IPS Journal of Drug Discovery Research and Reviews
IPS J Drug Discov Res Rev, 3(2): 38-43 (2025)
DOI: https://doi.org/10.54117/ijddrr.v3i2.33.

Comparative Assessment of the In vitro Antioxidant Activity and Phytochemical Analysis of *Vernonia amygdalina* Leaf and *Ocimum gratissimum* Leaf

C. C. Ilechukwu[1*], C. O. Ezenwelu[2], J. C. Ifemeje[1], J. N. Ilechukwu[3] and I. H. Iheukwumere[4]

[1]Department of Biochemistry, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State Nigeria.
[2]Department of Applied Biochemistry, Faculty of Bio- Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
[3]Department of Community Health and Primary Care, Faculty of Medicine, University of Lagos, Akoka Lagos.
[4]Department of Microbiology, Faculty of Natural Sciences, Chukwuemeka Odumegwu Ojukwu University, Anambra State Nigeria.

*Email for correspondence: chijiokeilechukwu@gmail.com

Abstract

Vernonia amygdalina and *Ocimum gratissimum* are a major part of the diet in many Nigerian families due to their wide culinary applications and appeal. This study, therefore, compared the in-vitro antioxidant activity and phytochemical content of the extracts of these two plants. The methanol extracts of freshly plucked leaves of *Vernonia amygdalina* and *Ocimum gratissimum* were obtained and extracted using standard procedures. The phytochemical composition and in-vitro antioxidant parameters (nitric oxide and hydrogen peroxide) were assessed. The results of the phytochemical screening of methanol leaf extracts of *V. amygdalina* and *O. gratissimum* showed the presence of tannins, glycosides, flavonoids, saponins, phlobatannins, and alkaloids, while anthraquinones were absent in *V. amygdalina* but present in *O. gratissimum*. The results showed that the methanol leaf extracts of *V. amygdalina* and *O. gratissimum* were able to scavenge nitric oxide in a dose-dependent manner. A concentration of 20 µg/ml gave the least percentage nitric oxide scavenging activity (34.45 ± 0.08 and 40.21 ± 0.22), while a concentration of 100 µg/ml gave the highest percentage scavenging activity (65.20 ± 0.11 and 77.31 ± 0.14), respectively. The results also showed that the methanol leaf extracts of *V. amygdalina* and *O. gratissimum* were able to scavenge hydrogen peroxide (H_2O_2) in a dose-dependent manner. A concentration of 20 µg/ml gave the least percentage scavenging activity (38.20 ± 0.32 and 50.21 ± 0.23), while a concentration of 100 µg/ml gave the highest percentage scavenging activity (69.36 ± 0.19 and 78.25 ± 0.28), respectively. *Ocimum gratissimum* showed better nitric oxide and H_2O_2 percentage scavenging activity when compared with similar doses of *Vernonia amygdalina*, but demonstrated lower nitric oxide and H_2O_2 scavenging ability when compared with the standard antioxidant, ascorbic acid. The study further validates the phytochemical constituents and antioxidant activity of the two plants, supporting their traditional use and suggesting potential leads for developing plant-based therapeutic drugs. Incorporating these vegetables into the diet is also encouraged to help mitigate the harmful effects of reactive oxygen species.

Keywords: *Vernonia amygdalina, Ocimum gratissimum, antioxidant activity, phytochemical screening, reactive oxygen species.*

Article History
Received: 07 May 2025
Accepted: 28 May 2025
Published: 31 May 2025

Scan QR code to view•

License: CC BY 4.0♦

Open Access article.

How to cite this paper: Ilechukwu, C. C., Ezenwelu, C. O., Ifemeje , J. C., Ilechukwu, J. N., & Iheukwumere, I. H. (2025). Comparative Assessment of the In vitro Antioxidant Activity and Phytochemical Analysis of Vernonia amygdalina Leaf and Ocimum gratissimum Leaf. *IPS Journal of Drug Discovery Research and Reviews*, *3*(2), 38–43. https://doi.org/10.54117/ijddrr.v3i2.33.

Introduction

Free radicals are particles such as atom, molecule and ion with an unpaired valence electrons making them highly reactive (Ilechukwu, 2022). Free radicals which include superoxide, hydroxide radicals and nitric oxide and other reactive species namely hydrogen peroxide, hypochloric acid, and peroxynitrite produced during aerobic metabolism in the body can cause oxidative damage to amino acids, lipids, proteins and DNA (Gutteridge, 1995). Free radicals are generated through metabolism of drugs, environmental chemicals, stress hormones example adrenalin and nor adrenalin (Nilesh, 2010). In the body free radicals are derived from two sources namely endogenous sources e.g. nutrient metabolism, ageing process etc. and exogenous sources e.g. tobacco smoking, ionizing radiation, alcohol, drugs, air pollution, organic solvents, pesticides etc (Buyukokuroglu, *et al.*, 2001). The most effective way to eliminate free radical which causes oxidative stress is with the help of antioxidants (Nilesh, 2010). Antioxidant, both exogenous and endogenous, whether synthetic or natural can be effective in preventing free radical

♦This work is published open access under the Creative Commons Attribution License 4.0, which permits free reuse, remix, redistribution and transformation provided due credit is given.

formation by scavenging them or promoting their decomposition (Ito *et al.,* 1983, Tiwari, 2001). Herbs and spices are recognized as sources of natural antioxidants that can protect man from oxidative stress and thus play an important role in the chemo-protection of diseases that have their etiology and pathophysiology in reactive oxygen species (Atawodi, 2005). In summary, an antioxidant is a synthetic or natural compound that has the ability to slow down lipid oxidation when present at low concentration compared to an oxidizable lipid. Most commercial food antioxidants work by scavenging free radicals or chelating metals. Free radical scavengers, such as tocopherols, butylated hydroxytoluene (BHT), and plant phenolics, inhibit lipid oxidation by reducing peroxyl and alkoxyl radicals to stable compounds. Through these pathways, free radical scavengers can inhibit chain propagation and formation of fatty acid decomposition products (e.g., aldehydes and ketones) that cause rancidity (Alamed, *et al.*, 2009). In the food industry, the attention of manufacturers has shifted from synthetic to natural antioxidants although so far the synthetic antioxidants have been economically used to control effectively oxidation and prolong the shelf life of foods, their effectiveness and safety have been questioned due to their high volatility and instability at elevated temperatures and their suspected carcinogenicity when consumed at excessively high levels of intake (Ramful *et al.,* 2011). Antioxidants were originally defined as "substrates that in small quantities are able to prevent or greatly retard the oxidation of easily oxidizable nutrients such as fats" (Skibsted, 2010). Antioxidants can prevent oxidative damage to food during processing, storage and preparation of meals. Antioxidants may accordingly help the development of healthier food with low levels of lipid and protein oxidation products. Antioxidants may also have more direct health effects as part of the diet, but methodological problems in assessing this have been identified since both vitamin antioxidants (vitamin E and C) and non-vitamin antioxidants (polyphenols and carotenoids) are multifunctional in biological systems and cannot be evaluated by "one dimensional" methods (Frankel and Meyer, 2000). The therapeutic effects of several medicinal plants are usually attributed to their antioxidant phytochemicals present in the plant (Iheukwumere *et al.,* 2025a; Iheukwumere *et al.,* 2025b). There is currently, widespread screening of plants with potential bioactive properties and concomitant isolation and characterization of these bioactive principles for prevention and combating a wide array of diseases (Farombi and Owoeye, 2011; Njan, 2012; Iheukwumere *et al.,* 2025c; Iheukwumere *et al.,* 2025d). Plants, herbs and spices contain phyto-nutrient or phytochemicals which possess antioxidant activity (Okpe *et al*., 2012; Anameze *et al.,* 2023). Plants, herbs and spices has been demonstrated to possess significant antioxidant properties in various studies suggesting its potential protective ability against oxidative stress and therefore play an important role in the chemo-prevention of diseases that have their etiology, progression and pathophysiology in reactive oxygen species (Ilechukwu *et al*., 2014; Ilechukwu and Okafor, 2020; Ilechukwu, 2022, Ifemeje *et al.,* 2025). Different plant parts used in various treatment regimens include leaves, barks, tubers and root that secrete phytochemicals such as alkaloids, terpenes and phenolic compounds. Their utilization may be in decoction, emulsion, apozems, liniments and powdered forms. Medicinal plants are also exploited in cosmetics, perfumery, pharmaceutical and food industries. Their successful exploitation depends on the identification, isolation and purification of desired phytochemicals (Belewu *et al.,* 2009).

Vernonia amygdalina commonly known as "bitter leaf" in Nigeria because of the bitter taste impacted by the leaves and the stem, is a major vegetable of the celebrated "bitter leaf soup" among the Nigerian people. Vernacular names include "ewuro" among the Yorubas and "onugbu" among the Igbos. It has a long history of use in folk medicine particularly among the people of sub-Saharan Africa. Several studies have reported that it possesses antimicrobial, antidiabetic, antimalarial, antiparasitic, insecticidal, anticancer, anti-inflammatory, antipyretic, analgesic, antihelminthic hepatoprotective, antioxidative and hypolipidaemic effects among others (Atangwho *et al.,* 2010; Yeap *et al.,* 2010; Danquah *et al.,* 2012).

Ocimum gratissimum, popularly known as "scent leaf", is an aromatic medicinal plant belonging to the family Lamiaceae. It is widely distributed in the tropics of Africa and warm temperate regions. In Nigeria, O. gratissimum is called "Efinrin" in Yoruba; "Nchoanwu" or "Ahigbo" in Igbo and "Aramogbo" in Edo. Scent leaf is used as a spice to enhance food flavor and in the production of dental care products (Awah and Verla, 2010; Akinjogunla *et al.,* 2011).

Foods of plant origin contain a variety of important bioactive compounds such as vitamins, carotenoids and phenolics. Vegetables are widely consumed by humans for their bioactive compounds in addition to their rich nutrients. Many vegetables have been reported to possess antioxidant capacities which enable them to scavenge reactive oxygen species, chelate metal ions, inhibit nitrosation and modulate certain enzymatic actions (Ola *et al*., 2009). *V. amygdalina* and *O. gratissimum* occupy reasonably prominent positions in the diets of several Nigerian communities. This study therefore set out to compare the antioxidant activity and phytochemical composition of these two widely consumed vegetables.

Materials and Methods

Reagents and chemicals

All chemicals and reagents used were pure and of analytical grade and products of British Drug house (BDH), England; May and Baker, England; Sigma Aldrich, USA.

Plant material

The fresh leaves of *Vernonia amygdalina* and *Ocimum gratissimum* were purchased from local market in Awka Anambra state, South East Nigeria. The samples were identified by Prof. C.G. Ukpaka a botanist in the department of Biological sciences Chukwuemeka Odumegwu Ojukwu University Anambra State Nigeria.

Preparation of sample and material

Plant samples were washed in tap water and rinsed with distilled water. It was allowed to dry with room temperature for 3weeks and then pulverized and filtered through a sieve (about 0.5mm pore size) to obtain a fine dry powder. The powdered sample were stored in the polythene bags and placed

at room temperature until they were used. The glass wares used were washed with detergents, rinsed in distilled water and dried.

Extraction

One hundred gram (100g) of powdered sample were weighed into a beaker and 500ml of 70% methanol was added. It was continuously stirred and left to stand for 72hours at room temperature to allow for extraction. The solution was filtered using Whatman filter paper and concentrated in water-bath. The extract obtained were stored in the refrigerator until required for use.

Phytochemical screening

The extracts thus obtained were subjected to phytochemical analysis following the methods of (Lee *et al*., 2003).

Test for flavonoids

Shinoda test: The extract (0.5g) was dissolved in 2 ml of 50% methanol. Metallic magnesium and four to five drops of concentrated hydrogen chloride were added. An orange colour indicates the presence of flavonoicaglycones.

Test for saponins

Frothing test: A quantity (0.5g) of the extract was dissolved in 10ml of distilled water. This was then shaken vigorously for 30 seconds and allowed to stand for 30 minutes. A honey comb formed for more than 30 minutes indicates saponins.

Test for tannin

Ferric chloride test: Exactly 0.5g of the extract was dissolved in 10ml of distilled water and then filtered. Few drops of ferric chloride solution were added to it. Formation of a blue-black precipitate indicates hydrolysable tannins and green precipitate indicates the presence of condensed tannins.

Test for glycosides

Concentrated sulphuric acid (5ml) was added to the extract and boiled for 15 mins. This was then cooled and neutralized with 20% KOH and was divided into two portions. Another part of extract was dissolved in distilled water, this was used as a control; no acid hydrolysis.

Fehlings Solution Test*:* Fehlings solution A and B was added to one portion of the mixture of concentrated sulphuric acid and extract as above and boiled for few minutes, red precipitate indicates the glycone portion as a result of hydrolysis.

Ferric Chloride Test*:* Ferric chloride solution (3 drops) was added to the other portion of the mixture of the extract and concentrated sulphuric acid. Green to Black precipitate indicates phenolic aglycone as a result of hydrolysis of glycoside (Trease and Evans, 1983).

Test for alkaloids

***Tannic Acid Test*:** Few drops of tannic acid were added to 1g of the extract. Black precipitate indicates the presence of alkaloids (Lee *et al.*, 2003).

Test for anthraquinones

***Bornirager's Test*:** One (1) gram of the extract was soaked in 10ml of benzene and filtered. Five millilitre of 10% of ammonia solution was added to the filtrate and stirred. The production of a Pink-red colour indicates the presence of free anthraquinones.

Hydrogen Peroxide Scavenging Effects

The ability of the leaf samples to scavenge hydrogen peroxide was assessed by the method of Ruch *et al*., (1989).

Reagents

1. Phosphate buffer (0.1M, pH 7.4)
2. H_2O_2 (40mM) in phosphate buffer

Procedure

A solution of H_2O_2 (40mM) was prepared in phosphate buffer. Leaf samples at the concentration of 10mg/10µl were added to H_2O_2 solution (0.6ml) and the total volume was made up to 3ml. The absorbance of the reaction mixture was recorded at 230nm in a spectrophotometer (Genesys 10-S, USA). A blank solution containing phosphate buffer, without H_2O_2 was prepared. The extent of H_2O_2 scavenging of the sample samples was calculated as

$$\% \text{ scavenging of hydrogen peroxide} = \frac{(A0 - A1) \times 100}{A0}$$

A0 - Absorbance of control

A1 - Absorbance in the presence of sample

Measurement of Nitric Oxide Scavenging Activity

The extent of inhibition of nitric oxide radical generation *in vitro* was followed as per the method reported by Green *et al.* (1982). Sodium nitroprusside in aqueous solution, at physiological pH, spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that are estimated spectrophotometrically at 546nm. The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of leaf samples (50mg) and incubated at 25ºC for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the samples. The absorbance was read at 546nm against the reagent blank, in a spectrophotometer (Genesys 10-S, USA).

Statistical Analysis

The data obtained from the study were analyzed using one-way ANOVA with the Statistical Package for the Social Sciences (SPSS, version 23) and expressed as mean ± standard error of the mean (SEM). Differences between means were evaluated using Duncan's Multiple Range post hoc test.

Results and Discussion

In recent years there have been an increased scientific interest in the study of antioxidants most especially those intended to prevent the harmful effects of free radicals in the human body and deterioration of fat and other food constituent. In all cases antioxidants from natural sources rather than synthetic are most preferred because they are easily accessible, affordable and with fewer or no side effects. The result of the phytochemical screening of methanol leaf extracts of *V. amygdalina* and *O. grattisimum* showed the presence of tannins, glycosides, flavonoids, saponins, phlobatannins and alkaloids while anthraquinones was absent for *V. amygdalina* but present in *O. gratissimum* (Table 1). This corroborates with the work of Kin *et al*. (2018) and Akinmoladun *et al.* (2007) which reported the presence of these phytochemicals.

Table 1: Quantitative phytochemical screening result of *V. amygdalina* and *O. gratissimum*

Phytochemicals	*V. amygdalina*	*O. gratissimum*
Tannins	+	+
Glycosides	+	+
Flavonoids	+	+
Saponins	+	+
Phlobatannins	+	+
Anthraquinones	-	+
Alkaloids	+	+

+ = present; - = not detected

An in-vitro hydrogen peroxide (H_2O_2) scavenging assay measures how well a substance can remove or neutralize H_2O_2 in a controlled laboratory environment. It is usually done by monitoring changes in in H_2O_2 concentration over time using a specific detection method like spectrophotometry or a colorimetric assay. In this study, methanol leaf extracts of *V. amygdalina* and *O. gratissimum* scavenge for free radicals was in a dose dependent manner with concentration of 20 µg/ml given the least effect while concentration of 100 µg/ml gave the highest effect (Table 2). The inhibition of hydrogen peroxide by the leaf extracts of *V. amygdalina* and *O. gratissimum* is in close agreement with the works of Odukoya *et al.* (2006) that reported antiperoxidative effect of *V. amygdalina* and *Ocimum gratissimum. Ocimum gratissimum* gave a better H_2O_2 scavenging ability when compared with similar doses of *V. amygdalina* but gave a lesser H_2O_2 scavenging ability when compared with the standard antioxidant ascorbic acid. This is in agreement with the works of Oriakhi *et al.* (2014) which reported that *Ocimum gratissimum* was able to scavenge more free radicals than *V. amygdalina* and less than ascorbic acid, the standard antioxidant.

Table 2: H_2O_2 scavenging activity of Methanol leaf extract of *Vernonia amygdalina* and *Ocimum gratissimum*

Conc. in µg/ml	% scavenging activity of *V. amygdalina*	% scavenging of *O. gratissimum*	% scavenging activity of standard (ascorbic acid)
20	31.25±0.32	50.21±0.23	55.40±0.14
40	47.30± 0.14	62.33±0.11	65.26±0.22
60	53.25±0.08	68.04 ±0.20	71.30±0.19
80	58.15±0.21	70.18±0.31	78.14±0.15
100	69.36±0.19	78.25±0.28	86.21±0.26

Values are expressed as mean ± SD (n = 3)

It was observed that increase in the concentration of the samples led to increased inhibition on hydrogen peroxide free radical. For the first concentration, 20 µg/ml, the ascorbic acid had the highest inhibitory effect of 55% when compared to the two samples of *V. amygdalina* and *O. grattisimum*, which had 30% and 50% inhibitory effects respectively. In subsequent concentration of 40, 60, 80 and 100 µg/ml. The ascorbic acid (standard) had the highest inhibitory effects when compared to the samples used. The extracts had the most effective scavenging potential at 100 µg/ml. The standard (Ascorbic acid) showed the highest inhibition (%) across all the concentration when compared to the samples of *V. amygdalina and O. grattisimum.*

The extent of inhibition of nitric oxide radical generation *in vitro* was measured spectrophotometrically and the result showed that *V. amygdalina* was able to inhibit nitric oxide generation in a dose dependent manner (Table 3). This is in conformity with the works of Odukoya *et al.,* 2006 which demonstrated similar dose dependent effect. *O. gratissimum* was able to inhibit nitric oxide generation more than *V. amygdalina*. This is in agreement with works of Oriakhi *et al.*, 2014 which observed also that *O. gratissimum* was a better nitric oxide inhibitor than *V. amygdalina.*

Table 2: Nitric oxide scavenging activity of methanol leaf extract of *V. amygdalina* and *O. gratissimum*

Conc. in µg/ml	% scavenging activity of *V. amygdalina*	% scavenging of *O. gratissimum*	% scavenging activity of standard (ascorbic acid)
20	34.45±0.08	40.21±0.22	45.06±0.18
40	44.46±0.18	48.65±0.21	54.23±0.23
60	50.41±0.20	55.33±0.30	62.14±0.18
80	58.32±0.14	66.20±0.28	74.08±0.26
100	65.20±0.11	77.31±0.14	86.15±0.14

Values are expressed as mean ±SD (n=3)

Conclusion

This study has demonstrated that the two plants *Vernonia amygdalina* and *Ocimum gratissimum* have antioxidant properties. These activities were found to increase as the concentration increased and hence dose dependent. The antioxidant properties of these two plants *Vernonia*

amygdalina and *Ocimum gratissimum* explains their wide and extensive use in folkloric medicines to prevent, manage and slow down the progression of oxidative stress implicated diseases. This study reveals that the widely used *Vernonia amygdalina* and *Ocimum gratissimum* are natural store for antioxidants which can be harnessed nutritionally and medically to produce natural and safe antioxidant drug and nutraceuticals with fewer or no side effects.

Competing interest

Authors declare no competing interest.

References

Akinjogunla, O. J, Ekoi, O. H and Odeyemi, A. T (2011). Phytochemical screening and in vitro antibacterial assessment of aqueous leaf extracts of *Vernonia amygdalina* (Asteraceae) and *Ocimum gratissimum* (Lamiaceae) on moxifloxacin resistant *Escherichia coli* isolated from clinical and environmental samples. *Nature Sci*. **9**(7): 42 – 52.

Akinmoladun, A. C. Ibukun, E.O., Afor, E. Obuotor, E.M. and Farombi, E. O. (2007). Phytochemical constituent and antioxidant activity of extract from the leaves of *Occimum grattisimum*. *Scientific Research and Essay*, **2**(5):163-166.

Alamed, J., Chaiyasit, W., McClements, D. J., and Decker, E. A. (2009). Relationships between free radical scavenging and antioxidant activity in foods. *Journal of Agricultural and Food Chemistry*, **57** (7), 2969–76.

Anameze, C. I., Emmy-Egbe, I. O., Anyaegbunam, L. C., Ogomaka, I. J., Uba, B. O., Odumodu, O. A., Ezeigwe, C., Kamalu, N. L., Chukwubude, C. B., Akaogu, O., Ezekwueme, E., Emmy-Egbe, C.C., Obiefoka, O. S., Ezenwata, S. I. and Ilechukwu, C. C. (2023). Qualitative and quantitative Phytochemical Analysis of *Gongronema latifolium* Leaf Extract. *IPS journal of Applied Microbiology and Biotechnology*, **2** (1), 16-19.

Atangwho, I. J, Ebong P. E, Egbung G. E and Obi, A. U (2010). Extract of *Vernonia Amygdalina* Del. (African Bitter Leaf) Can Reverse Pancreatic Cellular Lesion after Alloxan Damage in the Rat. *Aust J Basic Appl Sci*. **4** (5): 711-716.

Atawodi, S.E., (2005): Antioxidant potential of African Medicinal Plants. *African Journal of Biotechnology*, **4** (2): 128-133.

Awah, F. M and Verla A.W (2010). Antioxidant activity, nitric oxide scavenging activity and phenolic contents of *Ocimum gratissimum* leaf extract. *J Med Plants Res*. **4** (24): 2479-2487.

Belewu, M.A, Olatunde O.A and Giwa T.A (2009). Underutilized medicinal plants and spices: Chemical composition and phytochemical properties. *J Med Plant Res*. **3** (12): 1099-1103.

Buyukokuroglu, M.E., Gulcin I., Oktay M., and Kufrevioglu O.I. (2001). In vivo antioxidant properties of dentrolene sodium. *Pharmacological Resource*. **44** :491-494.

Danquah, J.A and Ofori, k. (2012). Variation and correlation among agronomic traits in 10 varieties of garden eggplant in Ghana. *I.J.S.N*., **3** (2) 373-379

Farombi E. O and Owoeye O (2011). Antioxidative and Chemopreventive Properties of *Vernonia amygdalina* and *Garcinia kola*. *Int J Environ Res Public Health*, **8**: 2533-2555

Frankel, E. N. and Meyer, A. S. (2000). The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture*, **80** (13):1925–1941.

Green, L.C., Wagner, D. A., Glogowski, J., Skipper, P.L., Wishnok, J.S. and Tannebaum, S. R. (1982). Analysis of Nitrate, Nitrite and [^{15}N] Nitrate in Biological Fluids. *Analytical Biochemistry*, **126**:131-138.

Gutteridge, J.M.C., (1995). Free radicals in disease processes: A compilation of cause and consequence. *Free radical Research Community*. **19**:141.

Ifemeje, J.C., Ilechukwu, C. C. and Obi, B.U. (2025). Effect of Ethanol Seed Extract of *Coriandrum sativum* on Kerosene Mediated Oxidative Stress Markers in Wistar Rats Fed with Kerosene Contaminated Diet. *IPS Journal of Toxicology*, **3** (1):11-18.

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025). Exploring the Phytochemical and Antimicrobial Properties of Fruit Vinegar: A Study on *Phoenix Dactylifera* and *Malus Sylvestris*. *IPS Journal of Applied Microbiology and Biotechnology*, **4**(1), 115–122. https://doi.org/10.54117/ijamb.v4i1.48.

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025). Safety Evaluation of Vinegar from *Phoenix Dactylifera* and *Malus Sylvestris*: Toxicity and Acetic Acid Content. *IPS Journal of Applied Microbiology and Biotechnology*, **4**(1), 123–131. https://doi.org/10.54117/ijamb.v4i1.49.

Iheukwumere, I. H., Ajeh, J. C., Iheukwumere, C. M., Ike, V. E., Obianom, A. O., Ihenatuoha, U. A., Igboanugo, E. U., Onwuasoanya, U. F., Okereke, F. O., Nnadozie, C. H., Agbaugo, C. F., Nwike, M. I., Nwakoby, N. E., & Ilechukwu, C. C. (2025). Microbial Quality and Sensory Assessment of Vinegar from Date Palm and Apple Fruits: Implications for Consumer Preference. *IPS Journal of Nutrition and Food Science*, **4**(2), 410–417. https://doi.org/10.54117/ijnfs.v4i2.100.

Iheukwumere, I. H., Iheukwumere, C. M., Obianom, A. O., Nnadozie, C. H., Onwuasoanya, U. F., Oduoye, O. T., Ike, V. E., Obiefuna, O. H., Igboanugo, E. U., Ejike, C. C., Udeagbara, O. E., Ochibulu, S. C., Onyemekara, N. N., Ihenatuoha, U. A., Nwakoby, N. E., Ilechukwu, C. C., & Destiny, E. C. (2025). Mitigating Newcastle Disease Virus-Induced Damage in Chicken Embryos Using Extracts of *Curcuma longa and Baphia nitida*. *IPS Journal of Basic and Clinical Medicine*, **2**(2), 58–63. https://doi.org/10.54117/ijbcm.v2i2.12.

Ilechukwu C.C, Ebenebe UE, Ubajaka CF, Ilika AL, Emelumadu OF and Nwabueze SA (2014). The Role of oxidative Stress in Diabetes Mellitus: A 24-year review. *Afrimedic Journal*. **5**(1): 1-7.

Ilechukwu, C. C. (2022). Hepatoprotective and antioxidant activities of methanol root extract of *Sphenocentrum jollyanum* on Rifampicin induced liver damage in Wistar Rats. *International Journal of Pharmacy and Pharmaceutical Research*, **24** (1): 251-261.

Ilechukwu, C. C. and Okafor, N. I. (2020). Hepatoprotective and antioxidant activities of methanol leaf extract of *Sphenocentrum jollyanum* on Rifampicin induced liver damage in Wistar Rats. *International Journal of Pharmacy and Pharmaceutical Research*, 19 (4): 99-113.

Ito N., Fukishima, S., Hassegawa, A., Slubata M. and Ogiso T., (1983). Carcinogenicity of Butylatedhydroxyanisole in F344 rats. Journal of National Cancer Institute, 70:343-347.

Kin, A., Abubakar, I., Musa, L. Y., Olusola, L.F. and Zubairu, R. (2018). Antibacterial activity of *Occimum grattissimum* (scent leaf) on some pathogenic gastrointestinal bacteria. *African Journal of Microbiology Research*, **12** (40):923-929.

Lee, S.E., Hwang, H.J. and Ha, J.S. (2003). Screening of medicinal plant extracts for Antioxidant activity. *Life Science*, **73**:167-179.

Nilesh, M. (2010). Drug-induced Hepatotoxicity. *Medscape: Drugs diseases and procedures*, **20**:1-7.

Njan, A. A (2012). Herbal Medicine in the Treatment of Malaria: *Vernonia amygdalina*: An Overview of Evidence and Pharmacology. In: Acree W, editor, Toxicity and Drug Testing Intech, Rijeka, Croatia. pp 167 – 186.

Odukoya, O. A., Inya-Agha, S. I., Segun, M.O., Sofidiya, M.O. and Ilori, O. O. (2006). Antioxidant activity of selected Nigerian green leafy vegetables. Medicinal plants and Research book of Abstracts. *Planta Medicinal*, **72**:10-14.

Okpe O., Abdullahi A.S, Nkeonye O.l, Ilechukwu C.C., Nweke O., and Ihuoma O. (2012). Hypoglycemic and Hypolipidemic Effects of Aqueous and Ethanolic Leaf Extracts of Vitex doniana (Verbenaceae) in Normoglycemic Albino Rats. *Global Advanced Research Journal of Microbiology*. 1(10): 173-179.

Ola S. S, Catia G, Marzia I, Francesco V. F, Afolabi A. A. and Nadia M (2009). HPLC/DAD/MS characterisation and analysis of flavonoids and cynnamoil derivatives in four Nigerian green-leafy vegetables. *Food Chem* **115**: 1568 -1574.

Oriakhi, K., Oikeh, E, I., Ezeugwu, N., Anoliefo, O., Aguebor, O. and Omorogie, E. S. (2014). In vitro antioxidant activities of extracts of *Vernonia amygdalina* and *Occimum gratissum* leaves. *Journal of Pharmacy and Bioresources*, 11 (2):58-65.

Ramful, D., Aumjaud, B., Neergheen, V. S., Soobrattee, M. A., Googoolye, K., Aruoma, O. I., and Bahorun, T. (2011). Polyphenolic content and antioxidant activity of *Eugenia pollicina* leaf extract in vitro and in model emulsion systems. *Food Research International*, **44**(5), 1190–1196.

Ruch, R.J., Cheng, S.J. and Klaunig, J.E. (1989). Carcinogenesis. **10**: 1003-1008.

Skibsted, L. H. (2010). Understanding oxidation processes in foods. In D. J. Decker, E.A., Elias, R.J., McClements (Ed.), Oxidation in Foods and Beverages and Antioxidant Applications: Understanding Mechanisms of Oxidation and Antioxidant Activity (1st ed., pp. 3–31). *Cambridge, U: Woodhead Publishing*

Sqaudriatto, G.I. and Pelor, W.A. (1998). Free radical. Oxidative chemistry of nitric oxide: The roles of superoxide, peroxynitrite and carbon dioxide. Biology and medicine, **25:**392–403.

Tiwari, A. (2001). Imbalance in antioxidant defense and human diseases: multiple approach of natural antioxidant therapy. *Current Science*, **81**:1179.

Trease, G.E. and Evans, W.C. (1983). Drugs of biological origin. In: *Pharmacognosy 12th edition UK, BalliereTindall,* pp. 3009-540.

Yeap, S.K., Ho, W. Y., Beh, B. K., Liang, W. S., Ky, H., Yousr, A. H. N. and Alitheen, N.B. (2010). *Vernonia amygdalina*, an ethnovertinary and ethnomedical used green vegetable with multiple bio-activities. *Journal of Medicinal Plants Research*, **4** (25): 2787-2812.

.

•*Thank you for publishing with us.*