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In vitro Assessment of Free Radical Scavenging and Antioxidant Capacity of Ethanol Extract of *Ageratum conyzoides* Leaves

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Abstract

This study determined the *in vitro* free radical scavenging capacity of ethanol extract of *Ageratum conyzoides* leaves and its phytochemical contents. Qualitative and quantitative phytochemical screening of the leaf revealed the composition of alkaloids (14.60%), flavonoids (6.00%), saponins (6.72%), cyanogenic glycosides (5.71%), and tannins (30.77%). The ethanol extract of *Ageratum conyzoides* DPPH radical scavenging presented a concentration-dependent effect, achieving 50% inhibition of DPPH radicals (IC_{50}) at 89.50 ± 4.48 µg/ml, inhibiting lipid peroxides with an IC_{50} value of 161.81 ± 6.47µg/ml, and inhibiting 25% of hydroxyl radicals at 273.28 ± 13.66 µg/ml. The ethanol extract of *Ageratum conyzoides* leaves also showed significant inhibitory capacity on nitric oxide radicals, reducing power property and total antioxidant capacity. The phytochemical composition of *Ageratum conyzoides* leaves and the free radical scavenging capacity support its traditional usage for the treatment of a wide range of health disorders. The results supported the age-long use of extracts of *Ageratum conyzoides* leaves in the treatment and management of free radical-induced oxidative and nitrosative cellular damage and biochemical derangements with naturally occurring secondary plant metabolites.

Keywords: Oxidizing-species, inhibitory capacity, flavonoids, oxidative stress, lipid peroxidation

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1. Introduction

Plants and their products have served as food and medicine for humans [1, 2, 3] and other species. The efficacy of plant extracts against diseases and disease conditions is attributed to their abundant bioactive nutrients and phytochemicals. These compounds elicit biochemical and physiological activities in the body of living organisms [4,5]. The plant *Ageratum conyzoides* is native to Central America, Southeast Asia, South China, India, and West Africa [6]. In Nigeria, Igedes of the Middle Belt, Yorubas of the Southwest, and Igbos of the Southeast call it "Ufuopioko", Imiesu," and Nriewu," respectively [7]. The bioactive constituents of the *Ageratum conyzoides* plant have shown effectiveness against numerous diseases as the constituents are exploited and deployed for the treatment and management of various ailments and physiological conditions, such as burns and wounds, pneumonia, inflammation, asthma, spasm, hemostatic effects, gynecological diseases, leprosy, and other skin diseases [8, 9]. The *Ageratum conyzoides* plant is used in the treatment of HIV/AIDS by Igede people in Nigeria [7]. *Ageratum conyzoides* has also shown insecticidal properties [10]. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the common free radicals generated physiologically by living organisms. At a normal physiological level, ROS and RNS are involved in the beneficial metabolism of cells, eliciting cell signaling and homeostasis. However, at certain elevated levels, free radicals actions become deleterious, and such a condition is termed oxidative stress. Oxidative stress is achieved when physiological pro-oxidant activities and the antioxidant

capacity for scavenging excess reactive oxygen species are not balanced, causing cellular damage [11,12].whereas nitrosative stress results when physiological production of RNS exceeds the cell's scavenging capacity [13, 14]. Oxidative and nitrosative stress conditions negatively affect the physiological and biochemical functions of living organisms. Oxidative and nitrosative stress are challenging conditions due to the overwhelming generation of free radicals. These free radicals can damage biomolecules, lead to metabolic derangement, and are essential in the etiopathogenesis of numerous diseases [15]. Free radicals cause cell damage through covalent binding and lipid peroxidation mechanisms, which can result in tissue injury [16]. Natural antioxidants of plant origin have been implicated as having the capacity to protect biomolecules from free radical damage [17]. These natural antioxidants are found in plant nutrients and phytochemicals. The use of crude and purified extracts of plants has shown significant importance in the amelioration and/or prevention of several diseases due to their antioxidant capacity. The aim of this study was to determine preliminary phytochemicals and the *in vitro* free radical scavenging capacity of an ethanol extract of *Ageratum conyzoides* leaves.

2. Materials and methods

2.1. Collection and preparation of plant sample

Fresh leaves of *Ageratum conyzoides* were collected in August, 2019 at the premises of the Federal University of Technology, Owerri, Imo State, Nigeria. The plant was identified by Prof. Cyril I. Duruigbo, a plant taxonomist in the Department of Crop Science, Federal University of Technology, Owerri, Imo State. The plant sample was given voucher specimen number FUTO/BCH/0049 and retained at the authors' laboratory. The leaves of *Ageratum conyzoides* were air-dried at room temperature (28 ± 2°C), pulverized to powder with an electric blender, and stored in sterile, airtight containers pending extraction.

2.2. Preparation of ethanol extract

An ethanol extract of *A. conyzoides* leaves was obtained by the modified method of Ujowundu [18]. Portions (200 g) of the powdered leaves of *A. conyzoides* were macerated in 1000 ml of 80% ethanol in a glass bottle and placed on an electronic shaker at room temperature (28± 2°C) for 72 hours. The setup was done in three batches and pooled together during filtration. Coarse filtration of the slurry was done using muslin cloth, and fine filtration was done with Whatman No. 1 filter paper. The extract was concentrated using a rotary evaporator at a mild temperature and reduced pressure. The extract was cooled, labeled ethanol extract of *A. conyzoides*, placed in an airtight sample container, wrapped in aluminum foil, and stored at 4°C in a refrigerator.

2.3. Phytochemical Screening

Qualitative and quantitative phytochemical compositions of the leaves of *Ageratum conyzoides were* carried out to determine the presence and quantity of alkaloids, flavonoids, saponins, cyanogenic glycosides, and tannins, according to standard protocols as described by Sofowora [19], Trease and Evans [20], and Harborne [21].

2.4. Determination of Radical Scavenging Properties of A. conyzoides

Free radical scavenging potential of *Ageratum conyzoides was* evaluated by measuring the DPPH radical potentials, inhibition of hydroxyl radical, inhibition of nitric oxide radical, inhibition of lipid peroxidation, reducing power property, and total antioxidant capacity.

2.5. 2, 2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) radical scavenging assay

The radical scavenging activity of an ethanol extract of *A. conyzoides* for 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was determined by the method of Blois [22] as described by Velazquez *et al.* [23]. The set-up consisted of 1.0 ml of ethanol extract of *A. conyzoides*(0–500 mg/ml) dissolved in methanol and mixed with 2 ml of 0.02 mg/ml DPPH in methanol. The set-up was incubated for 15 minutes at room temperature (28±2°C) and the absorbance was measured at 517 nm. Tannic acid (0–500 mg/ml) served as a positive control. Radical scavenging activity was calculated as follows:

$$\%\ DPPH\ radical\ scavenging = \frac{Blank\ absorbance - Absorbance\ test}{Blank\ absorbance} \times 100$$

The scavenging data was fitted into mathematical equations, with the highest correlation coefficient used to evaluate the IC_{50} (concentration causing 50% inhibition) values of the ethanol extract of *A. conyzoides* and the standard.

2.6. Hydroxyl radical scavenging assay

The hydroxyl radical scavenging capacity of the ethanol extract of *A. conyzoides* was determined by assessing 2-deoxyribose degradation by hydroxyl radicals generated from the Fe3+/ascorbate/EDTA/H_2O_2 system as described by Halliwell *et al.* [24]. The reaction mixture has deoxyribose (2.8 mM), $FeCl_3$ (0.1 mM), EDTA (0.1 mM), H_2O_2 (1 mM), ascorbic acid (0.1 mM), KH_2PO_4/K_2HPO_4-KOH buffer (20 mM, pH 7.4), and the ethanol extract of *A. conyzoides* (0–3000 µg/ml) in a final volume of 1.0 ml. After incubation at 37°C for 1 hr, deoxyribose degradation was measured as thiobarbituric acid reactive substances (TBARS) by the method of Ohkawa *et al*. [25], as modified by Liu *et al.* [26]. Furthermore, the set-up was treated with 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thiobarbituric acid (TBA), and 0.2 ml of 8.1% sodium dodecyl sulphate (SDS). After heating the mixture for 1 hr to 100°C, it was allowed to cool to room temperature, and 2 ml of TCA was added, vortexed, and

centrifuged at 3000 rpm for 10 min. The absorbance reading of the supernatant was taken at 532nm. Inhibition of deoxyribose degradation was calculated as follows:

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

2.7. Nitric oxide radical scavenging assay

The nitric oxide scavenging capacity of the ethanol extract of *A. conyzoides* was determined by the method of Marcocci *et al*. [27], as described by Alisi and Onyeze [28]. The analysis was done in a 4 ml volume with varying concentrations of ethanol extract of *A. conyzoides*(0–2000 µg/ml) and stock solution of quercetin (0–1000 µg/ml) in phosphate buffer (pH 7.2). Thereafter, 1 ml of 5 mM sodium nitroprusside solution in phosphate buffer (pH 7.2) was added into test tubes and incubated at 29°C for 2 hr. The nitrite formed was measured by reacting 2 ml of the incubation solution with 1 ml of Griess reagent (equal volume of 1% sulphanilic acid and 0.1% N-(1-naphthyl) ethylene diaminedihydrochloride (NED). Absorbance was taken at 550 nm in a spectrophotometer. Inhibition of nitrite formation by ethanol extract of *A. conyzoides* or quercetin was calculated relative to the control, which was void of ethanol extract of *A. conyzoides* sor quercetin.

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

2.8. Inhibition of hydrogen peroxide-induced lipid peroxidation in rabbit stomach Homogenate

The capacity of the ethanol extract of *A. conyzoides* to inhibit lipid peroxidation in rabbit stomach was studied by incubating rabbit stomach homogenate treated with hydrogen peroxide (10µM) with different concentrations of the ethanol extract of *A. conyzoides* (0 – 1000 µg/ml). A mixture of 200 µl rabbit brain homogenate (4% w/v), an ethanol extract of *A. conyzoides* (0 – 1000 µg/ml) and 200 µL hydrogen peroxide (10µM) were incubated at 28±2°C for 1 hr. The TBARS produced was measured according to Liu *et al.* [26]. The incubation mixture, bearing 0.75 ml of 20% acetic acid (pH 3.5), 0.75 ml of thiobarbituric acid (1.0%), and 0.2 ml of sodium dodecyl sulphate (8.1%), was heated at 100ºC for 1 hr, cooled to room temperature, and 2 ml of 10% TCA added. Furthermore, the mixture was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant was taken at 532nm. Quercetin served as standard. The inhibition of lipid peroxide formation by an ethanol extract of *A. conyzoides* or quercetin was calculated relative to the control.

$$\%\ Inhibition = \frac{Absorbance\ control - Absorbance\ test}{Absorbance\ control} \times 100$$

2.9. Reducing power determination

The reducing power of an ethanol extract of *A. conyzoides* was determined using Fe^{3+}/Fe^{2+} transformation in the presence of the test compound, as described by Oyaizu [29] and Hsu *et al*. [30]. This was carried out in 2.5 ml of a graded concentration of ethanol extract of *A. conyzoides*(0 – 1000 µg/ml) in 200 mM phosphate buffer (pH 6.6), to which 2.5 ml of 1% $K_3Fe(CN)_6$ was added, thoroughly mixed, and incubated at 50ºC for 20 min. Then, 2.5 ml of 1% TCA was added to the above mixture and centrifuged at 3000 rpm for 5 minutes. Furthermore, 2.5 ml of the upper layer of the solution was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$. The absorbance was read at 700nm. Reducing power was determined from the plot of optical density against the concentration of the ethanol extract of *A. conyzoides*. Reducing power (RP 0.5AU) was taken as the concentration of ethanol extract of *A. conyzoides* that produced a 0.5 absorbance reading.

2.10. Total antioxidant activity

The total antioxidant capacity of the ethanol extract of *A. conyzoides* was determined by the method described by Prieto *et al*. [31]. A portion (0.4 ml) of ethanol extract of *A. conyzoides* dissolved in methanol (1 mg/ml) was mixed with 4 ml of phosphomolybdenum reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The set-up was incubated in a water bath at 95ºC for 90 minutes, cooled, and the absorbance reading taken at 695 nm. The total antioxidant activity of the sample was determined using a standard curve prepared for ascorbic acid. The total antioxidant capacity of the ethanol extract of *A. conyzoides* was expressed as mg of ascorbic acid equivalents (AAE) per gram of ethanol extract of *A. conyzoides*.

2.11. Data Analysis

Data values obtained in this study were fitted into kinetic model equations: Log Norm Cum, logistic dose response, decay 1+1 kinetic sigmoid abcd, and sigmoid abc models, respectively. The iterative minimization of least squares was used to determine the parameters of the software Levenberg-Marquardt algorithm (Table Curve 2D SYSTAT USA) (Oyaizu, 1986).

3. Results and Discussions

The phytochemical screening (Table 1) shows that *Ageratum conyzoides* leaves contain phenolics (flavonoids and tannins), alkaloids, saponins, and cyanogenic glycosides (HCN). Tannins showed the highest amount, followed by alkaloids.

Threshold inhibitory concentration (IC) of A. conyzoides extract and Butylated hydroxytoluene on DPPH radicals

Results presented in Figure 1.0 show the DPPH radical scavenging effect of ethanol extracts of *A. conyzoides* and BHT. The scavenging of DPPH radicals by the extracts and BHT closely fits into a sigmoidal relationship (Sigmoid abc). The extract's DPPH radical scavenging effect was concentration dependent; achieving 5% inhibition of DPPH radicals at doses of 15.23 ± 0.76 µg/mland 20.18 ± 1.01 µg/ml for *A. conyzoides* and BHT, respectively (Table 3.0). Evaluation of the threshold inhibitory concentrations (Table 2) shows the concentration required for a corresponding scavenging effect of 5-80% DPPH radicals. *A. conyzoides* extract scavenged 50% of the DPPH radical with an IC_{50} value of 89.50 ± 4.48 µg/ml, compared to 606.61 ± 30.33 µg/ml for BHT, respectively.

Threshold inhibitory concentration of A. conyzoides and butylated hydroxytoluene on lipid peroxidation

Results presented in Figure 2.0 show the inhibitory effect of ethanol extracts of *A. conyzoides* and BHT on lipid peroxidation. The inhibition of *in vitro* lipid peroxidation in rabbit brain homogenate by *A. conyzoides* ethanol extract was significantly sigmoidal (Sigmoid abc), while BHT fitted into a logistic dose response equation; consistently inhibiting peroxidation with increasing concentration of the extract.

Threshold inhibitory concentrations (IC) of hydroxyl radicals by A. conyzoides and quercetin

Figure 3.0 and Table 4 present the inhibitory capacity of *A. conyzoides* ethanol extracts and quercetin on hydroxyl radicals. The hydroxyl radical is the most potent oxidant and one of the most reactive natural free radicals. Due to their very short half-life, hydroxyl radicals react with molecules at the site of generation [32]. The inhibition of hydroxyl radicals by *A. Conyzoides* and quercetin followed a logistic dose response model (LDR abc). The inhibition of hydroxyl radicals was tightly concentration-dependent.

Threshold inhibitory concentration (IC) of nitric oxide radicals by A. conyzoides ethanol extract and ascorbic acid

Results presented in Figure 4.0 and Table 5 show the nitric oxide-reducing potential of *A. conyzoides*ethanol extract and ascorbic acid. The scavenging of nitric oxide radicals by the extracts fitted into a sigmoidal relationship (Sigmoid abc), while scavenging by ascorbic acid was in a logistic dose response manner. Evaluation of the threshold inhibitory concentrations (Table 6.0) shows *A. conyzoides* extract scavenged 50% of nitric oxide radicals with an IC_{50} value of 5188.70 ± 207.55µg/ml, compared to 27976.63 ± 1119.07 µg/ml for ascorbic acid.

Reducing power equivalent and total antioxidant capacity of A. conyzoides ethanol extract and ascorbic acid

Figure 4 show the plot of reducing power equivalent of *A. conyzoides* ethanol extract and ascorbic acid. The reducing power of a compound has shown to be a significant indicator of the potential antioxidative activity [30,33]. The plot of absorbance against respective concentration Result showed that the ethanol extract of *A. conyzoides* possess significant ability to reduce ferric ions in a dose-dependent manner described by a sigmoidal relationship (Fig. 4). The reducing power is the concentration of extract that produces an absorbance of 0.5 at λ_{max}= 700nm, reducing Fe^{3+} to Fe^{2+}. The reducing power equivalent was 213.78 ± 8.55 µg/ml and 32.79 ± 1.31 µg/ml for *A. conyzoides* ethanol extract and ascorbic acid, respectively.

Discussion

These phytochemicals (Table 1) are bioactively involved in the metabolism of numerous diseases and conditions. On intake, phytochemicals are implicated in antioxidant metabolism [34] and free radical scavenging [35] to eliminate and/or reduce the emergence of degenerative diseases [36, 37]. Alkaloids are used as analgesic, antispasmodic, and bactericidal agents, while tannins are astringents that hasten the healing of wounds and inflamed mucous membranes [38]. Flavonoids exhibit antibacterial, anti-inflammatory, antiallergic, anti-mutagenic, antiviral, anti-thrombotic, and vasodilatory activity [39, 40, 41]. Saponins are cytotoxic, inhibiting the growth of a variety of cells, making them an anti-inflammatory and anti-cancer agent. The significant amount of tannins and appreciable saponin content of *Ageratum conyzoides* leaves could be attributed to their use in treating wounds, preventing blood loss, etc. [42]. *Ageratum conyzoides s*extract with an IC_{50} value of 89.50 ± 4.48 µg/ml for DPPH (Figure 1 and Table 2) showed stronger radical scavenging capacity than *Lagenariaspherica* (108.30±8.60 µg/ml), *Monodoramyristica* (229.60±10.56 µg/ml) *Mucunarostrata* (231.40±12.21 µg/ml) as reported by Nwaoguikpe *et al.* [43]. Similarly, *A. conyzoides*extract presented better radical scavenging capacity than honey from *Apismelliferaw*ithin the inhibitory concentration (IC_{50}) of DPPH radicals evaluated at 12.74 ± 0.71mg/ml [44]. The content of flavonoids and tannins in *A. conyzoides* extract may contribute to its antioxidant capacity because extracts rich in phenolics and flavonoids have shown excellent *in vitro* free radical scavenging capacity [45, 46].

Table 1: Qualitative and quantitative phytochemical compositions of the ethanol leaf extract of *A. conyzoides*

Phytochemicals	Qualitative Composition	Quantitative Composition (%)
Alkaloids	++	14.60± 2.01
Flavonoids	+	6.00± 0.10
Saponins	+	6.72± 0.24
Cyanogenic glycosides	+	5.71± 0.04
Tannins	++++	30.77± 0.32

Key: + = Presence of phytochemicals; Values are means ± standard deviation of triplicate determinations

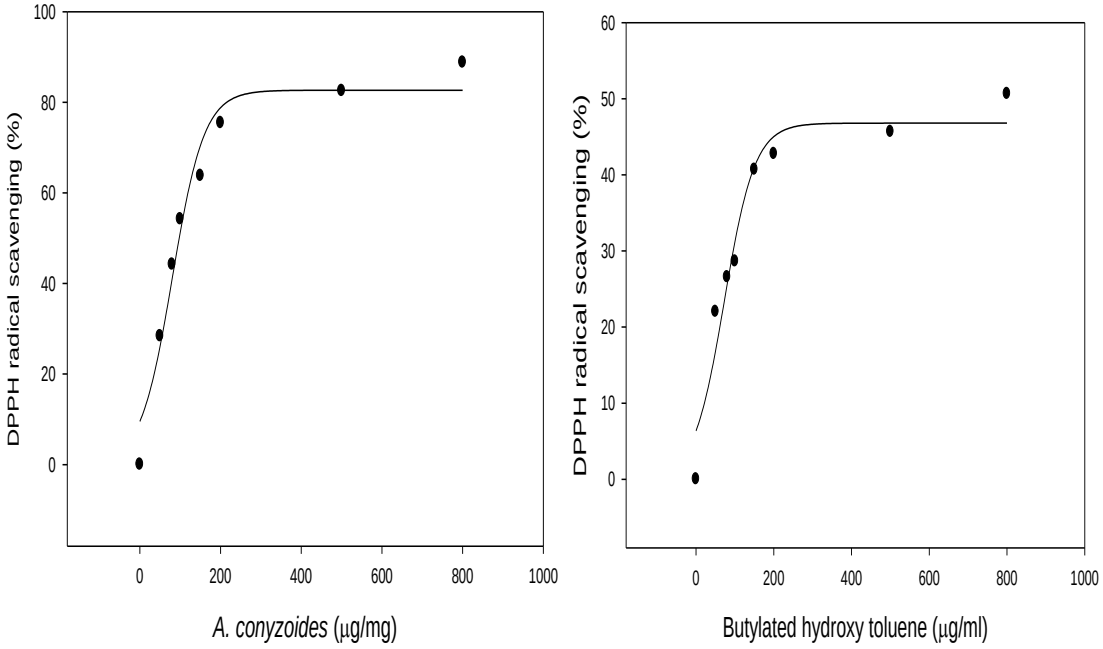

Figure 1: Effect of ethanol extract of *A. conyzoides* (A) and butylated hydroxytoluene (BHT) on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical

Table 2: Threshold inhibitory concentration (IC) of *A. conyzoides* ethanol extract and butylated hydroxyl toluene on DPPH radicals

Threshold Inhibition (%)	*A. conyzoides* (µg/ml)	Butylated hydroxytoluene (BHT) (µg/ml)
5	15.23 ± 0.76	20.18 ± 1.01
10	23.58 ± 1.18	32.76 ± 1.64
20	38.09 ± 1.90	58.57 ± 2.93
40	69.16 ± 3.46	162.01 ± 8.10
50	89.50 ± 4.48	606.61 ± 30.33
60	117.34 ± 5.87	ND
70	161.73 ± 8.09	ND
80	258.22 ± 12.91	ND
Model equation	Sigmoid abc $(y = \frac{a}{1+exp^{\left(-\frac{x-b}{c}\right)}})$	Sigmoid abc $(y = \frac{a}{1+exp^{\left(-\frac{x-b}{c}\right)}})$
R^2	0.967	0.949

Values are mean ± standard deviation of triplicate determinations. ND= Non determinable

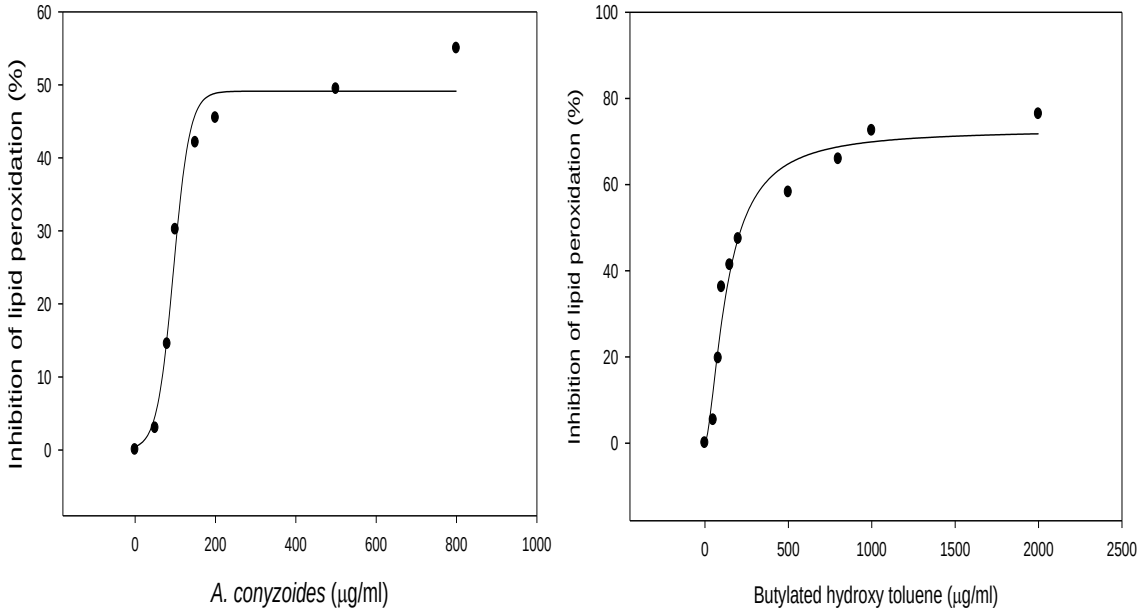

Figure 2: Inhibitory effect of ethanol extracts of *A. conyzoides* (A) and butylated hydroxytoluene (BHT) on lipid peroxidation

Table 3: Threshold inhibitory concentration of *A. conyzoides* and butylated hydroxytoluene on lipid peroxidation

Threshold Inhibition (%)	*A. conyzoides* ethanol extract (µg/ml)	Butylated hydroxytoluene (µg/ml)
5	56.89 ± 2.28	1.99 ± 0.10
10	68.15 ± 2.73	6.80 ± 0.34
20	84.39 ± 3.38	25.98 ± 1.30
25	91.92 ± 3.68	41.86 ± 2.09
40	119.66 ± 4.79	132.82 ± 6.64
50	161.81 ± 6.47	262.55 ± 13.13
60	ND	522.91 ± 26.15
70	ND	1122.67 ± 56.13
80	ND	2942.95 ± 147.15
Model equation	Sigmoid abc ($y = \frac{a}{1+exp^{(-x-b/c)}}$)	Logistic dose response abcd $y = a + \frac{b}{1+(x/c)^d}$
R^2	0.977	0.969

Values are mean ± standard deviation of triplicate determinations. ND= Non determinable

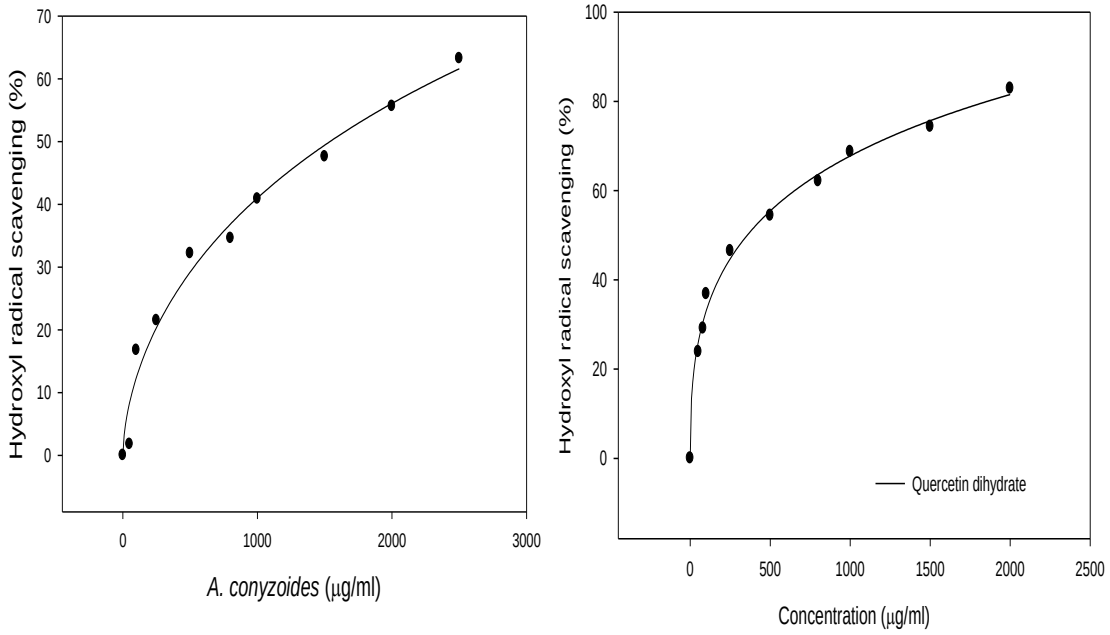

Figure 3: Effect of ethanol extracts of *A. conyzoides* (A) and quercetin dihydrate on hydroxyl radical

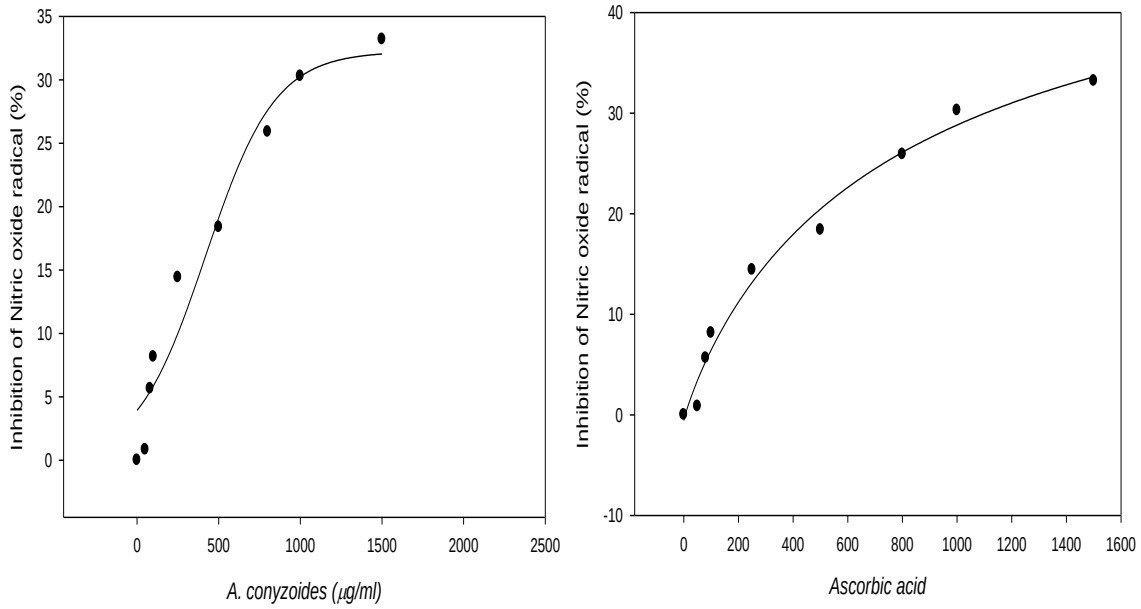

Figure 4: Nitric oxide radical scavenging properties of *A. conyzoides* ethanol extract and ascorbic acid

Table 4: Threshold inhibitory concentrations (IC) of hydroxyl radicals by *A. conyzoides* and quercetin

	Inhibitory concentration	
Threshold Inhibition (%)	*A. conyzoides* ethanol extract (µg/ml)	Quercetin (µg/ml)
5	5.58 ± 0.28	0.10 ± 0.00
10	28.86 ± 1.44	1.01 ± 0.05
20	156.05 ± 7.80	11.25 ± 0.56
25	273.28 ± 13.66	24.95 ± 1.25
50	1721.79 ± 86.09	332.30 ± 16.62
70	4629.68 ± 231.48	1300.32 ± 65.02
80	7062.23 ± 353.11	2308.02 ± 115.40
100	15125.43 ± 756.27	6385.07 ± 319.25
Model equation	Logistic dose response abc $y = \frac{a}{1 + (x/x_0)^b}$	Logistic dose response abc $y = \frac{a}{1 + (x/x_0)^b}$
R^2	0.980	0.991

Values are mean ± standard deviation of triplicate determinations

Table 5: Threshold inhibitory concentration (IC) of nitric oxide radicals by *A. conyzoides* ethanol extract and ascorbic acid

	Concentration (µg/ml)	
Threshold Inhibition	*A. conyzoides*	Ascorbic acid
5	26.88 ± 1.08	65.55 ± 2.62
10	116.09 ± 4.64	161.01 ± 6.44
20	534.29 ± 21.37	472.36 ± 18.89
40	2845.69 ± 113.83	3046.10 ± 121.84
50	5188.70 ± 207.55	27976.63 ± 1119.07
60	8812.31 ± 352.49	ND
70	14331.54 ± 573.26	ND
80	22721.45 ± 908.86	ND
Model equation	Sigmoid abc ($y = \frac{a}{1+exp\left(-\frac{x-b}{c}\right)}$)	Logistic dose response abcd $y = a + \frac{b}{1+(x/c)^d}$
R^2	0.952	0.985

Values are mean ± standard deviation of triplicate determinations. ND= Non determinable

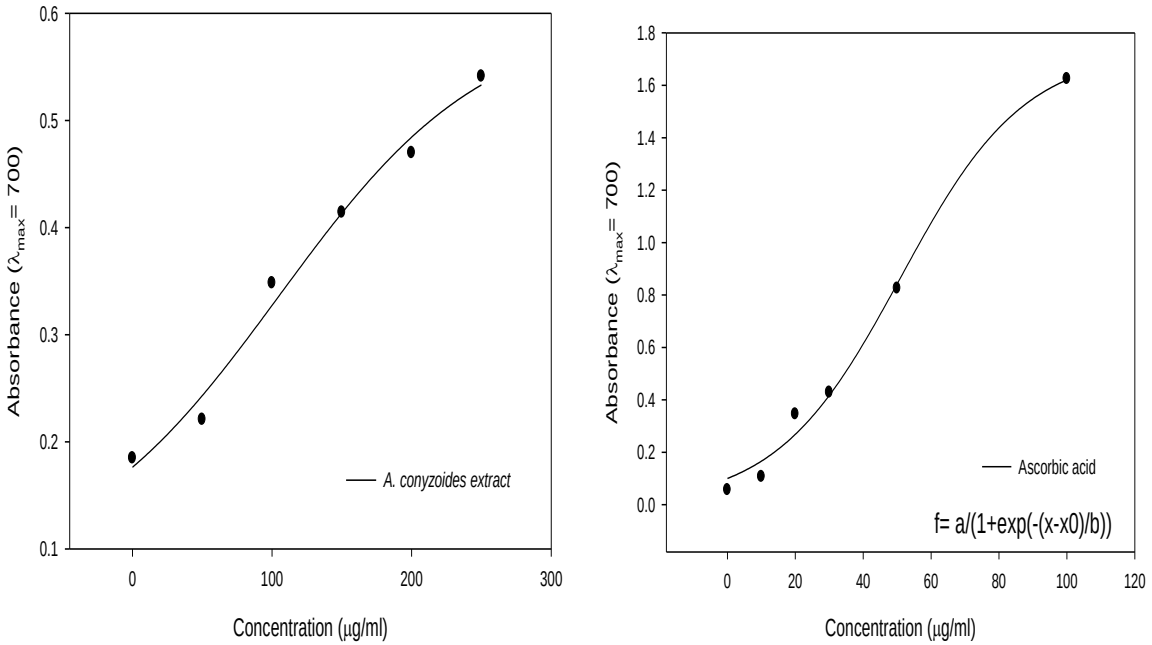

Figure 5: Reducing power property of *A. conyzoides* ethanol extract and ascorbic acid

Table 6: Reducing power equivalent and total antioxidant capacity of *A. conyzoides* ethanol extract and Ascorbic acid

	A. conyzoides extract (µg/ml)	Ascorbic acid (µg/ml)
Reducing power equivalent (O.D = 0.5)	213.78 ± 8.55	32.79 ± 1.31
Total antioxidant capacity (mg AAE/g extract)	32.33 ± 0.96	-

Values are Mean ± standard deviation of three determinations

Reducing power equivalent ≡ concentration at absorbance 0.5

The maximum scavenging activity obtained for *A. conyzoides* extract was 80% at 258.22 ± 12.91µg/ml, while beyond 50%, the BHT scavenging effect was non determinable. The inhibition of lipid peroxidation (Figure 2 and Table 3) in the brain is neuroprotection against loss of brain barrier function [47, 48]. The inhibition of lipid peroxidation by *A. conyzoides* extract exhibited saturation at 200 µg/ml; beyond this concentration, further increases resulted in no net change in inhibition. The evaluation of the threshold inhibitory concentrations (Table 3) shows the concentration required for a corresponding inhibition of 5–50% peroxidation by *A. conyzoides* and BHT. The extract inhibited 50% of lipid peroxides with an IC_{50} value of 161.81 ± 6.47µg/ml, compared to 262.55 ± 13.13µg/ml for BHT, respectively. The IC_{50} value of *A. conyzoides* extract indicates a strong capacity to inhibit membrane lipid peroxidation. Lipid peroxidation commonly occurs in membranes that are rich in unsaturated fatty acids, with linoleic acid and arachidonic acid being the most susceptible to oxidative metabolism [49]. The maximum scavenging activity obtained for *A. conyzoides* extract was 50%, beyond which inhibition was non determinable. *Ageratum conyzoides* extract compared with quercetin in hydroxyl radical scavenging was effective but less potent than quercetin (Figure 3 and Table 5). *A. conyzoides* ethanol extracts inhibited 25% of the hydroxyl radical at 273.28 ± 13.66 µg/ml and this was within the 250 µg/ml at 25% for *Gnetum africanum* Welw reported by Iheme *et al*. [50]. The *A. conyzoides* extract inhibited 50% of hydroxyl radicals with an IC_{50} value of 1721.79±86.09 µg/ml, compared to 332.30 ± 16.62 µg/ml for quercetin. This indicates that *A. conyzoides* extract can inhibit hydroxyl radical-induced damage to DNA molecules. Hydroxyl radicals ($OH^{\bullet}$) have been shown to induce DNA fragmentation and DNA strand breaks [51, 52]. This inhibition of hydroxyl radicals can be attributed to *A. conyzoides* appreciable flavonoid content, but especially the high tannin content (30%, Table 2). Tannins are water-soluble flavonoid polymers [53] reported to possess anticarcinogenic and antimutagenic potentials [54]. Furthermore, complete scavenging of hydroxyl radicals was observed at 15125.43±756.27µg/ml of *A. conyzoides* extract. *Ageratum conyzoides* extract scavenged 15% of nitric oxide radicals with an IC_{15} value of 245µg/ml (Figure 4 and Table 5) and within the range scavenged by *G. africanum* Welw reported by Iheme *et al*. [50]. Nitric oxide (NO) actively reacts with the superoxide anion, forming peroxynitrite (ONOO), a cytotoxic molecule, that induces the nitration or hydroxylation of aromatic compounds [55]. Peroxynitrite can also induce physiological oxidative damage to proteins [56, 57]. The scavenging of nitric oxide radicals at the rate observed in this study serves as a significant indicator of *A. conyzoides* extract's potential antioxidant activity [52, 58]. The nitric oxide scavenging activity of phenolics (flavonoids and tannins) in *A. conyzoides* extract) might be responsible [59]. The maximum scavenging activity obtained for *A. conyzoides* extract was 80% at 22721.45 ± 908.86µg/ml, while beyond 50%, the ascorbic acid scavenging effect was non determinable. The reducing power of 213.78 ± 8.55 µg/ml presented by the extract of *A. conyzoides* (Figure 5) was within the value of 210.22 µg/ml for extracts of *Chromoleana odorata* [44]. However, *C. dolichopentalum* leaf extract presented a reducing power of 29.95 µg/ml [60]. The observed reducing power and/or antioxidative power can be attributed to the appreciable content of antioxidant phytochemicals [61]. A comparative determination of total antioxidant capacity (Table 6) showed that the antioxidant capacity of A. *conyzoides* ethanol extract was estimated at 32.33 ± 0.96 mg ascorbic acid equivalent/g extract (Table 6). The result of reducing power showed that *A. conyzoides* ethanol leaf extracts contain antioxidants capable of donating electrons [62, 63] to cause reduction of oxidized intermediates produced during lipid peroxidation. Adetuyi *et al*. [9] reported that methanol extracts of *A. conyzoides* can be used as a potent natural antioxidant against free radicals and for the management of erectile dysfunction caused by oxidative stress.

4. Conclusions

The results of this study have shown that *Ageratum conyzoides* contains phytochemical components that support its traditional usage for the treatment of a wide range of health disorders. In the present study, the plant was proven to possess good free radical scavenging activity. This study also corroborates the use of the plant in ethnomedicine to cure several infections. These promising properties may be due to the presence of various phytochemicals such as tannins, saponins, alkaloids, and flavonoids.

Conflict of interest

The authors declare no conflict of interest.

Authors contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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