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Research Article

Phenolic Profile and Antioxidant Potential of Aqueous Extracts of Selected Traditional Anti-cough Plants

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Abstract



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The recent surge in attention towards plant-based antioxidants can be attributed to their perceived advantageous impact on human health. The present study assessed the phenolic profile, antioxidant potential, and free radical scavenging ability of hot aqueous leaf extracts obtained from *Vernonia amygdalina*, *Piper guineense*, and *Gongronema latifolium*. High-Performance Liquid Chromatography (HPLC) was used to examine phenolic profile. Antioxidant capacity was conducted using total flavonoid content (TFC), total phenol content (TPC), total antioxidant capacity (TAC), polyphenol oxidase (PPO) activity, while ferric reducing antioxidant potential (FRAP), nitric oxide (NO), hydroxyl (OH), and 2, 2-diphenyl-2-picrylhydrazyl (DPPH) were analysed for their radicals scavenging activities. *G. latifolium* displayed notable phenolic compounds, such as lunamarin (76.32mg/ml), ribalinidine (64.08 mg/ml), galocatechin (48.58mg/ml), aglycone (41.69 mg/ml), flavonone (33.45mg/ml), and flavan-3-ol (30.49mg/ml). The plant extracts contained resveratrol, kaempferol, and ribalinidine in a consistent manner across all samples. The findings suggest that the plants exhibits noteworthy antioxidant potential, with *G. latifolium* displaying the highest antioxidant capacity ($96.18 \pm 0.11 \mu\text{g/mL}$) and total phenolic content ($28.50 \pm 0.04 \mu\text{g/mL}$). *V. amygdalina* showed highest polyphenol oxidase activity, at $7.14 \pm 0.10 \mu\text{g/mL}$ *G. latifolium* as well showed high $\bullet\text{NO}$ scavenging radicals and FRAP activity, while exhibiting the least efficacy in $\bullet\text{OH}$ scavenging radicals. The results indicate that all three samples demonstrated a noteworthy increase in DPPH scavenging activity, which was statistically significant at $p < 0.05$ compared to standard. Results reveals that plant extracts showed significant phenolic compounds, antioxidant and free radicals property. These observations provides a rationale for the traditional use of these extracts in folk medicine for management and treatment of cough.

Keywords. *Gongronema latifolium*, *Veronia amygdalina*, *Piper guineense*, Phenolic profile, Antioxidant capacity, Free Radical, cough.

INTRODUCTION

In recent times, there has been a significant surge in the attention given to the potential of complementary and alternative medicines in managing acute and chronic illnesses.¹ The escalating prevalence of diseases, emergence of drug resistance, and the need for medications with minimal adverse effects have prompted researchers to investigate the optimal plant-based sources of medicine using contemporary scientific and technological approaches.² According to traditional healers, certain medicinal plants exhibit greater efficacy in treating infections when compared to synthetic compounds. Interest has been directed towards the different categories of phytochemicals such as the Free radical Scavenging and antioxidant properties of polyphenols. Botanical agents are present in diverse sources. The utilisation of plants, vegetables, and spices in folk and traditional medicine has been widely acknowledged as a significant avenue for the discovery and development of prophylactic and chemopreventive drugs.³ Fruits and plants are known to be abundant sources of phenolic compounds, which exhibit diverse properties such as antioxidant, antibacterial, anti-inflammatory, hepatoprotective, and anticarcinogenic activities. The biological functions of flavonoids and phenolic

compounds have been ascribed to their abilities to scavenge free radicals, chelate metal ions, and exhibit antioxidant activities³. Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS), commonly referred to as free radicals, have been identified as agents that can induce harm to lipids, proteins, enzymes, and nucleic acids, ultimately resulting in cellular or tissue impairment.⁴ Phytochemicals present in plants possess the ability to act as free radical scavengers or antioxidants, which can either eliminate the free radicals or impede their generation. Plants are widely acknowledged as primary origins of antioxidants, encompassing phytochemicals like flavonoids and non-flavonoids such as phenolic acids, lignins, stillbenes, terpenoids⁵. Numerous studies have provided evidence that various plant extracts with the ability to scavenge free radicals display antibacterial and antimicrobial properties, thereby establishing a correlation between the antioxidant and antibacterial characteristics of plant specimens.^{6,7}

Vernonia amygdalina is classified taxonomically as a member of the Asteraceae family, which is a group of angiosperms belonging to the Asterales order. It is cultivated in tropical regions and holds significant economic value. The plant commonly referred to as "Bitterleaf" in Africa is characterised

by a bitter taste. The presence of this phenomenon is also observed in tropical regions of Africa, specifically in West Africa¹⁰. The indigenous tribes of Nigeria, namely Igbo, Yoruba, and Hausa, refer to this certain plant as "Olugbu," "Ewuro," and "Fetefete," respectively, in their respective languages. The shrubs exhibit a soft texture and attain a vertical stature ranging from 1 metre to 6 metres in height. This particular plant has the ability to endure various weather conditions. The nomenclature "bitterleaf" was assigned to a certain plant species owing to its acrid flavour and anti-nutritive characteristic.¹¹ The foliage of the plant exhibits a dark green color and possesses a diameter of 6 millimetres, while measuring a length of 20 centimetres. In Africa, the plant leaves are also commonly consumed as delicacies¹².

Piper guineense, a member of the Piperaceae family, is a perennial shrub. This species is indigenous to West Africa. *Piper guineense* is recognised by various names such as climbing black pepper, West African black pepper, Ashanti pepper, Guinea cubeb, and Benin pepper. The foliage and seeds of this plant are utilised as a seasoning agent, food preservative, insect repellent, herbal remedy, and as a scent in the cosmetic industry within the Southern region of Nigeria. It is noteworthy, however, that its cultivation is only partially practised in this area.¹³ *P. guineense* is a medicinal herb that is utilised for the treatment of rheumatism, bronchitis, diarrhoea, and cough. The study conducted¹⁴. The observed effects include heightened activity of digestive enzymes, decreased levels of lipid peroxidation, and protection against oxidative stress and inflammation¹⁵. The biological effects of this substance include insecticidal, antimicrobial, antifungal, and antioxidant properties.¹⁶

Gongronema latifolium is a prevalent plant species found in the equatorial rainforests of West African countries such as Nigeria, Côte d'Ivoire, Sierra Leone, Ghana, and Senegal. The plant belongs to the taxonomic classification of the *Asclepiadaceae* plant family. The plant species, commonly grown in the southern region of Nigeria, is recognised by the locals as utazi and arokeke in the Igbo and Yoruba languages, respectively. Empirical investigations have demonstrated that the entirety of the plant manifests a diverse range of herbal actions, including but not limited to analgesic, antitumor, broad spectrum antimicrobial (namely antibacterial, antifungal, antiparasitic, and antiviral), antipyretic, antioxidant, anti-inflammatory, antiulcer, anti-sickling, anti-asthmatic, mild expectorant, hypoglycemic, hypolipidemic, hepatoprotective, digestive tonic, and laxative properties. Furthermore, the plant possesses medicinal properties whereby the consumption of its raw leaves or infusion with hot water can promptly alleviate symptoms such as catarrh, congestion, runny nose, and cough¹⁷. According to¹⁸, *G. latifolium* is purportedly utilised for the treatment of cough in Nigeria. The leaves of *G. latifolium* are reported to possess properties that may alleviate wheezing in individuals with asthma when chewed. Additionally, the roots of the plant may be cold-macerated and ingested as a potential remedy for asthma.¹⁹.

The utilisation of various plants has been widely practised in traditional settings. This study was designed to investigate the phenolic content, antioxidant properties, and free radical scavenging effect of hot aqueous leaf extracts of *P. guineense*, *G. latifolium*, and *V. amygdalina*. The aim was to explore the potential therapeutic benefits of these plants in the treatment of cough, given their wide traditional use for this purpose.



Figure 1: *Piper guineense*



Figure 2: *Vernonia amygdalina*



Figure 3: *Gongronema latifolium*

MATERIALS AND METHOD

Plant Materials

Fresh leaves of *P. guineense*, *G. latifolium*, and *V. amygdalina* from Relief Market, a reputable marketplace located in the Owerri Municipal Local Government Area of Imo State. Mr. Francis Iwueze, a plant taxonomist associated with the Department of Wildlife and Forestry at the Federal University of Technology Owerri (FUTO) in Imo State, identified them subsequently with voucher numbers FUTO/FWT/ERB/2022/88, FUTO/FWT/ERB/2022/89, and FUTO/FWT/ERB/2022/90.

Extraction and Plant Sample Preparation

After the harvest, the plants underwent a two-week period of air-drying at ambient temperature, while being arranged in a spread-out manner. The specimen was comminuted into a fine particulate morphology using an industrial-grade grinding machine, and subsequently transferred into containers that

were clearly marked for the purpose of easy identification and storage. The samples were subjected to standardized preparation methods in order to obtain pure aqueous extracts. 450 grams of ground plant samples were individually dissolved in a 2.5 litre volumetric flask with distilled water that had been heated to a boiling point. Subsequently, the solution was adjusted to the intended volume and subjected to boiling for a duration of 30 minutes then solution was decanted and filtered. The specimens were placed in properly marked containers and kept refrigerated at a temperature of 4°C, while being protected from light and humidity. The infusions were prepared in triplicate, adhering to standard scientific methodology.

Antioxidant Parameters

Total Flavonoid Content

The Total Flavonoid Content (TFC) was quantified using the Aluminium chloride method Babu *et al*²⁰ as outlined . A

solution was prepared by adding 1 milliliter of plant extract with a concentration of 1 milligram per milliliter to 4 milliliters of distilled water. To this solution, 0.3 milliliters of a 5% sodium nitrite solution was added and allowed to react for a duration of 5 minutes. Subsequently, 0.3 milliliters of a 10% solution of Aluminium chloride was introduced, and this was succeeded by the addition of 2 milliliters of a 1 Molar concentration of sodium hydroxide. The measurement of absorbance was conducted at a wavelength of 510 nm utilizing a UV-Vis spectrophotometer. The experiment was conducted with three replicates for each assay and the results were presented as the mean value with standard deviation.

Total Phenolic Content (TPC)

The total phenolic content (TPC) was assessed using the Folin-Ciocalteu (FC) method Pourmorad *et al*²¹, as outlined. A volume of 1 milliliter of the sample was combined with 5 milliliters of distilled water and an additional 1 milliliter of Folin reagent. Following a 5-minute incubation period, 1 milliliter of 10% Na₂CO₃ was introduced and subsequently incubated for one hour under dark conditions at ambient temperature. The measurement of absorbance was conducted at a wavelength of 725 nm utilizing a UV spectrophotometer. The experimental procedure was conducted in triplicate and the resulting data was presented as the mean ± standard deviation.

Total Antioxidant Capacity

The method described by Farombi *et al.*²² was employed to determine the overall antioxidant activity of the extracts derived from *V. amygdalina*, *P. guineense* and *G. latifolium*. During the experiment, a mixture of 1 ml of 2 mM ABTS and 1 ml of 0.1 mM hydrogen peroxide was combined with 5 mg of each extract in 50 mM glycine-HCl buffer with a pH of 4.5. 0.5 ml of 0.25 mM peroxidase was introduced into the reaction mixture. The spectrophotometric measurement of the solution's absorbance was conducted at a wavelength of 414 nm, following a 10-minute interval. The study employed Garlic Acid as a benchmark and quantified the overall antioxidant capacity in terms of mM Garlic Acid equivalent.

Polyphenol Oxidase Activity

The measurement of polyphenol oxidase activity was conducted at a temperature of 40°C, utilizing the methodology outlined by Kim and Kim²³. The experimental setup comprised of a reaction mixture containing 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.8), 0.2 mL of 20 mM pyrogallol, and 0.2 mL of enzyme extract. The measurement of absorbance was conducted at a wavelength of 430 nanometers. The activity of polyphenol oxidase (PPO) was quantified as the rate of oxidation of 1 micromole of pyrogallol per minute per milligram of protein, denoted as "Unit mg-1(protein)".

Free Radical Scavenging parameters

Nitric Oxide Scavenging Activity

A 10 mM solution of Sodium nitroprusside (SNP) in 20 mM phosphate buffer at pH 7.4 was prepared. Subsequently, 50 µL of the prepared solution was combined with 50 µL of a 400 µg/mL extract solution. The concoction underwent incubation at a temperature of 27 degrees Celsius for a duration of 150 minutes. Subsequently, 100 µL of griess reagent was introduced with agitation, and the absorbance was promptly assessed at 542 nm utilizing the Epoch microplate spectrophotometer (23). A solution comprising of 50 µL of each extract and control was prepared, while a control solution was created by combining 50 µL of SNP and 50 µL of methanol without extract. The experimental procedure involved conducting each test in triplicate and utilizing Garlic

Extract as a positive control, which is a recognized standard antioxidant. The determination of Nitric Oxide free radical scavenging activity was conducted by employing the subsequent equation.

$$\% \text{ Nitric Oxide Scavenging Activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

Where, Abs control = Absorbance of control

Abs sample = Absorbance of sample

Ferric Reducing Antioxidant Power (FRAP)

The methodology employed by Peixoto *et al.*²⁴ was utilized to determine the reducing power of the plant extracts. Each sample was mixed with 2.0 ml of 0.2 M phosphate buffer (pH 6.6) and 2.0 ml of 10 mg/l potassium ferricyanide (0.1% (w/v)) solution, resulting in a total volume of 2.0 ml. The solution was subjected to incubation in a temperature-controlled water bath set at 50 degrees Celsius for a duration of 20 minutes. Subsequently, a volume of 2.0 ml of trichloroacetic acid solution with a concentration of 100 mg/l (10% w/v) was introduced. A 2.0 ml aliquot of the mixture was blended with 2.0 ml of distilled water and 0.4 ml of a 0.1% (w/v) solution of ferric chloride (FeCl₃.6H₂O). The spectrophotometric analysis was conducted to determine the absorbance of the reaction mixture at a wavelength of 700 nm, following a 10-minute reaction period. The extract's ferric reducing antioxidant power was quantified in terms of gallic acid equivalent per gramme (GAE/g).

$$\text{FRAP} = A/A_0 \times M_0/M$$

Where A= sample absorbance

A₀= mean absorbance of the standard

M₀= mass of standard

M= mass of sample

Hydroxyl scavenging radical activity

The method described by Yu W²⁵ was utilized to measure the scavenging activity of the extracts on hydroxyl radical. To each 1.5mL of the diluted extract, 60 µL of FeCl₃ (1mM), 90 µL of 1,10-phenanthroline (1mM), 2.4mL of phosphate buffer (0.2M; pH 7.8), and 150 µL of H₂O₂ (0.17M) were added. Subsequently, the amalgamation underwent homogenization via a vortex and was subjected to an incubation period of 5 minutes at ambient temperature. The measurement of absorbance was taken at a wavelength of 560nm in comparison to the blank. The radical scavenging activity percentage of every extract was computed using the following equation:

$$\% \text{ Scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

Where, Abs control = Absorbance of control

Abs sample = Absorbance of sample

2,2-diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity

The DPPH free radical scavenging activity was conducted following the protocol outlined by Irshad *et al.*²⁶, with minor adjustments. A volume of 2 milliliters of extract solution at varying concentrations was combined with 2 milliliters of DPPH solution. Subsequently, the sample was subjected to a period of 30 minutes under dark conditions, after which the absorbance was determined at a wavelength of 517 nm utilizing a UV-Vis Spectrophotometer. The experiment was conducted with three replicates for each assay. The determination of radical scavenging activity was conducted through the utilization of the subsequent equation. The

percentage of scavenging activity was plotted against the concentration.

$$\% \text{ DPPH Scavenging activity} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100\%$$

Where Abs control = Absorbance of control

Abs sample = Absorbance of sample

Analysis of Phenolic Compounds by HPLC

The separation and detection of phenolic compounds in extracts is commonly achieved through the use of High Performance Liquid Chromatography (HPLC) with UV detection, which allows for the quantification of compounds. The samples were solubilized in distilled water at a proportion of 0.3 g per 10 mL and subjected to centrifugation at 4706 revolutions per minute for a duration of 10 minutes. The liquid portion of the sample was subjected to filtration using a cellulose acetate membrane filter (with a pore size of either 0.20 µm or 0.45 µm, manufactured by Schleicher & Schuell) and subsequently utilized for analytical purposes. An examination was conducted utilizing an Agilent Technologies 1200 HPLC system that was equipped with a SUPELCO SIL LC-18 column, which had a length of 250 mm, a diameter of 4.6 mm, and a packaging size of 5 mm. The temperature of the column was established at 20°C. The composition of the mobile phase comprised of an aqueous solution of acetic acid (0.5% v/v) denoted as "A" and acetonitrile denoted as "B". The elution process involved an initial 2-minute use of 100% solvent A, followed by a 58-minute period during which a mixture of 40% solvent A and 60% solvent B was utilized. The flow rate was established as 1 milliliter per minute and the volume of injection was 25 microliters. Polyphenols were identified utilizing a UV detector set at a wavelength of 280 nm. The study measured the retention times of the identified polyphenolic compounds using a single standard solution with a concentration of 100 mg/L.

Statistical Analysis

The statistical analysis was conducted using the SPSS 11 statistical package, and the results were presented as mean ±

standard deviation. The Student's t-test was utilized for the analysis. The study employed a one-way analysis of variance (ANOVA) to evaluate distinctions among the samples, and subsequently conducted post hoc comparisons utilizing Tukey's honestly significant difference (HSD) test. Statistical significance was determined at a significance level of $p < 0.05$. The experimental procedure was conducted in triplicate and the resulting data was analysed accordingly.

RESULTS

Table 1 displays the total antioxidant content, as well as the total phenolic and flavonoid compound contents, of various aqueous plant extracts. The results of the assays indicated a significant presence of antioxidants in the samples, along with substantial quantities of total flavonoid and moderate levels of total phenolic content, with gallic acid serving as the benchmark. The quantification of polyphenol oxidase activity revealed its presence in significant quantities.

The assays for evaluating the capacity of free radical scavenging, as presented in Table 2, typically rely on the deactivation of radicals, including hydroxyl (OH) and nitric oxide (NO) radicals. The study found that the aqueous ethanol extract of *G. latifolium* exhibited a significantly higher Nitric Oxide Scavenging Activity ($33.91 \pm 0.13b$) and Ferric Reducing Antioxidant potential ($6.64 \pm 0.07b$) compared to the standard. On the other hand, *Vernonia amygdalina* had a higher content of Hydroxyl Scavenging radical ($76.98 \pm 0.25c$) and 2,2-diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity ($94.08 \pm 0.11c$). The present study provides evidence that *V. amygdalina*, *P. Guineense*, and *G. latifolium* possess scavenging properties against DPPH•, OH•, NO•, and FRAP radical.

Table 3 presents the results of the HPLC analysis, which indicate the levels of identified phenolic content. The plant extracts were found to contain multiple phenolic compounds, including Ribalinidine, kaempferol, and Resveratol, with varying concentrations observed across all samples. The findings pertaining to the different peaks achieved through the utilization of aqueous plant extract have been illustrated in Figures 4 and 5.

Table 1: Antioxidant Capacity in Aqueous extracts of selected traditional anti-cough plants

Parameter	<i>Piper guineensis</i>	<i>Gongronema latifolium</i>	<i>Vernonia amygdalina</i>	Standard (Garlic acid)
Total Flavonoid Content(TFC)	11.28± 0.11 ^a	96.18± 0.11 ^b	10.83± 0.39 ^a	13.63± 0.67 ^c
Total Phenol content(TPC)	20.37± 0.06 ^a	28.50± 0.04 ^b	19.83± 0.04 ^c	38.84± 0.09 ^d
Total Antioxidant Capacity (TAC)	11.28± 0.11 ^a	96.18± 0.11 ^b	10.83± 0.39 ^a	13.63± 0.67 ^c
Polyphenol oxidase activity	0.05± 0.002 ^a	0.26± 0.04 ^b	7.14 ± 0.10 ^c	0.27 ± 0.02 ^b

Table 2: Free Radical Scavenging potential on aqueous extracts of selected traditional anti-cough plants

Antioxidant	<i>Piper guineensis</i>	<i>Gongronema latifolium</i>	<i>Veronia amygdalina</i>	Standard (Garlic acid)
Nitric Oxide Scavenging Activity	10.81± 0.10 ^a	33.91± 0.13 ^b	7.31± 0.03 ^c	9.75 ± 0.07 ^d
Ferric Reducing Antioxidant potential (FRAP)	2.15± 0.03 ^a	6.64± 0.07 ^b	2.13± 0.24 ^a	2.14± 0.06 ^a
Hydroxyl Scavenging radical	72.98± 0.62 ^a	32.94± 1.43 ^b	76.98 ± 0.25 ^c	71.88± 0.68 ^a
2,2-diphenyl-2-picrylhydrazyl (DPPH) Scavenging activity	92.19± 0.07 ^a	90.69± 0.11 ^b	94.08± 0.11 ^c	98.44± 0.01 ^d

Table 3: Phenolic profile of aqueous extracts of selected traditional *anti-cough plants*

Compounds	Concentrations(mg/ml)		
	<i>Piper guineense</i>	<i>Gongronema latifolium</i>	<i>Vernonia amygdalina</i>
Proanthocyanin	2.8995	-	2.998
Ribalinidine	5.8944	64.0834	3.0609
Ellagic acid	6.8944	-	15.6101
Sparteïn	2.5994	-	3.3288
Ferullic acid	25.8994	-	2.0014
Epicatechin	8.9944	-	6.4064
Sapogenin	7.3899	-	3.1998
Vanillic acid	14.6773	-	5.4591
Anthocyanin	4.3899	-	2.9737
Tannin	4.8993	-	-
Tyrosol	13.8993	-	-
kaempferol	2.3788	27.9606	3.7812
Naringenin	18.7884	-	1.1100
rutin	2.8995	-	1.3494
Resveratol	2.8994	11.384	2.3649
Protocatechuic	1.0884	-	-
Coumaric acid	-	-	15.4968
Ephedrine	-	-	0.6444
hydroxytyrosol	-	-	5.1641
Catechin	-	22.5308	-
Flavonone	-	33.4475	-
Flavan-3-ol	-	30.4883	-
Flavone	-	12.6566	-
Aglycone	-	41.6918	-
Lunamarin	-	76.3193	-
Gallocatechin	-	48.5752	-
Isoflavonoid	-	7.2342	-

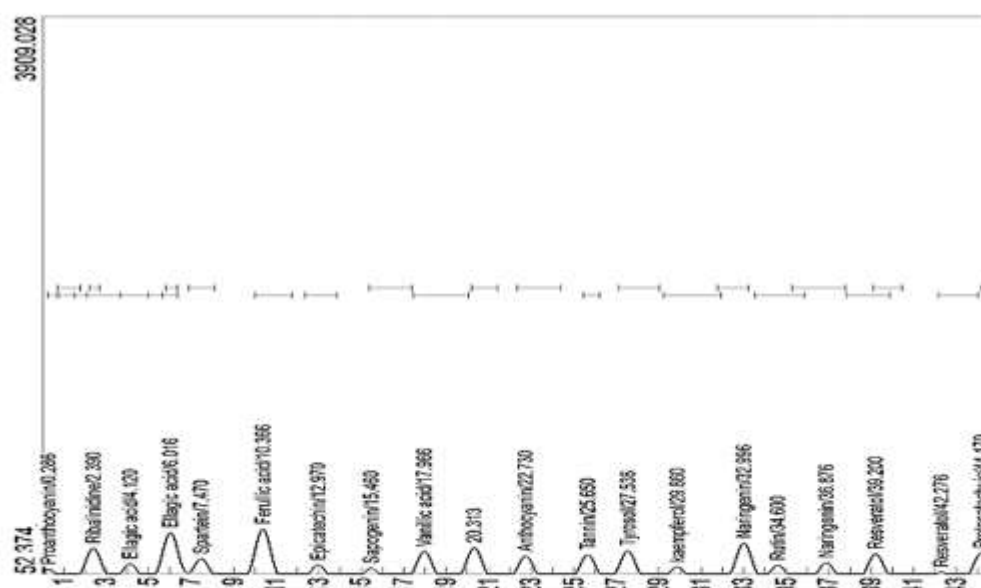


Figure 5: HPLC chromatogram of Phenolic profile of aqueous extracts of *Piper guineense*

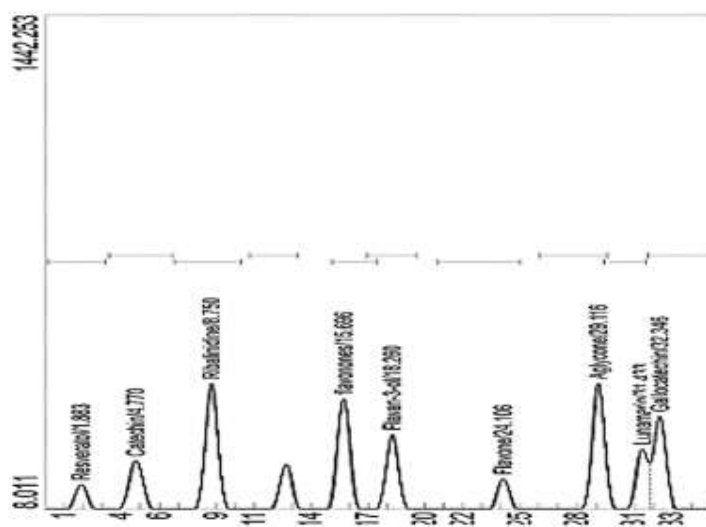


Figure 6: HPLC chromatogram of Phenolic profile in aqueous extracts of *Gongronema latifolium*

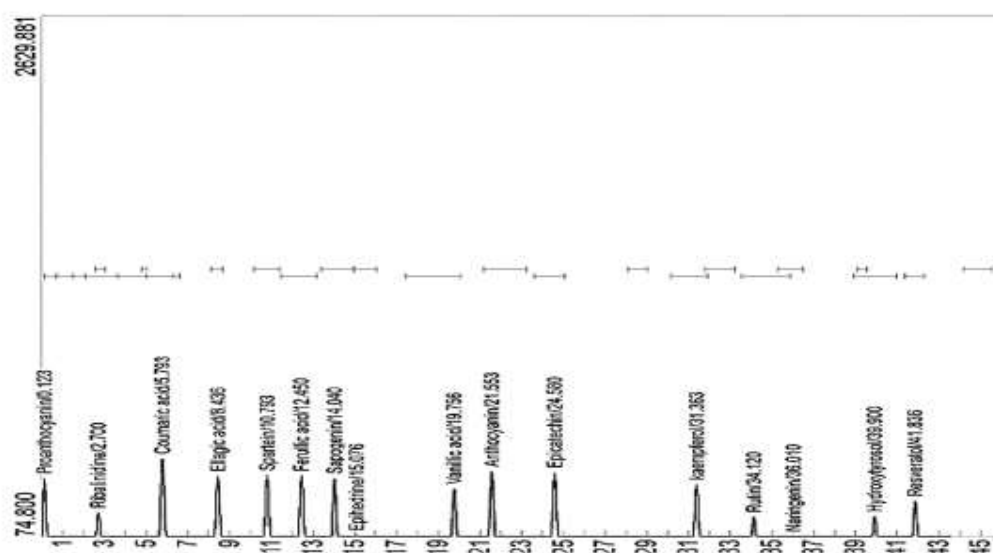


Figure 7: HPLC chromatogram of Phenolic profile in aqueous extracts of *Vernonia amygdalina*

DISCUSSION

Antioxidant Capacity

The flavonoid levels exhibited a range of variability, with values ranging from $10.83 \pm 0.39\%$ to $96.18 \pm 0.11\%$. The results indicate that *Gongronema latifolium* exhibited higher levels than the standard. This finding is consistent with the study conducted²⁷ which demonstrated that the antioxidant efficacy of *G. latifolium* leaves surpasses that of *Ocimum gratissimum*. This phenomenon could potentially be attributed to the synergistic interactions among multiple bioactive compounds that are present within the leaves. The results of the study indicate that *G. latifolium* exhibited the highest phenolic content ($28.50 \pm 0.04\%$) among the selected plants, while *V. amygdalina* displayed the lowest phenolic content ($19.83 \pm 0.04\%$). The measured values exhibited a moderate decrease in comparison to the standard of Garlic acid. The findings are consistent with²⁸ report on the total phenol content of aqueous and methanol in *G. latifolium*, which indicated a low phenol content and a prevalence of flavonol. The investigation involved an analysis of the total antioxidant capacity of aqueous extract derived from specific anti-cough plant species. The results indicated a range of

values, with the lowest being $10.83 \pm 0.39\%$ and the highest being $96.18 \pm 0.11\%$. *G. latifolium* showed high concentration where as *V. amygdalina* had the least total antioxidant capacity. This finding aligns with the research conducted by²⁹ regarding the total antioxidant property. The study found that *V. amygdalina* exhibited the highest level of polyphenol oxidase activity ($7.14 \pm 0.10\%$), while *G. latifolium* and *P. guineense* demonstrated the lowest levels when compared to the standard. The efficacy of polyphenols in scavenging radicals is dependent on their molecular structure, the arrangement of hydroxyl groups, the accessibility of phenolic hydrogen, and the potential for stabilization of HO and NO radicals through hydrogen donation or electron delocalization expansion, as noted³⁰.

Free Radical scavenging Property

The extract of *G. latifolium*, which exhibited a value of $33.91 \pm 0.13\%$, demonstrated the most potent inhibition of Nitric Oxide free radical. This was followed by the extract of *P. Guineense*, which exhibited a value of $10.81 \pm 0.10\%$. The extract of Vernonia amygdalina exhibited a moderate level of inhibition towards the NO radical, as evidenced by a value of $7.31 \pm 0.03\%$, in comparison to the standard. The study

observed a notable variation in the Ferric Reducing Antioxidant potential activity among the aqueous plant extracts. Notably, *G. latifolium* exhibited the highest Frap activity of $6.64 \pm 0.07\%$ in comparison to *P. guineense* and *V. amygdalina*. The hydroxyl scavenging radical effects of aqueous extract of *Vernonia amygdalina* were reported³¹. The results of our study indicate that *V. amygdalina* and *P. guineense* exhibit higher scavenging properties ($76.98 \pm 0.25\%$ and $72.98 \pm 0.62\%$, respectively) compared to *G. latifolium* ($32.94 \pm 1.43\%$) and the standard Garlic acid, with statistical significance at $p < 0.05$. The assessment of the antioxidant potential of naturally derived foods and plants is commonly conducted through the utilization of the scavenging ability of DPPH free radical. According to the data presented in the table, all of the extracts exhibited inhibitory potential against the DPPH free radical. The study evaluated the free radical scavenging activity of extracts obtained from *V. amygdalina*, *P. guineense*, and *G. latifolium* on DPPH. The results indicated that the extracts exhibited moderate scavenging power, with values of $94.08 \pm 0.11\%$, $92.19 \pm 0.07\%$, and $90.69 \pm 0.11\%$ for *V. amygdalina*, *P. guineense*, and *G. latifolium*, respectively. Among the tested extract samples at various concentrations, *V. amygdalina* exhibited the most noteworthy ($p < 0.05$) inhibitory potential with respect to DPPH scavenging activity compared to the other extracts and the standard. The moderate phenolic content present in *V. amygdalina* may have undergone oxidation due to exposure to external factors such as light, heat, and moisture, as observed²⁴.

Phenolic compounds

A fair correlation between antioxidant, free radical scavenging activity and phenolic content was observed among the three (3) plants. Hence, result showed that similar phenolic compounds Reveratrol, Ribalinidine and kaempferol were all present in plants extracts but in different concentrations. Following the twenty-seven identified compounds, significantly higher peak areas in band to varying peak values was noticed highest in *P. guineense* > *V. amygdalina* > *G. latifolium*. The findings are consistent with prior research that has exhibited the antioxidative capacity of botanical phytochemicals³². The concentration-dependent classification of these molecules can be categorised into six distinct groups. Polyphenol (Proanthocyanin, Ellagic acid, Epicatechin, Anthocyanin, Tannin, Resveratrol), Flavonoid (kaempferol, Naringenin, rutin, Catechin, Flavonone, Flavan-3-ol, Flavone Gallo catechi, Isoflavonoid), Glycoside (Aglycone, Lunamarine), Steroids, or saponin (Sapogenin), Phenol (Ribalinidine, Ferullic acid, Vanillic acid, Tyrosol, Protocatechuic, Coumaric acid, hydroxytyrosol), Alkaloid (Sparteine, Ephedrine).

CONCLUSION

The findings of the current investigation indicate that the plant extracts of *V. amygdalina*, *P. guineense*, and *G. latifolium* possess notable free radical scavenging properties and antioxidant capacity, which may be attributed to their high phenolic content. The traditional use of plants as anti-cough remedies is substantiated by the additional findings obtained through the analysis of the phenolic profile of the extracts

Conflict of Interest

No conflict of interest exist

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Authors' contributions

The work was carried out in collaboration among all Authors. Author O.L.O. carried out the analysis of the study and wrote the manuscript. Authors C.U.I and C.P.N conceptualized and designed the work. Author O.C.N managed the literature searches. Author C.U.I and J.C.E managed the statistical analysis. All Authors read and approved the final manuscript.

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