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Phytochemical and Antioxidant Evaluations of *Chromolaena odorata* and *Huntaria umbellata*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

With the increasing demand for herbal medicine, it is imperative to validate the presence of the most anticipated antioxidants present in plants, which motivated the aim of this study. Hence, the efficacy of *Chromolaena odorata* and *Hunteria umbellata* were validated. Natural antioxidants in medicinal plants, such as polyphenols and flavonoids, mitigate oxidative stress and serve as

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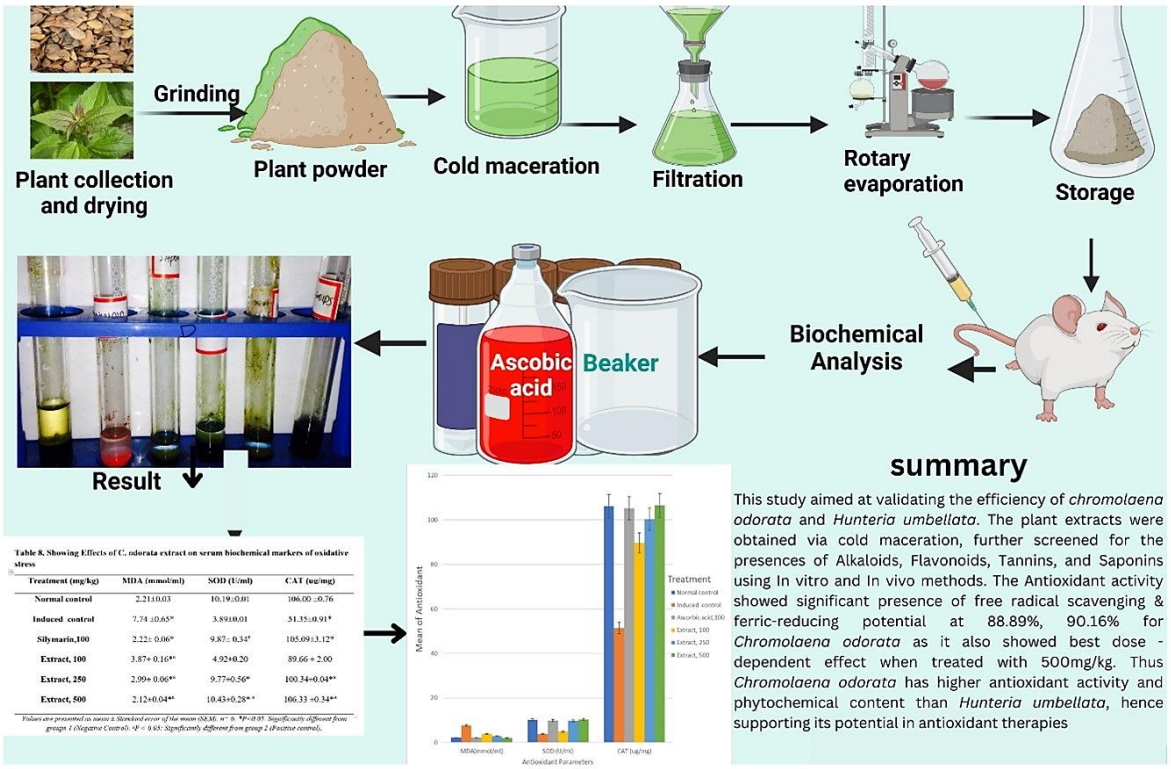
alternative treatments for various diseases. We obtained crude extracts through cold maceration and qualitative and quantitative phytochemical screenings for alkaloids, flavonoids, tannins, and saponins. Antioxidant profiles were assessed using DPPH and FRAP assays for *In vitro* and MDA, SOD, and CAT assays for *In vivo*. The liquid (ethanol) extracts from these plants were tested to confirm the presence of alkaloids, flavonoids, saponins, tannins, and glycosides in the ethanol extracts of *Chromolaena odorata* leaves and *Hunteria umbellata* seeds. The decreasing effect of DPPH experienced in both plant extracts validates the presence of free radical scavenging activities and ferric-reducing antioxidant potential in a concentration-dependent manner. *Chromolaena odorata* demonstrated superior inhibition in both DPPH (88.89 ± 0.26%) and FRAP (90.16 ± 0.76%) assays compared to *Hunteria umbellata*. *In vivo* studies showed dose-dependent antioxidant effects, with 500 mg/kg of *Chromolaena odorata* exhibiting the best results across MDA, SOD, and CAT indices. This study concludes that *Chromolaena odorata* leaves possess higher antioxidant activity and phytochemical content than *Hunteria umbellata* seeds, supporting their potential use in antioxidant therapies.

Graphic Abstract



Keywords: Phytochemical; antioxidant; Chromolaena odorata; Hunteria umbellate; In vitro; In vivo; acute toxicity; oxidative stress.

1. INTRODUCTION

Ancient civilizations skillfully utilized plants to treat and cure a wide range of ailments long before the advent of Western medicine and pharmaceutical manufacturing. This knowledge was meticulously passed down through generations and is now known by various terms such as "alternative therapies" and "folk medicines."

The drawbacks and complications associated with allopathic medicine have spurred a significant global increase in the development of herbal remedies. The scientific community is profoundly researching medicinal plants for new health-giving applications. Medicinal plants are rich in phytochemicals with biological activity, which play a meaningful role in protecting humans from numerous diseases and complications, explaining their widespread use.

The indigenous populations of various regions are particularly familiar with the use of medicinal plants. Plants have played vital roles as the primary source of many contemporary drugs, which involve isolating and modifying active compounds from specific medicinal plants. Historically, herbal medicine has been integral to healing practices across all cultures. Traditional medicine is gaining mainstream recognition as improvements in analytical methods, quality control, and clinical research highlight its efficacy in disease control, cure, and prevention (Kraft, 2009).

Natural medicine targets remedies that enhance subjective well-being, inhibit diseases, and proffer solutions to ailments mainstream medicine often fails to treat (Blackman, 2008). Traditional medical systems, both structured and unstructured (herbal drugs), yield outstanding benefits to indigenous people in developed countries using herbal plants as raw materials (Bannerman, 1979; Rastogi & Dhawan, 1982).

Two (2) decades ago, the World Health Organization proposed that about 80 percent of the global population depends on natural medicine for some aspect of their primary health care. A similar percentage of the Nigerian population uses traditional healthcare practices (Sofowora, 1991; Ajibesin et al*.,* 2008). Modern therapies are confronted with fresh, challenging conditions, and given the comprehensive care approach of traditional medicine, the prominence of traditional practices is increasing. In most developed and prosperous nations, there is a growing reliance on preventive or palliative medicine. For example, the record has it that in France, 75% of the population has tried the use of traditional medicines more than once, and 77% of pain management clinics in Germany incorporate acupuncture. In the United Kingdom, approximately $2.3 million is spent annually on complementary and alternative medicine. In the US, public displeasure with the high cost of orthodox medicine and a preference for natural or organic remedies have fueled a rise in herbal medicine use (Moquin et al*.,* 2009). The worldwide demand for herbal medicine is estimated at around USD 60 million (NNMDA, 2006).

The use of phytomedicine is prevalent globally, including Nigeria, and has been documented among adults (Eisenberg et al., 1993), cancer patients (Ezeome & Anarado, 2007), pre-surgical patients, asthma patients, individuals with hypertension and diabetes, pregnant women, breastfeeding mothers, children with chronic health conditions, the general pediatric population, medical inpatients and outpatients, HIV patients, and the broader public (Fakeye et al*.,* 2011; Yusuff & Tayo, 2011; Oreagba et al*.,*2011).

Traditional medicine knowledge is typically passed orally within communities, families, or individuals, though such information is rarely archived. Ethnobotanical studies have led to the isolation of 122 chemical compounds, with 80% of this blend or mixture being applied for the same or related medicinal purposes across 94 plant species (Farnsworth et al*.,* 1985).

A current issue is that the custodians of indigenous plant use knowledge are often older generations and traditional healers in Nigeria and other developing countries. With the decline in their numbers due to aging and other unforeseen events, and the younger generation's disinterest due to urbanization and technological progress, there is a risk that this valuable knowledge could disappear. Additionally, the heritage of herbal medicine is threatened by secrecy, superstition, and a lack of adequate records.

In recent decades, there has been renewed severe interest in the importance of traditional medical knowledge in plant research (Newman & Crag, 2007). This resurgence is driven by several factors, including unmet therapeutic needs, the notable diversity of chemical and biological activities of secondary metabolites, and the utility of novel bioactive natural compounds as biochemical samples. The field of natural product chemistry has gained significant interest due to the structural complexity of active constituents and advancements in addressing the supply-demand challenges of these complex natural products (Clark, 1996).

The development of cheap and reliable diagnostics is pivotal in controlling infectious diseases. Currently, one of the most effective strategies for combating infectious diseases involves using natural products of plant origin. This is due to the belief that long-term use of Western medicine can lead to serious complications and to curb the prevalence of antibacterial infections. (Chibuzor, J. V. et al*.,* 2024). Folk medicine has long been the foundation for treating numerous ailments in industrialized and developing economies, and its significance is widely acknowledged. This study

emphasizes the antioxidant potential of medicinal plants, highlighting their vital role in Nigeria's healthcare sector. However, traditional medical specialists often need to be recognized and marginalized.

2. MATERIALS AND METHODS

2.1 Plant Materials

On 24th March 2019, leaves of *Chromolaena odorata* (L.) R.M. King & H. Rob. (Asteraceae) and *Hunteria umbellata* (K. Schum.) Hallier f. (Apocynaceae) were collected from the Botanical Garden University of Ibadan, Oyo State, in sub-Saharan Africa. This plant (*Chromolaena odorata*) is known as "Awolowo" or "Siam Weed." Prof. G.E. Osuagwu, a renowned Professor of Plant Physiology in the Department of Plant Science and Biotechnology at Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria, identified the leaves. Subsequently, the leaves were weighed and then dried at room temperature.

2.2 Apparatus and Equipment

The apparatus and equipment used in this study are of analytical grade. These include; Spectrophotometer, Append off tube, Glass column, flasks, beakers, test tubes, measuring cylinders, rotary evaporator, Analytical weighing balance (Metler H30, Switzerland), Spectrophotometer (B. Bran Scientific &Instrument Company, England), Water Bath (Techmel & Techmel, Texas, USA), and National Blender (Japan), and Micropippete (Finnipipette® Labsystems, Finland). (Motic B3, Motic Carlsbad, CA, USA) at x 100 magnification.

2.3 Reagents and Chemicals

The following chemicals, reagents, and drugs were used: an EDTA container, a micro-hematocrit centrifuge, a Superoxide Dismutase test protocol (India), and a ThioBarbituric Acid Reactants test protocol (India).

2.4 Preparation of Plant Material

The leaves were dipped in water to remove dust and unwanted particles. They were weighed and air-dried at room temperature for two weeks. The dried leaves were pulverized with an analytical milling machine and sieved to control the particle size. Then, they were stored in an airtight container for further analysis (Bruce et al., 2016).

2.5 Extraction

The powdered leaves (600 g) were extracted with ethanol (2500 ml) by cold maceration for 72 hours with occasional stirring. The mixture was then sieved using a porcelain cloth and filtered with filter paper. The filtrate dried in vacuo at 40°C, and the extract was stored in a refrigerator for later use (Onyegbule et al., 2019).

2.6 Animals

A total of thirty-three (33) male albino mice, weighing between 20-35g, were obtained from the Laboratory Facility at the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka. The mice were then transferred to the animal house at the Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu Campus, for the experiment. They were housed in clean metal cages, provided with pelleted feed and water, and handled following the stipulated ethical policy of the N.I.H.G. as a set of comprehensive standards and recommendations that outline the proper care and ethical use of laboratory animals in research and testing—8th edition (2011).

2.7 Qualitative Phytochemical Screening

The extracted crude plants were tested using standard methods for the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, phenols, and glycosides (Evans, 2002).

2.8 Test for Alkaloids

Two (2) grams of the extract were combined with 20 mL of 30% sulfuric acid in 50% ethanol and heated in a boiling water bath for 10 minutes. After cooling, the mixture was filtered. A 2 mL portion of the strain was administered with a few drops of Mayer's reagent, which is a 1% solution of potassium mercuric iodide. The remaining filtrate was then transferred into a 100-mL separatory funnel and made alkaline using a dilute ammonia solution. The aqueous alkaline layer was separated, and the extract was tested with Mayer's and Wagner's reagents.

2.9 Test for Saponins

Twenty milliliters of distilled water were added to 0.25 g of the extract and heated in a hot water bath for two minutes. The mixture was then hot-

filtered and allowed to cool, and the resulting filtrate was used for subsequent testing.

2.10 Emulsion Test

A 5 ml filtrate was diluted with 15 ml distilled water and shaken vigorously.

2.11 Test for Tannins

One gram of the leaf powder was boiled with 20 mL of water, then filtered, and the filtrate was used for the subsequent test: **Ferric Chloride Test:** Three milliliters of the filtrate were mixed with two drops of Ferric Chloride. The formation of a greenish-black precipitate confirmed the presence of tannins.

2.12 Test for Flavonoids

A 10 ml ethyl acetate was added to 0.2 g of the extract and heated in a water bath for 3 minutes. The mixture was cooled, filtered, and used for the following tests:

1. Ammonium test: A 4 ml filtrate was mixed with 1 ml dilute ammonia solution and stirred. After allowing the layers to separate, the yellow color in the ammoniacal layer indicated the presence of flavonoids.

2. 1% Aluminium chloride test

A 4 ml filtrate stirred with 1 ml of 1 % Ammonium Chloride solution. The layers were allowed to separate. The yellow color in the aluminum chloride layer indicates the presence of a flavonoid.

2.13 Test for Steroids and Terpenoids

A 9 Ml of ethanol was added to 1 g of the ethanol crude extract, and the mixture was refluxed briefly before being filtered. The filtrate was then concentrated using a boiling water bath. Five milliliters of hot distilled water were added to the concentrated solution, which was allowed to stand for one hour, after which the waxy matter was filtered out. A 2.5 Ml of chloroform using a separatory funnel was used for the extraction. In a test tube, 0.5 mL of the chloroform extract was gently layered with 1 mL of conc. H_2SO4, resulting in the formation of a reddish-brown interface, which suggests the presence of steroids. In a separate procedure, 0.5 mL of chloroform was evaporated to dryness in a water bath and then heated with 3 mL of concentrated sulfuric acid for 10 minutes. The development of a grey color signified the presence of terpenoids.

2.14 Test for Glycosides

A 5 ml of the alcoholic extract was stirred with 1 mL of water in a test tube, followed by the addition of two drops of aqueous sodium hydroxide. The identification of a yellow coloration indicated the presence of glycosides.

2.15 Test for Reducing Sugars

A 0.5 g sample of leaf powder was mixed with distilled water and filtered. The resulting filtrate was then treated with 5 ml of a combined solution of Fehling's A and B. The appearance of a red precipitate, indicating the presence of cuprous oxide, confirms the presence of reducing sugars.

2.16 Test for Proteins

Xanthoproteic reaction test: A 5 ml volume filtrate was obtained from boiling 2 g of the powdered plant with two (2) drops of concentrated nitric acid; the yellow color changing to orange on adding alkali indicates the presence of protein.

2.17 Quantitative Phytochemical Analysis

Edeoga and Gomina, 2000, tested the coarse powder of the plant material to determine the quantity of Alkaloids, Flavonoids, Saponins, and Tannins using the following methods.

2.18 Alkaloid Determination

In 250 ml and 200 ml of 10% acetic acid, 5 g of powdered sample was added, and an ethanol solution was introduced. The mixture was covered and left to stand for 2 hours. Afterward, the extract was filtered and reduced to a quarter of its original volume. The precipitate was collected after the solution had settled, washed with diluted ammonium hydroxide, and filtered again. The remaining residue was dried, weighed, and quantified as alkaloids.

2.19 Flavonoid Determination

The total flavonoid content was determined as quercetin. A 1 g sample of the material was extracted using methanol. To 25 ml of the extract, 20 ml of acetone, 2 ml of 25% HCl, and 1 ml of 0.5% hexamethyl methylene tetramine were added, and the mixture was refluxed at 56°C for 30 minutes. The extract was then filtered and re-extracted twice with 20 ml of acetone (designated as the basic sample solution, BSS). From the BSS, 20 ml was combined with 20 ml of water and subsequently

extracted with ethyl acetate in two stages: first with 15 ml and then with three 10 ml portions. The ethyl acetate extracts were water-washed, filtered, and brought up to 50 ml with ethyl acetate (referred to as S1). To 10 ml of S1, 0.5 ml of 0.5% sodium citrate solution and 2 ml of AlCl3 solution were added. This mixture was then adjusted to 25 ml with a 5% methanolic acetic acid solution (designated as the sample solution, SS). A similar procedure was followed for a blank sample solution, omitting the AlCl3. After 45 minutes, the yellow solutions were filtered, and absorbance was measured at 425 nm. The total flavonoid content was assessed based on three independent analyses, with the yield calculated as a percentage of quercetin using the specified formula.

$$g\% = A \times 0.772 / b$$

Where A is absorbance and b represents the mass of dry powdered sample in grams.

2.20 Saponin Determination

In a conical flask, 20 g of powdered sample and 100 ml of 20 % ethanol were mixed and concentrated at 55°C for 4 hours with continuous stirring in a water bath. Residue from the filtered mixture was further extracted with 200 ml of 20 % ethanol. The combined concoctions (extracts) were evaporated to 40 ml over a water bath at 90 ^{o}C, then turned over to a 250 ml separating funnel and further extracted twice (2) with 20 ml diethyl ether and vigorously shaken. The aqueous layer was retained, and the ether was discarded. 60 ml of butanol was added to the aqueous solution or layer. 10 ml of 5 % aqueous sodium chloride was used to wash the butanol extract twice (2). The result was heated, concentrated in a thermostatic bath, and concentrated in an oven to a constant weight at 40 ^{o}C, after taking the initial weight of the sample, the Saponin content was calculated.

2.21 Tannin Determination

Exactly 500 milligrams of the powdered sample was measured and placed into a plastic bottle with a capacity of 50 milliliters. In a mechanical shaker, 50 ml of distilled water was introduced and agitated for one hour. The liquid was passed through a filter and collected in a flask with a volume of 50 ml. The flask was then filled to the designated mark. Next, 5 ml of the filtered solution was then turned over into a test tube using a pipette. Then, it was combined with 2 ml of a solution containing 0.1 M FeCl in 0.1 N HCL and 0.008 M. The measurement of absorbance was conducted at a wavelength of 120nm and completed within 10 minutes.

Median Lethal Dose Evaluation (Acute Toxicity)

Determination of LD50 of the extracts, as described by Lorke's 1983

Phase 1: Nine adult albino mice were weighed, marked and randomized into three groups of three mice each. Groups 1, 2, and 3 were given doses of the extract at 10 mg/kg, 100 mg/kg, and 1000 mg/kg, respectively. The mice were then monitored for 24 hours for indicators of toxicity and mortality.

Phase 2: Four adult albino mice were used in this phase, randomized into four groups. Each of them were weighed and marked. Dose selection was based on result obtained in phase 1. Observation for obvious toxicity and death were recorded accordingly. The LD50 was calculated in the formula below (Lorke's, 1983).

$$LD50 = \sqrt{(D0 \times D100)} \text{ – equation} \quad (1)$$

D0 = Highest dose that gave no mortality,
D100 = Lowest dose that produced mortality.

Dosage selection: Dosage of extract administered to animals was determined from 1/5th, 1/10th and 1/20th of the estimated LD50 as described by Neharkar and Galkwad (2011).

Table 1. Various groups and treatment received

GROUPS	MICE CONDITION	TREATMENTS	DOSE (MG/KG)
GROUP 1	Normal standard	Distilled H_2O	10ml/kg
GROUP 2	Negative standard	Sodium chloride diet	16% salt diet only
GROUP 3	Positive standard	Silymarin + 16% salt diet	100mg/kg
GROUP 4	Small dose	Extract + 16% salt diet	100mg/kg
GROUP 5	Medium dose	Extract + 16% salt diet	250mg/kg
GROUP 6	High dose	Extract + 16% salt diet	500mg/kg

Antioxidant activity assays FRAP (Ferric Reducing Antioxidant Power): According to Sutharsingh et al. (2011), the ferric depleting power of the plant extracts was estimated with some modifications. A stock solution of the extracts and standard ascorbic acid (≥99.0%, Merck®) was prepared in the concentration of 10 mg/ml. In the ferric-depleting antioxidant power assay, 1 ml of a test sample from both the leaf of C. odorata and H. umbellate, at different concentrations, was combined with 1 ml of 0.2 M Na2SO4 buffer (pH 6.6) and 1 ml of 1% C6N6FeK3 in different test-tubes. The mixtures were incubated in a temperature-controlled water bath at 50 C for about 20 mins, subsequently the addition of 1 ml of 0.1 (10%) trichloroacetic acid (≥99.0%, Merck®). The combination was further centrifuged for 10 min at room temperature. The precipitate obtained (1 ml) was added to 1 ml of deionized water in a test tube, and 0.2 ml of 0.1% FeCl3 (≥99.99%, Merck®) was also added. The blank was made ready in the same manner as the samples, except that the extract was replaced by deionized water. The optical density of a blend of chemicals, also known as a mixture of reactions, was noted at 700 nm. The depleting strength was expressed as an increase in A700 after blank subtraction.

DPPH (2, 2'-diphenyl-1-picrylhydrazyl) assay: The free radical scavenging power of the plant extracts was also estimated using the DPPH radical scavenging method (Sutharsingh et al*.,* 2011) with little modifications. DPPH solution (0.04% w/v) was prepared in 95% ethanol (≥99.8%, Merck®). A stock solution of the extracts and ascorbic acid was made ready at 10 mg/ml as a standard. From the stock solution, 10ml, 8ml, 6ml, 4ml, and 2ml of this solution were taken in five (5) test tubes, respectively. The last volume of each test tube was made up to 10 ml to give conc. of 100 mg/ml, 80 mg/ml, 60 mg/ml, 40 mg/ml and 20 mg/ml respectively. Furthermore, 2 ml of freshly prepared DPPH solution (0.04% w/v) was added to every test tube. The compound was spawned in darkness for 15 minutes, and later, the optical density was recorded against the blank at 523 nm. For the blank, 2 ml of DPPH solution in ethanol was mixed with 10 ml of ethanol, and the absorbance of the solution was noted after 30 min. The antioxidant activity, expressed as percentage inhibition (% IP) of the DPPH radical, was recorded by measuring the decrease in absorbance of DPPH upon the addition of the test samples compared to the control.

Comparative studies on crude extract and various fractions of C. odorata:

Twenty (20) laboratory mice were randomized into 5 groups of 5 mice each as follows:

Biochemical Assays of Markers of Oxidative Stress: MDA content was determined according to the earlier method reported by Aydin (2011). CAT activity was assayed from the rate of decomposition of H2O2by the method of Sinha (1972). SOD activity was estimated using the method previously reported by (Assady et al*.,* 2011).

2.22 Statistical Analysis

The insight from the study were expressed statistically with the help of Statistical software application known as SPSS - 27 (Statistical Package for Social Sciences). The output were expressed as the mean ±, and the standard error of the mean (SEM) of the sample replicates. The raw data were subjected to One-Way ANOVA for the variance analysis, followed by a post hoc Tukey's test. A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Result

The phytochemical screening showed that the ethanol leave extract of *Chromolaena odorata* contains phyto-constituents as listed in the Table 3.

Percentage Phytochemical Content of *Chromolaena odorata*: The phytochemical screening showed that the ethanol leave extract of *Huntaria umbellata* contains phyto-constituents as listed in the Table 4.

3.2 Proximate Analysis

3.2.1 *In vitro* antioxidant activity

Tables 6 and 7 represents the results of the various antioxidant activities studied. The values of the various antioxidant activities ranged from 20mg/ml - 100mg/ml. *Chromolaena odorata* ethanol leaf extract has (88.96 ± 0.26%) at 100 mg/ml as the highest activity and the lowest activity was recorded at 20 mg/ml (18.52 ± 0.52%) for the leaf and Hunteria umbellata

ethanol seed extract has (77.19 ± 0.49%) at 100mg/ml as the highest activity, and lowest activity was recorded (15.77 ± 0.34%) at 20mg/ml in FRAP assay in (Table 6).

Chromolaena odorata ethanol leaf extract at 100 mg/ml showed best percentage DPPH scavenging activity with (90.16 ± 0.76%) and the lowest activity was recorded at 20 mg/ml (61.21 ± 2.36%), whereas, Hunteria umbellata ethanol seed extract has (79.89± 0.18%) at 100mg/ml as the highest activity and (45.77 ± 0.32%) (Table 7) in DPPH assay.

Table 2. The phytochemical result of the ethanol leaf extract of *Chromolaena odorata*

S/N	Phytochemicals	Crude extract
1	Alkaloids	++
2	Saponins	+++
3	Tannins	++
4	Flavonoids	+
5	Steroids	+++
6	Terpenoids	+
7	Cardiac glycosides	++
8	Proteins	+
9	Fats and oils	++
10	Reducing Sugars	++

(-): Not Present
(+): Weak concentration
(++): Moderately concentration
(+++): High concentration

Table 3. Showing Percentage Phytochemical content of *Chromolaena odorata*

Parameters	% Phytochemical compound
Alkaloids	5.2
Saponins	8.6
Tannins	8.1
Flavonoids	4.2

Table 4. Showing the phytochemical screening result of the ethanol leaf extract of *Huntaria Umbellata*

S/N	Phytochemical	Crude extract
1	Alkaloids	+
2	Saponins	++
3	Tannins	-
4	Flavonoids	++
5	Steroids	-
6	Terpenoids	++
7	Cardiac glycosides	+
8	Proteins	-
9	Fats and oils	+
10	Reducing Sugars	-

(-): Not Present
(+): Weak concentration
(++): Moderate concentration
(+++): High concentration

Table 5. Showing Proximate analysis of Hunteria umbellata leaves

Parameter	% Composition
Alkaloids	2.4
Saponins	5.1
Tannins	1.7
Flavonoid	5.4

Table 6. Showing antioxidant activity (% inhibitions) of the ethanol extracts in DPPH assay

Plant (extracts)	20 mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml
Ascorbic Acid	73.17± 1.81	79.17 ± 0.54	81.22 ± 1.29	89.74 ± 0.33	100.09± 0.42
H. umbellata	45.77± 0.32	50.14 ± 0.51	63.63 ± 1.33	69.90±0.23	79.89 ± 0.18
C. odorata	61.21± 2.36	78.00 ± 0.52	80.91 ± 0.65	87.99 ± 0.25	90.16 ± 0.76

Results are mean ± SEM for three determinations

Table 7. Showing antioxidant activity (% Inhibitions) of the ethanol extracts in FRAP assay

Plant (extracts)	20 mg/ml	40 mg/ml	60 mg/ml	80 mg/ml	100 mg/ml
Ascorbic Acid	21.90 ± 2.12	35.87 ± 0.32	48.67 ± 0.44	68.88 ± 0.56	90.07 ± 0.33
H. umbellata	15.77 ± 0.34	27.16 ± 0.44	47.54 ± 0.66	59.39 ± 1.87	77.19 ± 0.49
C. odorata	18.52 ± 0.52	29.16 ± 0.36	56.18 ± 1.42	67.65 ± 0.61	88.96 ± 0.26

Results are mean ± SEM for three determinations

Table 8. Showing Effects of C. odorata extract on serum biochemical markers of oxidative stress

Treatment (mg/kg)	MDA (mmol/ml)	SOD (U/ml)	CAT (ug/mg)
Normal control	2.21±0.03	10.19±0.01	106.00 ±0.76
Induced control	7.74 ±0.65*	3.89±0.01	51.35±0.91*
Silymarin,100	2.22± 0.06*	9.87± 0.34[a]	105.09±3.12*
Extract, 100	3.87± 0.16*[a]	4.92±0.20	89.66 ± 2.00
Extract, 250	2.99± 0.06*[a]	9.77±0.56*	100.34±0.04*[a]
Extract, 500	2.12±0.04*[a]	10.43±0.28* [a]	106.33 ±0.34*[a]

*Values are presented as mean ± Standard error of the mean (SEM). n= 6; *P<0.05: Significantly different from groups 1 (Negative standard). [a]P < 0.05: Significantly different from group 2 (Positive standard).*

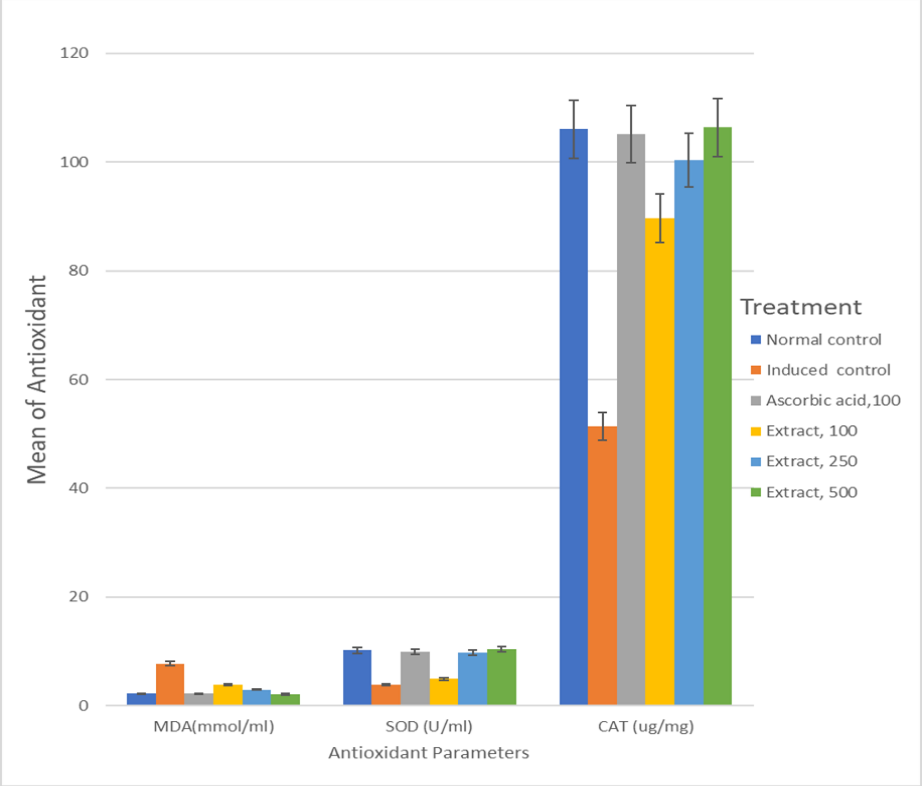


Fig. 1. Effects of *Chromolaena odorata* extract on Antioxidant parameters

4. DISCUSSIONS

The current study demonstrates that the distinctive characteristics obtained from antioxidant and phytochemical analyses can serve as markers for identifying crude drugs in both fresh and dried forms, specifically from the leaves and seeds of *Chromolaena odorata* and Hunteria umbellata. Plants contain numerous phytochemical constituents, many of which are biologically active and responsible for a variety of pharmacological activities. Preliminary phytochemical screening aids in predicting the nature of crude drugs and identifying the phytoconstituents they contain.

Key phytochemical groups identified in the leaves and seeds of these plants include alkaloids, anthraquinones, phenolics, saponins, tannins, and glycosides. The presence of these important phytochemical groups in different parts of these medicinal plants underscores their therapeutic properties and partially validates their extensive ethno medicinal uses. Among secondary metabolites, phenolics are particularly significant due to their therapeutic importance, and these polyphenolic compounds, including simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives, and flavonoids. These classes of phenolics are notable for their wide range of physiological functions, including free radical scavenging, anti-mutagenic, anti-carcinogenic, and anti-inflammatory effects (Manthey, 2000; Bandoniene & Murkovic, 2002).

It is well-established that phenolics, flavonoids, and tannins are highly effective against various types of inflammation, wounds, and pain (Adedapo et al*.,* 2008; Alia et al*.,*2003). The significant presence of these therapeutically active compounds suggests that this plant could be a valuable source of anti-inflammatory drugs.

The antioxidant activities of ethanol extracts from selected plant parts were evaluated using the DPPH, FRAP, and CAT assays, which are widely recognized methods for assessing the antioxidant activity of phytochemicals. Plant extracts rich in phenolics and other antioxidant compounds demonstrated significantly high inhibition percentages.

In the DPPH radical scavenging assay, *C. odorata* leaves exhibited the highest inhibition percentage (88.96 ± 0.26%), surpassing other plant parts studied (with the seeds showing 77.19 ± 0.49%). Similarly, in the FRAP radical scavenging activity study, *C. odorata* leaves again showed the highest inhibition value (90.16 ± 0.76%), compared to other plant parts. These variations in inhibition activities are due to the unequal distribution of antioxidant molecules such as phenolics and flavonoids in different plant parts.

The results indicate that the ethanol extracts of *C. odorata*have greater antioxidant activity across all three methods compared to *H. umbellata*. This superior antioxidant activity of *C. odorata* correlates with its higher contents of phenolics, flavonoids, and tannins. Different phenolic groups have functional groups, including hydroxyls, responsible for their radical scavenging activity. This confirms the importance of phenolics as potential antioxidant agents (Adedapo et al*.,* 2008; Alia et al*.,* 2003; Osawa et al., 1994).

The antioxidant activity of C. odorata leaves aligns well with the total phenolic, flavonoid, and tannin contents of H. umbellata seeds, highlighting these species as potent sources of antioxidant substances and encouraging further investigation into novel antioxidants and therapeutic natural products. It has been proven that *Chromolaena odorata* is more potent in terms of phytochemical content and antioxidant activity than Hunteria umbellata.

Further scientific studies on these two shrubs, particularly their leaves and seeds, are highly recommended to standardize valuable antioxidant phytochemicals. The pharmacognostic characteristics identified in this study will serve as markers for correctly identifying crude drugs derived from the leaves and seeds of *Chromolaena odorata* and Hunteria umbellata, and will aid in detecting adulterants.

5. CONCLUSION

This study shows that ethanol extracts of *Chromolaena odorata* and Hunteria Umbellata show high antioxidant activity. The data affirm that both extracts are good antioxidants for plants. This implies that traditional healers using aqueous or ethanol solvents to extract C. odorata leaf and H. umbellata seed would pull good antioxidants from the plant, which would help combat diseases caused by free radicals. Based on data generated from this study, one could explore the potential application of these extracts in total cancer therapy.

CONSENT AND ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1. Source materials sourced through google and reviewed.
2. Did not use OpenAI, ChatgPT 3 or 4 prompt for my writings.
3. Used Quill Bot for paraphrasing some context

DISCLAIMER

The products utilized in this research are widely and predominantly used in our field of study and our country. There is no conflict of interest between the authors and the product manufacturers, as our objective is to advance knowledge rather than pursue litigation. Additionally, the research was independently funded through the personal efforts of the authors, without any financial support from the producing company.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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