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PHYTOCHEMICAL, PROXIMATE AND IN-VITRO ANTIOXIDANT ANALYSES OF *Ageratum conyzoides* LEAF

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ABSTRACT

Before the emergence of pharmaceuticals, different cultures worldwide depended on medicinal plants to combat diseases. *Ageratum conyzoides*, an annual herb that possesses a rich record of medicinal use has been recognized for its pharmacological properties. This study aimed to examine the proximate content analysis, phytochemical components plus *in-vitro* antioxidant potentials of ethanol extract of *A. conyzoides* leaf (ELEAC). Standard procedures were employed to conduct proximate and mineral composition analysis as well as qualitative and quantitative phytochemical assessments, while *in vitro* antioxidant assays were done using 1,1- Diphenyl2-picryl hydrazyl (DPPH) radical scavenging, Ferric Reducing Antioxidant Power (FRAP), Total Phenol Content (TPC) assays. Qualitative phytochemical assessment recorded the presence of saponins, tannins, terpenoids, alkaloids, phenols, and flavonoids. Proximate and mineral analysis revealed high carbohydrate content (84.97%), along with crude protein (1.53%), crude fat (3.27%), moisture (9.00%), Magnesium (42.30 mg/L), Sodium (73.21 mg/L) and Iron (4.30 mg/L). The extract exhibited a % DPPH radical scavenging activity of 44.63 at 200 µg/ml and 56.05 at 1000 µg/mL while the FRAP assay indicated a concentration-dependent increase in absorbance from 0.544 at 20 µg/mL to 0.756 at 100 µg/ml. TPC of the extract was quantified as 1.40 µg gallic acid equivalent/ mg of residue at 200 µg/mL and 2.22 µg gallic acid equivalent/ mg of residue at 1000 µg/mL. In conclusion, this study affirms the presence of medicinal phytochemicals in *A. conyzoides*. The identified phytochemicals, nutrients, and minerals with the demonstrated antioxidant activities, lend credibility to the traditional use of *A, conyzoides* in herbal medicine.

Keywords: *Ageratum conyzoides, antioxidant activity, herbal medicine, medicinal plant, phytochemicals, proximate analysis*

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INTRODUCTION

The scientific exploration of the traditional knowledge of the use of herbs in treating diseases is a key focus of research because herbal medicines are highly sought after in both developed and developing countries for primary healthcare due to their broad medicinal activities, enhanced safety margins, and cost-effectiveness (Qato *et al.,* 2008; Cohen and Ernst, 2010).

Phytochemicals are naturally occurring compounds within plants that serve as protective agents against diseases and damage, despite lacking nutritive value (Mathai, 2000; Prakash *et al.,* 2020). These compounds, known as secondary plant metabolites, highlight the medicinal properties of plants by exhibiting diverse biological characteristics depicting potential chemotherapeutic principles for prospective drug development (Alabi *et al.,* 2011; Egamberdieva *et al.,* 2017). Phytochemicals exist in several major groups such as phenols, flavonoids, carotenoids, terpenoids, alkaloids, saponins, and tannins with distinct chemical structures (Adebo and Gabriela Medina-Meza, 2020; Loi *et al.,* 2020). These plant chemicals exhibit diverse attributes such as antimicrobial, anti-inflammatory, antioxidant, anticarcinogenic, anthelmintic, antiproliferative, and antigenotoxic effects, providing therapeutic efficacy against ailments or pathogens (Makhafola *et al.,* 2017; Lahlou *et al.,* 2019).

Antioxidants, naturally present in both food and the body system, play a crucial role in preventing cell damage caused by oxidative destruction resulting from free radical generation (Mititelu *et al.,* 2020; Sharifi Rad *et al.,* 2020). Literature has outlined various pathways through which antioxidants act, including impeding the spread of free radicals and lessening or suppressing free radical generation with the assistance of metal-chelating agents thereby decreasing cellular reactive oxygen species creation, and influencing the overall antioxidant mechanism (Padureanu *et al.,* 2019; Calina *et al.,* 2020). Scientists worldwide study natural plants, herbal medicines and nutrient-dense foods, revealing substantial therapeutic capabilities such as antidiabetic, anticancer, lipid-lowering, anti-inflammatory, and antibacterial activities (Rouhi *et al.,* 2017; Chen *et al.,* 2018).

The therapeutic potentials of indigenous plant products for both medicinal and nutritional purposes have sparked the search for bioactive compounds within these resources. Medicinal plants contain crucial nutritional elements like carbohydrates, proteins, and fats, vital for various physiological, metabolic, and morphological functions in the human body (Radha *et al.,* 2021). In addition to this, the diverse array of phytochemicals and elemental compositions present in plants significantly influences their medicinal effectiveness. Hence, delving into the phytoconstituents, nutritional, and mineral components within medicinal plants becomes crucial for evaluating their therapeutic capabilities.

One such frequently employed medicinal plant found in Africa, including Nigeria, is *Ageratum conyzoides*. Popularly known as goat-weed, *A. conyzoides* earned its name due to its odor resembling that of male goats in Australia (Igoli *et al.,* 2005). This plant has been famous from early ages for its healing attributes and is being employed in the management of a variety of ailments including burns/wounds, and bacterial infections. arthrosis, headaches, gynecological diseases, and skin conditions (Kamboj and Saluja, 2008). The goal of this investigation

was to explore the proximate composition, phytochemical constituents as well as *in-vitro* antioxidant capabilities of the ethanol extract of *A. conyzoides* leaf (ELEAC).

MATERIALS AND METHODS

COLLECTION OF PLANT

Newly harvested *A. conyzoides* collected from a community in the Odo-ona area of Ibadan, Nigeria was verified at the herbarium of Forestry Research Institute of Nigeria with Herbarium number 113595.

PLANT EXTRACT PREPARATION

Freshly harvested leaves of A. *conyzoides* were air-dried at ambient temperature for two weeks after which it was milled in an electric mill. The powdered sample was soaked in ethanol for 72 hours and filtered using a Whatman filter paper. The filtrate was concentrated by evaporating it in a rotary evaporator resulting in a dark green substance, which was utilized as the extract in the study.

PHYTOCHEMICAL ANALYSIS

Qualitative and quantitative phytochemical analysis using different reagents and test procedures was carried out according to Sofowora (2008) and Krishnaiah *et al.,* (2009).

PROXIMATE TESTING

Proximate analysis was conducted following the protocol of the Association of Official Analytical Chemists (AOAC, 1990). The ethanol extract of *A.conyzoides* leaf was examined for nutrient composition of carbohydrate, protein, fat, crude fiber, moisture, and ash contents.

EVALUATION OF MINERAL COMPONENTS

The extract was subjected to incineration to obtain ash, which was then dissolved in 1 ml of 2M HCl and made up to a volume of 100 ml using deionized water. The resultant mixture was utilized to measure the quantities of Na, Mg, P, Fe, Zn, Cr, Pb, Ni, and Cu using an atomic absorption spectrophotometer (Buck Scientific AAS 200 A), following the method outlined by Igwe *et al.,* (2007). Phosphorus levels were assessed utilizing the vanadiumolybdate colorimetric technique as detailed by Pearson (Pearson, 1976).

ANTIOXIDANT ASSAYS

Assessment of Total Phenolic Content

The total phenolic contents were assayed by the Folin-Ciocalteau technique (Singleton *et al.,* 1999). Different dilutions of ELEAC were homogenized with Folin Ciocalteu reagent for 5 min, afterward aq. Na_2CO_3 (4 ml, 1 M) was added. Following a 15-minute incubation period, phenols were measured using a colorimetric (UV/Visible spectrophotometer (Perkin Elmer, Singapore) method at a wavelength of 765 nm. An appropriate standard curve was generated with various concentrations of Gallic acid dissolved in ethanol: water (50:50, v/v) in µg/ml solutions. The total phenolic compound contents were reported in terms of µg GAE (Gallic Acid Equivalent) per milligram of dry residue.

Determination of DPPH radical scavenging activity

To assess the antioxidant capability as a result of free radical hunting by the extract, the activity of DPPH radicals was observed as specified by the technique of Manzocco *et al.,* (2008). The extract was prepared with ethanol in five different concentrations starting from 200-1000 µg/mL. 1mL of the different concentrations was transferred into a test tube and 1mL DPPH solution (0.3 mM) was included. At the end of 30 minutes, the absorbance was quantified at 517 nm by utilizing a UV/Visible spectrophotometer (Perkin Elmer, Singapore). To compute the proportion of the DPPH radical hunting by ELEAC, the following formula was utilized:

% inhibition of DPPH radical = ([Abb – Aba] / Abb) x 100 (1)

Here, Abb represents the absorbance before the reaction (blank), and Aba signifies the absorbance after the reaction. The IC_{50} value, indicating the sample concentration considered necessary to neutralize 50% of the DPPH free radical, was computed from the inhibition (%) plotted against the concentration of the extracted graph.

Determination of Ferric Ion Reducing Antioxidant Power (FRAP)

The FRAP protocol, as detailed by Oyaizu (1986), was followed. The extract was combined with 2.5 ml phosphate buffer (20 mM) and 2.5 ml of 1% potassium ferricyanide at various concentrations. After incubating the mixture for 30 mins at 50°C, 2.5 ml of 10% trichloroacetic acid and 0.5 ml of 0.1% ferric chloride were introduced. The solution was allowed to stand for 10 mins, and its absorbance was considered at 700 nm utilizing a UV/Visible spectrophotometer (Perkin Elmer, Singapore).

STATISTICAL ANALYSIS

The procedures were replicated three times, and the outcomes were presented as mean ± SD (standard deviation), calculated using EXCEL software.

RESULTS AND DISCUSSION

The outcomes of the qualitative and quantitative phytochemical assessments in this study are shown in Tables 1 and 2. Qualitative phytochemical results indicated the occurrence of tannins, flavonoids, cardiac glycosides, anthraquinones, phenols, saponins, terpenoids, and alkaloids. In addition, the quantitative phytochemical assessments showed that saponins were present in the highest concentration (2.00 %), followed by alkaloids (1.15 %), tannins (1.02%) and phenols (0.70%). This aligns with reports and findings of other researchers such as Kambooj and Saluja (2008) and Kaur and Dogra, (2013) who had similar results and can be well correlated with the many medicinal uses of the plant in disease therapy. The detected phytochemicals in this study are known for their medicinal and physiological activities, highlighting the continuous significance of the herb. Saponins exhibit antimicrobial and act as natural antibiotics that enhance the body's ability to combat infections, possess anti-inflammatory activities, lower cholesterol and improve vaccine effectiveness (Okwu and Ndu, 2006; Odeleye et al., 2014). Flavonoids, alkaloids, and phenols possess antibacterial, anti-inflammatory, and vasodilatory activities. Flavonoids, in particular, show potential in scavenging radicals and act as antioxidants in biological systems, protecting against inflammation, platelet aggregation, and tumors (Amadi et al., 2012).

Table 1: Qualitative phytochemical assessment of ethanol leaf extract of *A. conyzoides*

Phytochemical	Quality
Saponins	++
Tannins	+
Flavonoids	+
Cardiac Glycosides	+
Anthraquinones	+
Terpenoids	+
Steroids	-
Alkaloids	++
Phenol	+

Key: + = slight; ++ = abundant - = absent

Table 2: Quantitative phytochemical assessment of ethanol leaf extract of *A. conyzoides*

Phytochemical	% Chemical w/w
Saponins	2.00
Tannins	1.02
Flavonoids	0.57
Terpenoids	0.50
Alkaloids	1.15
Phenols	0.70

The proximate analysis of *A. conyzoides* (Table 3) indicated a carbohydrate content of 84.97%, higher than the reported value for dried *A. conyzoides* leaf (36.84%) by Agbafor *et al.,* (2015). Carbohydrates are essential organic compounds crucial for sustaining life and providing energy both in plants and animals (Oladele and Oshodi, 2007). The fat content, relatively higher at 3.27%, compared to dried *A. conyzoides* leaf (2.27%), suggests that the ethanol extract may be more palatable because nutritional fats function to improve food palatability by enhancing flavor absorption and retention in food (Rehman and Adnan, 2018). The presence of crude fiber may contribute to the plant's medicinal applications, as clinical studies suggest that fiber exerts various benefits related to bowel functionality, gut well-being, and immunity (Hameed and Hussain, 2015). The moisture content of 9.00% aligns favorably with the reported values for dried *A. conyzoides* leaf and root (10.02% and 13.35%, respectively), indicating that the leaf extract is less prone to deterioration, as high moisture content in food correlates with deterioration due to microbial contamination growth (Adegbolagun *et al.,* 2021).

The elemental study of the plant revealed the accumulation of various minerals signifying the plant's rich mineral content (Table 4). The observed zinc content (0.31mg/ml) is lower than the value reported by Nwankpa (2015). The presence of zinc may also explain the plant's utilization in wound care, given its suppressive effect on bacterial

growth and involvement in immune responses. However, the high sodium content (73.21 mg/ml) may present a potential drawback due to its direct correlation with hypertension, as reducing sodium intake can mitigate the development of hypertension (Morgan, 1999).

Table 3: Proximate analysis of ethanol leaf extract of *A. conyzoides*

Constituent	**(% content w/w)**
Ash content	0.48±0.03
Crude fibre	0.75±0.07
Fat content	3.27±0.06
Moisture content	9.00±0.14
Crude protein	1.53±0.02
Carbohydrates	84.97±0.58

n=3, mean±SD.

Table 4: Mineral content ethanol leaf extract of *A. conyzoides*

Nutrients	**Content (mg/L)**
Magnesium	42.30
Phosphorus	1.77
Sodium	73.21
Iron	4.30
Copper	0.24
Zinc	0.31
Chromium	0.43
Lead	0.00
Nickel	0.00

Cells and tissues produce free radicals internally through processes like inflammation, and metabolism, or they can stem from external sources such as irradiation, certain foods, or drugs. Additionally, a reduction in the body's protective capacity can lead to the generation of these unstable molecules. Elevated production of free radicals can potentially cause oxidative damage within the system (Ghiselli *et al.,* 2000; Kohen *et al.,* 2002). Therefore, in current times, there is a keen focus on assessing the antioxidant activity of plant extracts to explore potential therapeutic characteristics (Ghani *et al.,* 2019).

The DPPH method of evaluating antioxidant potential is one of the most efficient and consistent *in vitro* approaches. This method exhibits the capacity to capture and neutralize free radicals, providing valuable insights into the

antioxidant capabilities of the tested extracts (Bruck de Souza *et al.*, 2020). In this study, ELEAC presented a concentration-dependent rise in radical scavenging capacity (Figure 1) of 42.3% at 200 µg/mL to 57.4% at 1000 µg/mL and IC_{50} value of 595.56 µg/mL, indicating a low antioxidant capacity of the ELEAC. Patel and Modi (2021) reported moderate antioxidant activity for the ethanol extract of the root and stem of *A conyzoides* with IC_{50} values of 248.99 µg/mL and 229.38 µg/mL respectively. The difference in these values could be as a result of differences in locations which may cause variations in the content of secondary metabolites possessed by these plants (Li *et al.*, 2020). Moreover, prior studies have established a positive relationship between the antioxidant capabilities of plant extracts and the presence of phenolics and flavonoids (Prakash *et al.*, 2007; Piluzza and Bullitta, 2011) and in this present work, ELEAC showed a moderate presence of phenolics and flavonoids. Additionally, it has been suggested that the combined action of multiple phytochemicals might lead to alterations in both the physiological effects and the bioavailability of each constituent in relation to antioxidant levels (Phan *et al.*, 2016). Moreover, the TPC of the extract (Table 5) also followed the same pattern corroborating the fact that the occurrence of phenolic constituents in the extract corresponds with the antioxidant effect of the extract because the hydroxyl group of phenolic compounds neutralises radicals and advances the antioxidant effect (Duh, 1994).

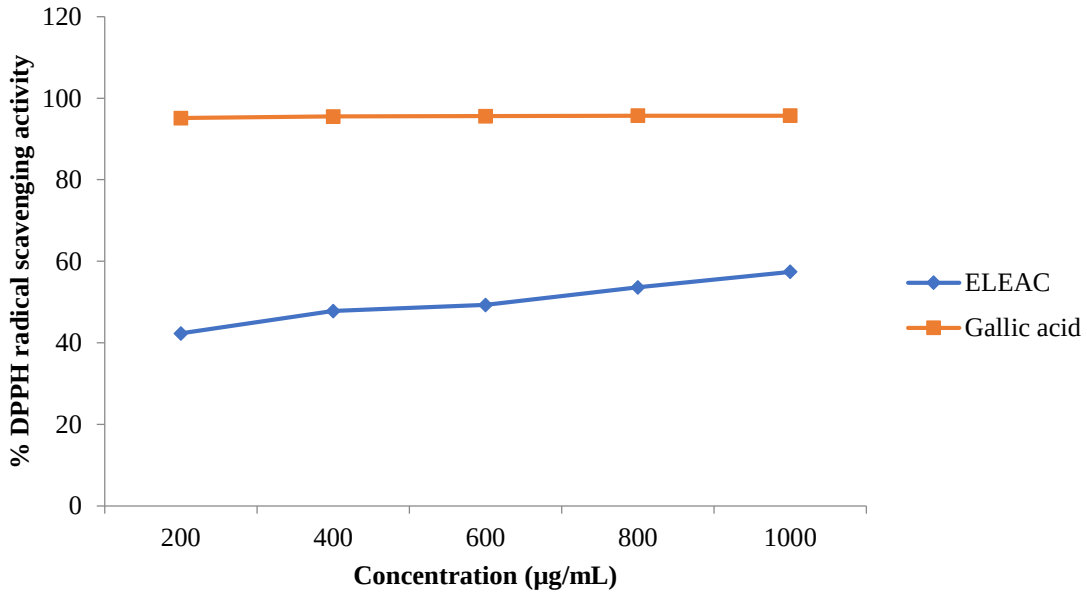

Figure 1: DPPH radical scavenging activity of ethanol leaf extract of *A. conyzoides* (ELEAC) and the reference standard (Gallic acid). (n=3, mean±SD).

Table 5: Total phenol content (TPC) of ethanol leaf extract of *A. conyzoides*

Concentration (µg/mL)	TPC µgGAE/mg residue
200	1.40
400	1.55
600	1.82
800	2.06
1000	2.22

The FRAP technique was further utilized to assess the antioxidant capacity of ELEAC. This assay involves the reduction of Fe^{3+} to Fe^{2+}, changing its coloration to blue with the occurence of antioxidant substances where increases in absorbance point towards an increase in reducing power (Dewan *et al.*, 2013). The outcomes of this study demonstrated a concentration-dependent rise in absorbance values (Figure 2) of *A conyzoides* indicative of appreciable reducing power similar to the findings of Hossain et al. (2013). This reducing attributes are related to the occurrence of antioxidants, which have demonstrated the ability to execute their antioxidant power by donating a hydrogen atom (Duh *et al.*, 1999).

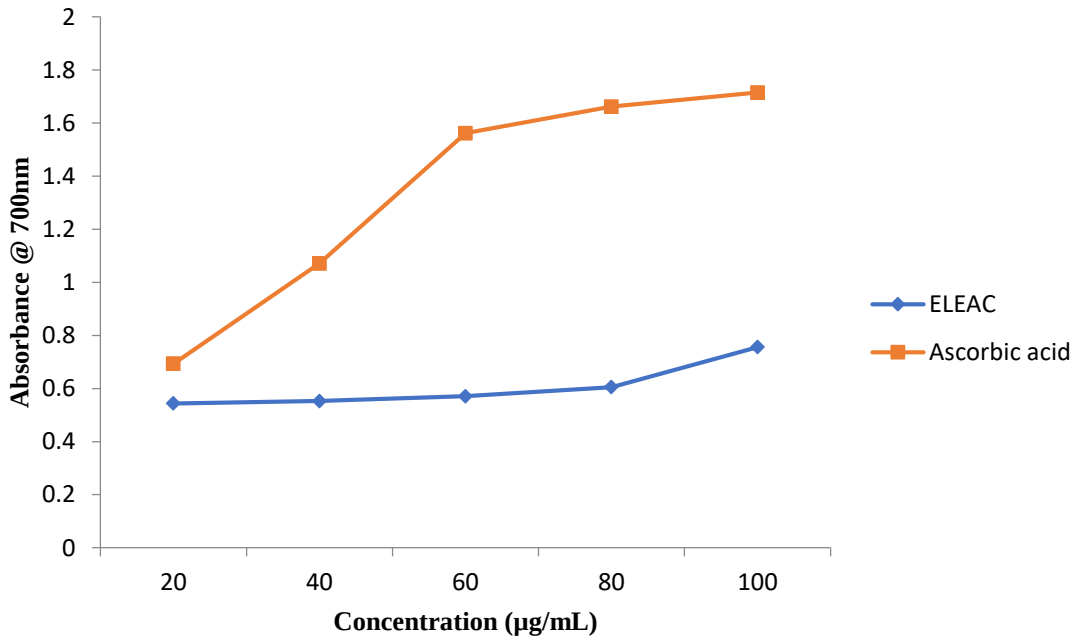

Figure 2: Ferric ion reducing antioxidant power of ethanol leaf extract of *A. conyzoides* (ELEAC) and the reference standard (Ascorbic acid). (n=3, mean±SD).

CONCLUSION

This study affirms the presence of advantageous minerals, nutrients, and medicinal phytochemicals in *A. conyzoides*. The identified phytochemicals, coupled with the demonstrated antioxidant activities that correlate with the flavonoids and phenol content, lend credence to the traditional use of *Ageratum conyzoides* in herbal medicine.

CONFLICT OF INTEREST

The authors affirm that there are no conflicting interests in the presentation of this research.

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