPH BIOAUTOGRAPHY ANALYSIS

The TLC results confirmed the presence of antioxidant compounds in both extracts. The ethanol extracts(plate 1) exhibited larger and more intense yellow spots(on a purple background) compared to the ethyl acetate extracts (plate 2.), indicating a higher concentration of antioxidant compounds. This can be attributed to the higher polarity of ethanol, which facilitates the extraction of a broader range of polar antioxidant compounds compared to ethyl acetate. This is also consistent with the result obtained with 20 mg/mL in the DPPH inhibition analysis (Table 1, Fig 1).

Plate 1.TLC-DPPH EtOH extract

Plate 2. TLC-DPPH for EtOAc extract

CONCLUSION

This study confirms a strong antioxidant property of *O. gratissimum* leaf grown in Jos, with ethanol extracts exhibiting the highest activity. The various leaf extracts of the plant demonstrated promising antioxidant properties that could be explored in nutraceuticals, pharmaceuticals, and functional foods. The extracts exhibited strong antioxidant activity, although the specific bioactive compounds responsible for this effect were not identified in this study. Further research is recommended to explore and investigate the major compounds responsible for the high activity observed in this locally cultivated plant.

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