ECOLOGICAL STUDY AND PHYTOCHEMICAL ANALYSIS OF *Ageratum conyzoides* IN THREE SELECTED STATES OF SOUTH-EAST NIGERIA.

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ABSTRACT

Ageratum conyzoides is an annual herbaceous plant of American origin with a pantropical distribution. It belongs to the *Asteraceae* family. It owns a reputed history of indigenous remedial uses, including as a wound dressing, an antimicrobial, and mouthwash as well as in treatment of dysentery, diarrhea, skin diseases, etc. The plant has unique biological attributes and a raft of miscellaneous chemical compounds that render it a pharmacologically important herb. *Ageratum conyzoides* was gotten from three different states namely: Anambra, Enugu, and Imo state. They were air dried for seven days and taken to the laboratory for proper phytochemical screening. Ecological study on *Ageratum conyzoides* was also carried out. When the test for phytochemical constituent was carried out, the result of preliminary phytochemical analysis of *Ageratum conyzoides* showed that saponin, flavonoid, alkaloid, tannin, terpenoids, glycosides, carbohydrates, anthraquinones, and phenol were present in the three states while steroids, proteins, oil and resin, and reducing sugar were absent in the three states. The result for the ecological study of *A. conyzoides* in the three states showed that *A. conyzoides* has the highest species diversity, followed by Enugu, and then Imo state. The mean value of the species diversity is 2.09. The analysis of variance from the photochemical screening showed a significant difference in the percentage quantitative phytochemical composition between states and between extracts ($p<0.05$), while the species diversities are not significantly different from one another which show the homogeneity of the three states.

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Commented [U3]: The phrase "air dried for seven days" could also be refined. Perhaps: "air-dried for seven days before being subjected to laboratory phytochemical analysis."

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For example: These compounds are known to exhibit antimicrobial, anti-inflammatory, and wound-healing properties, supporting the plant's traditional medicinal uses.

INTRODUCTION

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Medicinal plants constitute an effective source of both traditional and modern medicines. Herbal medicine has been revealed to have valid utility and about 80% rural population depends on its efficacy for their primary health care. Scientist from divergent fields in a similar effort are investigating flora anew with an eye to their therapeutic worth. A sense of urgency accompanies the search as the pace of species extinction continues. Over the years, the WHO advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and nonmicrobial origins (WHO 1978). As a part of such study, an annual herb, *Ageratum conyzoides* (Family-*Astreracae*) was focused on. *Ageratum conyzoides* is erect, 30-80cm tall with fine white hairs on the stem and pink flowers (Amarpreet *et al*., 2023). It is a weed, commonly called Billygoat-weed, Goat weed etc., generally found in cultivated fields and other ecosystems such as pastures,

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grasslands, wastelands and even forest areas. The plant is known to have originated from tropical America and now spread to various tropical and subtropical parts of the world (Juliana *et al*., 2010). This plant is widely utilized in traditional medicine wherever it grows. It has been long known in herbal or folk medicine as a remedy for diverse ailments in Africa and worldwide. Various pharmacological investigations have verified its antibiotic efficacy, analgesic effect in rats, antioxidative effect, hepatoprotective effects and as a blood booster (Fatema, 2013). The phytochemical screening showed that *A. conyzoides* contains alkaloids, resins, saponins, tannins, Glycosides and flavonoids. Many different compounds have been isolated and identified in A. conyzoides; such as kaempferol and glycoside (rhamnoside); quercetin, scutellarein, eupalestin, chromene, stigmas-7-en-3-Ol, sitosterol, stigmasterol, fumaric acid, caffeic acid, saponin, pyrrolizidinic alkaloids, ageratochromene derivatives and alkane (Nyuna *et al*., 2010). Furthermore, Ageconyfavones A, B, and C; Hexametoxyflavone; three coumarinic compounds, including 1-2 benzopirone; 1,2- desifropirrolizidinic and licopsamine have been identified in A. conyzoides (Amarpreet *et al*., 2023).

Ageratum conyzoides (Family *Asteraceae*) is an aromatic annual herb native to Central and South America. The genus "*Ageratum*" refers to the Greek term "ageras", signifying the seemingly non-ageing quality of this species (referring to its long lifespan), and the species epithet "konyz" refers to the Greek name of the plant species, *Inulahelenium,* which the weed resembles (Kaur *et al.,* 2012). The common name, "goat weed" or "Billy goat weed", is derived from an Australian male goat due to a close resemblance in odor. It has two subspecies: "*latifolium*", found within the American continent, and "*conyzoides*", with a distribution throughout the tropical and subtropical regions of the world (Kaur *et al*., 2012). The plant was initially distributed across different continents owing to its ornamental value, but it has now naturalized and spread in nearly all types of ecosystems, colonizing aggressively, and presenting management issues to environmentalists, ecologists, conservation managers, and agronomists (Yadav *et al.,* 2019). Apart from its invasive abilities, the plant is well known for its strong phytochemical composition, unique biological attributes, and versatile applications in agriculture and industry. Fast growth, a short life cycle, early reproductive maturity, prolific seed production, and vegetative reproduction enable *A. conyzoides* to establish itself in an alien environment. The large production of small, lightweight seeds with a wide range of dispersal enables its fast spread and colonization. A study conducted in China revealed that *A. conyzoides*

dispersed at a minimum speed of 2.4 km year−1, mainly through human or wind-mediated dispersal (Horvitzet *et al.*, 2017). A single plant is reported to produce 40,000–95,000 seeds with a germination rate of 50%. Additionally, the weed may also proliferate quickly through vegetative reproduction by stolon production (Kaur *et al.*, 2023).

The plant is widely spread all over the world (shown in plate 1). It is very common in West Africa and some parts of Asia and South America. It is softly hairy, erect and branched annual weeds up to 80-90 cm in height. The flowers are purple to white; the fruits are achenes and easily dispersed. It has a peculiar odor that is similar to male goat in Australia and hence called 'Goat Weed' or 'Billy Goat Weeds'. It is not eaten by men due to its bad odor. The whole plant is used for medicinal purposes. The plant has insecticidal and other biological properties which may be used in Agriculture (Kamboj and Saluja, 2011). The plant has long history of traditional uses in many countries in the world, especially in tropical and subtropical regions. The genus *Ageratum* consists of approximately 30 species but only few species have been phytochemically investigated. This plant is a common weed easily available in field and various regions for large scale production for its commercialization. The leaves extract has been potentially active against bacterial infections, fungal derived skin disease and cancer of cervix, eczema, itchiness of eye and to kill lice.

Environmental Suitability and Adaptability

Ageratum conyzoides is highly adaptable to different temperatures, moisture conditions, soil textures, and altitudinal ranges. Its growth is best suited to temperatures ranging from 20–25°C, but it also survives well at 15–30°C. This explains its prevalence at higher altitudes (i.e., temperate climates) as well as on the plains (i.e., tropical climates). Its growth is not affected by soil fertility status, and the weed acclimatizes well to high light intensity and severe salinity stresses (Kaur *et al*., 2023). High phenotypic plasticity allows the plant to settle in novel surroundings via suitable biomass allocation.

Ecological Range

Within its natural geographic range, *A. conyzoides* is only considered to be an agricultural weed, but in invaded areas, its thickets can be spotted in agricultural lands, grasslands, wastelands, natural forests, wetlands, plantations, vegetable gardens, pastures, orchards, tea plantations, alongside water channels, disturbed sites, sites of fresh landslides, and roadsides (plate 1). The plant is an early colonizer of abandoned fields or shifting cultivation sites, and sometimes it dominates as a pioneer community. It can easily colonize available gaps in widely spaced annual crops or plantations (Osman *et al.*, 2021).

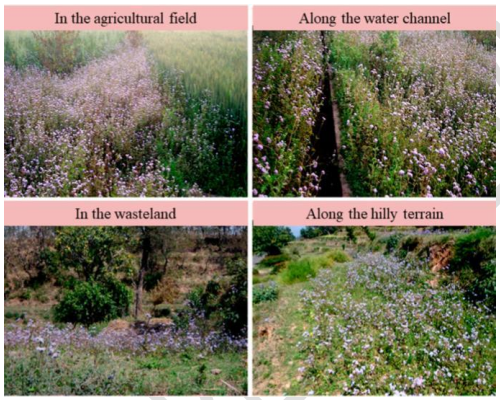

Plate 1: The spread of *Ageratum conyzoides* in various habitats (Amarpreet *et al.*, 2023).

Ecological Impacts

Ageratum conyzoides damages ecosystems both economically and ecologically, either directly competing with the native plants for resources, and/or indirectly by altering ecosystem processes and ecological functioning such as soil nutrient cycling, pollination, etc. Nevertheless, there is a scarcity of research in this domain, specifically in terms of studies that offer empirical data to accurately assess the magnitude of the harm inflicted. While *Ageratum conyzoides* has beneficial uses in medicine, it is also considered a problematic weed in agricultural settings. Its ability to rapidly colonize disturbed areas and compete with crops has led to efforts to manage its spread in certain regions.

Aim of study

This work is aimed at carrying out the ecological study and photochemical analysis of *Ageratum conyzoides* collected from Anambra, Enugu, and Imo state.

Objective of study

- Carrying out an ecological study on *Ageratum conyzoides* to know the species diversity, species richness, and species evenness in the three states the plant was collected.
- Carrying out a preliminary phytochemical screening on *Ageratum conyzoides* to establish the presence of bioactive constituents.
- Carrying out a quantitative phytochemical analysis on *Ageratum conyzoides* to know the quantity of each of the bioactive constituents.

MATERIALS AND METHODS

ECOLOGICAL STUDY

Study Area

The ecological study of *Ageratum conyzoides* was carried out in three states located at the Southeastern region of Nigeria. These states include: Anambra, Enugu, and Imo state. The ecological study was carried out at a large area of plant community for each state.

Sample Area Description

Sample area description for Anambra state

The ecological study of *Ageratum conyzoides* in Anambra stste was carried out at The Botanical Garden at Nnamdi Azikiwe University, Awka. It is characterized by high temperatures and significant rainfall throughout the year. The average temperature typically ranges from 24°C to 32°C (75F to 90F). The wet season usually spans from April to October, while the dry season runs from November to March. This abundant rainfall supports the lush vegetation within the garden.

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Sample area description for Enugu state.

Enugu state experiences a savanna climate with a distinct wet season (April to October) and a dry season (November to March). Average temperatures range from 20°C to 30°C, supporting a diverse array of plant and animal life. The ecological study of *Ageratum conyzoides* in Enugu state was carried out at Emene. Emene is a suburban area located within Enugu state, Nigeria. It features a mix of urban and rural environments, characterized by varied vegetation, including secondary forests and agricultural lands. The region experiences a humid tropical climate, with distinct wet and dry seasons, influencing its biodiversity.

Sample area description for Imo state.

Imo state is located in the Southeastern region of Nigeria. It is bordered by Abia state to the south, Ebonyi state to the east, Enugu state to the North, and Anambra state to the west. The state's capital is Owerri. The ecological study of *Ageratum conyzoides* in Imo state was carried out at Amakohia, Owerri, Imo state, Nigeria. Amakohia experiences a tropical climate typical of the region, with a distinct wet season (April to October) and a day season (November to March). Average temperatures range from 22°C to 31°C, with significant rainfall during the wet months, often exceeding 2000mm annually. The area is characterized by rolling hills and gentle slopes, contributing to a varied topography. It is primarily covered by secondary rainforest and cultivated land.

Experimental Equipments/ Materials

Equipments used include: Cutlass, peg, line, measuring tape, quadrat (1m by 1m), recording sheet.

Sampling Procedure

The sampling method used in the study is the random sampling method. An area of 600m^2 (30m by 20m) was mapped out in each sample area with the aid of a measuring tape and pegs. A cutlass was used to make small holes where the pegs were inserted using a 1m by 1m quadrat. Before sampling, the sampling intensity was calculated which gave approximately 16%.

Two coordinates, AB (30m to represent 30units) and BC (20m to represent 20units), were measured out in each sample area. Quadrat points were determined by selecting random numbers from a pack of card for each of the coordinates. Care was taken to discard numbers that fall at the edge of the mapped out area. At each random point, the quadrat was placed in such a way that the point of intersection is in the center of the quadrat. The species found in the quadrat were identified, counted, and recorded. Only plants that were rooted within the quadrat were counted. This procedure was carried out until 15 randomly selected points (intersects) were sampled for each of the sample area 1, 2, and 3.

PYTOCHEMICAL ANALYSIS

Sample collection

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In sample collection, show some photographs of sample and its collection from study area. Photographs related to phytochemical analysis of the samples, confirmation tests could have been added for more scientific clarity.

The medicinal plant used in this study was *Ageratum Conyzoides*. They were obtained from Anambra, Imo and, Enugu State, Nigeria and identified at Botany department of Nnamdi Azikiwe University, Awka. The samples were immediately transported to the laboratory for use.

Sample preparation

The samples were sliced into very small sizes and sun dried for 7 days to constant weight. This was later ground into fine powder and stored in an air tight plastic container for extraction.

Extraction of plant.

Water extract

The plant material collected (1000g) was dried and further used for water extraction. It was soaked with 1 L of water allowed to stand for 48 h and then filtered. The filtrate was evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Ethanol extract

The plant material collected (1000g) was soaked with 2.5 L of Ethanol, and was allowed to stand for 48 h and then filtered. The filtrate was also evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Preliminary Phytochemical Screening

The extracts will be subjected to preliminary chemical screening for their presence or absence of active phytochemical constituents by the following methods according to (AOAC. 2010).

Test for Alkaloids

The extracts were treated with dilute (10%) hydrochloric acid and filtered. The filtrates were treated with various alkaloidal reagents.

a. Mayer's test: The extracts were with Mayer's reagent (Potassium mercuric iodide). Appearance of cream colour indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

b. Dragendroff's test: The extracts were treated with the Dragendorff's reagent (Potassium bismuth iodide), the appearance of reddish brown precipitate indicates the presence of alkaloid in chloroform, methanolic and aqueous extracts.

c. Hager's test: The extracts were treated with the Hager's reagent (Picric acid), the appearance of yellow colour precipitate indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

d. Wagner's test: The extracts were treated with the Wagner's reagent (Iodine solution) the appearance of brown colour precipitate indicates the presence of alkaloids in chloroform, methanolic and aqueous extracts.

Test for Carbohydrates

a. Molisch's test: To the extracts, 2 drops of 1 % alcoholic α-Naphthol was added and 2 ml of conc. Sulfuric acid was added through the sides of test tubes. A violet colour ring at the junction of two layers was observed in methanolic and aqueous extracts.

b. Barfoed's test: Small portions of the different extracts were treated with Barfoed's reagent ($CuSO_4$ + Acetic acid). A red colour precipitate formed in methanolic and aqueous extracts indicating the presence of monosaccharides.

c. Fehling's test: Extracts were treated with Fehling's solution A and B, warmed on a water bath for 5 minutes. A brick red precipitate was formed in methanolic and aqueous extracts indicating the presence of reducing sugar.

Test for Cardiac Glycosides

a. **Keller-Killani test :** When a pinch of the extracts were dissolved in the Glacial acetic acid and few drops of ferric chloride solution was added, followed by the addition of concentrated Sulphuric acid, formation of red ring at the junction of two liquids indicates the presence of glycosides in methanolic and aqueous extracts.

Test for Flavonoids

a. Shinoda's test: The extracts were dissolved in alcohol, to that one piece of magnesium followed by conc. hydrochloric acid were added drop wise and heated. Appearance of magenta color shows the presence of flavonoids in methanolic and aqueous extracts.

b. Ferric Chloride test: To the extracts, few drops of neutral ferric chloride were added. Blackish red colour was observed in methanolic and aqueous extracts.

Test for Saponins

a. Foam test: The extracts were diluted to 20 ml with distilled water and shaken well in a graduated cylinder for 15 minutes. The formation of foam in the upper part of the test tube indicates the presence of saponins in methanolic and aqueous extracts.

Test for Steroids

a. **Salkowski reactionc** To 2 ml of extract, add 2ml chloroform and 2 ml concentrated H2SO4. Shake well. Chloroform layer showed red color and acid layer showed greenish yellow fluorescence.

b. **Liebermann-Burchard test:** When the extracts were treated with concentrated sulphuric acid, few drops of glacial acetic acid, followed by the addition of acetic anhydride, absence of green colour indicates the absence of steroids in all extracts.

Test for Tannins

a. **Lead acetate solution**: When the extracts were treated with 10% lead acetate solution, appearance of white precipitate indicates the presence of tannins in methanolic and aqueous extracts.

b. Ferric Chloride Solution: When the extracts were treated with ferric chloride solution, NaOH, & AgBr Solution appearance of green colour precipitate indicates the presence of tannins in methanolic and aqueous extracts.

Test for Proteins

Millon's test: When the extract was treated with Millon's reagent (Mercuric Sulphate), appearance of pink colour indicates the presence of proteins in methanolic and aqueous extracts.

Test for phenol

Ferric chloride test: 2 drops of neutral ferric chloride solution was added to 1ml of diluted aqueous solution of the test sample. A greenish purple color indicates the presence of phenolic compounds.

Test for the presence of Terpenoids (Salkowski test)

The presence of terpenoids was tested in 0.2 g of the extract of the plant sample and mixed with 2 mL of chloroform and concentrated sulphuric acid (3 mL H_2SO_4) was added carefully to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoid.

Test for the presence of Anthraquinone (Borntrager's test)

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About 0.5 g of the extracts was boiled with 10% HCl for a few minutes in a water bath. It will be filtered and allowed to cool. An equal volume of chloroform was added to the filtrate. Few drops of 10 per cent ammonia will be added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinones.

Quantitative phytochemical screening

Tannin

Analytical method for quantitative determination of tannin was according to (Ejikeme *et., al.,* 2011]. By dissolving 50 g of sodium tungstate (Na_2WO_4) in 37 cm^3 of distilled water, Folin-Denis reagent was made. To the reagent prepared above, 10 g of phosphomolybdic acid ($H_3PMo_{12}O_{40}$) and 25 cm^3 of orthophosphoric acid (H_3PO_4) were added. Two-hour reflux of the mixture was carried out, cooled, and diluted to 500 cm^3 with distilled water. One gram of each wood powder (sample) in a conical flask was added to 100 cm^3 of distilled water. This was boiled gently for 1 hour on an electric hot plate and filtered using number 42 (125 mm) Whatman filter paper in a 100 cm^3 volumetric flask. Addition of 5.0 cm^3 Folin-Denis reagent and 10 cm^3 of saturated Na_2CO_3 solution into 50 cm^3 of distilled water and 10 cm^3 of diluted extract (aliquot volume) was carried out after being pipetted into a 100 cm^3 conical flask for colour development. The solution was allowed to stand for 30 minutes in a water bath at a temperature of 25°C after thorough agitation. With the aid of a Spectrum Lab 23A spectrophotometer optical density was measured at 700 nm and compared on a standard tannic acid curve. Dissolution of 0.20 g of tannic acid in distilled water and dilution to 200 cm^3 mark (1 mg/cm^3) were used to obtain tannic standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which Folin-Denis reagent (5 cm^3) and saturated Na_2CO_3 (10 cm^3) solution were added and made up to the 100 cm^3 mark with distilled water. The solution was left to stand for 30 minutes in a water bath at 25°C. Optical density was ascertained at 700 nm with the aid of a Spectrum Lab 23A spectrophotometer. Optical density (absorbance) versus tannic acid concentration was plotted.

Determination of Alkaloids

Quantitative determination of alkaloid was according to the methodology by (Harborne, 2006). Exactly 200 cm^3 of 10% acetic acid in ethanol was added to each sample (2.50 g) in a 250 cm^3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide drop-wise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm^3 of 0.1 M of ammonium hydroxide and then filtered using Gem filter paper (12.5 cm). Using electronic weighing balance Model B-218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as

Determination of Flavonoid

Flavonoid determination was by the method reported by (Ejikeme *et., al.,* 2011). Exactly 50 cm^3 of 80% aqueous methanol added was added to 2.50 g of sample in a 250 cm^3 beaker, covered, and allowed to stand for 24 hours at room temperature. After discarding the supernatant, the residue was re-extracted (three times) with the same volume of ethanol. Whatman filter paper number 42 (125 mm) was used to filter whole solution of each wood sample. Each wood sample filtrate was later transferred into a crucible and evaporated to dryness over a water bath. The content in the crucible was cooled in a desiccator and weighed until constant weight was obtained. The percentage of flavonoid was calculated

Determination of Saponin

Saponin quantitative determination was carried out using the method reported by (Ejikeme *et., al.,* 2011). Exactly 100 cm^3 of 20% aqueous ethanol was added to 5 grams of each sample in a 250 cm^3 conical flask. The mixture was heated over a hot water bath for 4 hours with continuous stirring at a temperature of 55°C. The residue of the mixture was reextracted with another 100 cm^3 of 20% aqueous ethanol after filtration and heated for 4 hours at a constant temperature of 55°C with constant stirring. The combined extract was evaporated to 40 cm^3 over water bath at 90°C. 20 cm^3 of diethyl ether was added to the concentrate in a 250 cm^3 separator funnel and vigorously agitated from which the aqueous layer was recovered while the ether layer was discarded. This purification process was repeated twice. 60 cm^3 of n-butanol was added and extracted twice with 10 cm^3 of 5% sodium chloride. After discarding the sodium chloride layer

the remaining solution was heated in a water bath for 30 minutes, after which the solution was transferred into a crucible and was dried in an oven to a constant weight. The saponin content was calculated as a percentage:

Steroids

One gram (1 g) of the extract will be macerated with 20 ml of ethanol. Two milliters (2 ml) of chromagen solution will be added to 2 ml of the filtrate and allowed to stand for 30 minutes. Absorbance will be read at 550 nm. A standard will be made following the same procedure at different concentrations using steroid hormone, a standard curve of absorbance vs concentration will be plotted and the concentration of steroid in the extract extrapolated from the standard curve.

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Determination of Phenols

2 g sample was carried out for 2 hours in 100 cm^3 of ether using a soxhlet apparatus. The defatted sample (0.50 g) was boiled for 15 minutes with 50 cm^3 of ether for the extraction of the phenolic components. Exactly 10 cm^3 of distilled water, 2 cm^3 of 0.1 N ammonium hydroxide solution, and 5 cm^3 of concentrated amyl alcohol were also added to 5 cm^3 of the extract and left to react for 30 minutes for colour development. The optical density was measured at 505 nm. 0.20 g of tannic acid was dissolving in distilled water and diluted to 200 mL mark (1 mg/cm^3) in preparation for phenol standard curve. Varying concentrations (0.2–1.0 mg/cm^3) of the standard tannic acid solution were pipetted into five different test tubes to which 2 cm^3 of NH_3OH, 5 cm^3 of amyl alcohol, and 10 cm^3 of water were added. The solution was made up to 100 cm^3 volume and left to react for 30 minutes for colour development. The optical density was determined at 505 nm. A standard curve of absorbance against concentration will be plotted and the concentration of phenol extrapolated from the standard curve.

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Quantification of Terpenoid Content

The determination of terpenoid was carried out according to the method of [AOAC 2010]. A quantity (0.lg) of the extract was weighed out separately, macerated with 20ml of ethanol and filtered through whatman No. 1 filter paper. The filtrates (1ml) were pipette out and 1 ml of 0.5% phophorylbdic acid solution was added and shaken. Gradually 1ml of concentrated H2SO4 was

added to each. The mixtures were left to stand for 30 minutes. Ethanol (2ml) was added and absorbance was measured at 700nm.

Concentration of Terpenoid = $\frac{\boldsymbol{Abs \times D.F}}{\mathbf{100} \times \boldsymbol{weight of sample used}}$

Determination of Cyanogenic Glycoside

Cyanogenic glycoside quantitative determination methodology used in this research is that reported by (Ejikeme *et., al.,* 2011]. It was weighed into a 250 cm^3 round bottom flask and about 200 cm^3 of distilled water was added to one gram of each sample and allowed to stand for 2 hours for autolysis to occur. Full distillation was carried out in a 250 cm^3 conical flask containing 20 cm^3 of 2.5% NaOH (sodium hydroxide) in the sample after adding an antifoaming agent (tannic acid). Cyanogenic glycoside (100 cm^3), 8 cm^3 of 6 M NH_4OH (ammonium hydroxide), and 2 cm^3 of 5% KI (potassium iodide) were added to the distillate(s), mixed, and titrated with 0.02 M $AgNO_3$ (silver nitrate) using a microburette against a black background. Turbidity which was continuous indicates the end point.

Data analysis

From the records obtained from the ecological study of *Ageratum conyzoides* in the three states, the following will be calculated:

- Abundance of plant: Abundance will be calculated by counting the total number of individual of a species in all quadrats and dividing it by total number of quadrat in which the species occurred.
- Relative abundance = $\frac{\text{Abundance of one species}}{\text{Total abundance of all species}} \times \frac{100}{1}$
- Density = $\frac{\text{Total no. of individual of species}}{\text{Total area of the plots sampled}}$
- Relative density = $\frac{\text{Density of one species}}{\text{Total density of all species}} \times \frac{100}{1}$
- Frequency = $\frac{\text{Number of occurrence of one species}}{} \times 100$

Number of time sampled 1

- Relative frequency = Frequency of one species x 100
  Total frequency of all species 1
- Importance value index = Relative density of a species + Relative frequency of the same species + Relative abundance of the same species

Species diversity will be calculated using Shannon Wienner index formula:

$Hs = \sum Pi\ (In\ Pi)$

Hs = Shannon Wienner index.

Pi = Proportion of total sample belonging to the species.

In = natural logarithm.

Species richness will be calculated using:

$$\text{Species richness} = \frac{S - 1}{\text{Log } N}$$

S = number of species present in community.

N = number of individual in community.

Species evenness will also be calculated using:

$$C = \frac{H}{\text{Log } S}$$

C = species evenness

H = Shannon Wienner index

S = number of species present in community.

Statistical analysis

The experimental results were presented in mean ±SD of the mean of three replicates. The sample means were compared using Analysis of Variance (ANOVA) to determine the level of significance. Difference in mean values were considered significant at $P<0.05$.

RESULTS

RESULTS ON ECOLOGICAL STUDY

Quadrat size: 1m by 1m

Table 1: Abundance values obtained from Quadrat samples for Anambra state.

Species	NO of individuals	A	RA (%)	D (M^{-2})	RD (%)	F (%)	RF (%)	IVI	Pi	Pi(InPi)
Calopogonium mucunoides	11	0.10	9.90	0.73	9.69	53.33	12.70	32.29	0.10	-0.230
Mimosa pigra	21	0.19	18.81	1.40	18.59	60	14.29	51.69	0.19	-0.316
Ageratum conyzoides	17	0.15	14.85	1.13	15.00	53.33	12.70	42.55	0.15	-0.285
Sida linifolia	13	0.12	11.88	0.87	11.55	46.67	11.11	34.54	0.12	-0.254
Phyllanthus nirurii	11	0.10	9.90	0.73	9.69	53.33	12.70	32.29	0.10	-0.230
Cyperus esculentus	10	0.09	8.91	0.67	8.90	40	9.52	27.33	0.09	-0.217
Alterananthera brasiliana	9	0.08	7.92	0.60	7.97	33.33	7.94	23.83	0.08	-0.202
Assystasia gangetica	6	0.05	4.95	0.40	5.31	33.33	7.94	18.20	0.05	-0.150

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Imperata cylindrical	15	0.13	12.87	1.00	13.28	46.67	11.11	37.26	0.13	-0.265
TOTAL	**113**	**1.01**		**7.53**		**419.99**				**-2.149**

A= Abundance

RA= Relative abundance

D = Density

RD = Relative density

F = Frequency

RF = Relative frequency

IV = Important value

Pi = Proportion of total sample belonging to the species

Pi InPi = Shannon - Wienner index

Table 1 above shows that *Mimosa pigra* has the highest density, followed by *Ageratum conyzoides* while *Assystasia gangetica* has the lowest density. *Mimosa pigra* also has the highest frequency followed by *Calopogonium mucunoides*, *Ageratum conyzoides* and *Phyllanthus nirurii* with the same frequency, while *Altherananthera brasiliana* and *Assystasia gangetica* has the lowest and the same frequency. The table also shows that *Mimosa pigra* has the highest important value while *Assystasia gangetica* has the lowest important value.

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Quadrat size: 1m by 1m

Table 2: Abundance values obtained from Quadrat samples for Enugu state.

Species	**NO of individuals**	**A**	**RA (%)**	**D (M^{-2})**	**RD (%)**	**F (%)**	**RF (%)**	**IVI**	**Pi**	**Pi(InPi)**
Mimosa pigra	18	0.16	15.84	1.20	15.67	53.33	12.70	44.21	0.16	-0.293
Tridax procumbens	8	0.07	6.93	0.53	6.92	40	9.52	23.37	0.07	-0.186
Ageratum conyzoides	14	0.12	11.88	0.93	12.14	60	14.29	38.31	0.12	-0.254
Paspalium scrobiculatum	9	0.08	7.92	0.60	7.83	40	9.52	25.27	0.08	-0.202
Imperata cylindrical	26	0.23	22.77	1.73	22.58	66.67	15.87	61.22	0.23	-0.338
Calopogonium mucunoides	21	0.18	17.82	1.40	18.28	60	14.29	50.39	0.18	-0.309
Chromolaena odorata	10	0.09	8.91	0.67	8.75	53.33	12.70	30.36	0.09	-0.217
Eluesine indica	9	0.08	7.92	0.60	7.83	46.67	11.11	26.86	0.08	-0.202
TOTAL	**115**	**1.01**		**7.66**		**420**				**-2.001**

From table 2 above, the results shows that *Imperata cylindrica* has the highest density followed by *Calopogonium mucunoides* while *Tridax procumbens* had the lowest density, followed by *Eluesine indica. Imperata cylindrica* also has the highest frequency, followed by *Ageratum conyzoides* and *Calopogonium mucunoides* with the same frequency while *Tridax procumbens*

and *Paspalium scrobiculatum* has the lowest and the same frequency. The table also showed that *Imperata cylindrica* has the highest important value, followed by *Calopogonium mucunoides* while *Tridax procumbens* has the lowest important value.

Quadrat size: 1m by 1m

Table 3: Abundance values obtained from Quadrat samples for Imo state.

Species	**NO of individuals**	**A**	**RA (%)**	**D (M^{-2})**	**RD (%)**	**F (%)**	**RF (%)**	**IV**	**Pi**	**Pi(InPi)**
Imperata cylindrical	22	0.20	20	1.47	19.65	60	14.06	53.71	0.20	-0.322
Eluesine indica	8	0.07	7	0.53	7.09	46.67	10.94	25.03	0.07	-0.186
Ageratum conyzoides	19	0.17	17	1.27	16.98	60	14.06	48.06	0.17	-0.301
Sida acuta	13	0.12	12	0.87	11.63	46.67	10.94	34.57	0.12	-0.254
Gomphrena celosioides	12	0.11	11	0.80	10.70	46.67	10.94	32.64	0.11	-0.243
Chromolaena odorata	10	0.09	9	0.67	8.96	40	9.37	27.33	0.09	-0.217
Euphorbia hirta	7	0.06	6	0.47	6.28	40	9.37	21.65	0.06	-0.169
Assystasia gangetica	6	0.05	5	0.40	5.35	33.33	7.81	18.16	0.05	-0.150
Calopogonium mucunoides	15	0.13	13	1.00	13.37	53.33	12.50	38.87	0.13	-0.265
TOTAL	**112**	**1.00**		**7.48**		**426.67**				**-2.107**

Table 3 above shows that *Imperata cylindrica* has the highest density, followed by *Ageratum conyzoides*, then *Calopogonium mucunoides* while *Assystasia gangetica* has the lowest density. *Ageratum conyzoides* and *Imperata cylindrica* also has the highest and the same frequency, followed by *Calopogonium mucunoides*, then *Eluesine indica*, *Sida acuta* and *Gomphrena celosioides* with the same frequency, while *Assystasia gangetica* has the lowest frequency. The table also showed that *Imperata cylindrica* has the highest important value while the lowest important value is seen in *Assystasia gangetica.*

Table 4: Species diversity, Species richness, and Species evenness of the three states.

States	Species diversity	Species richness	Species evenness
Anambra state	2.149	3.90	2.25
Enugu state	2.001	3.40	2.22
Imo state	2.107	3.90	2.21

From the table 4 above, it was observed that Anambra state has the highest species diversity, followed by Enugu state, and Imo state. The species diversities are not significantly different from one another which show the homogeneity of the three states. Also, Anambra and Imo have the highest and the same species richness, followed by Enugu state. Also from the table, Anambra state has the highest species evenness, followed by Enugu state, then Imo state. No significant difference occurs between the species richness and evenness. In general, the mean value of the species diversity is 2.09.

RESULTS ON PHYTOCHEMICAL ANALYSIS

TABLE 5: QUALITATIVE PHYTOCHEMICAL (ETHANOL EXTRACT)

PHYTOCHEMICALS	Anambra	Enugu	Imo

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Given that the study involves both quantitative (phytochemical composition) and
qualitative (species diversity) data, the inclusion of figures (such as bar charts or pie
charts for the chemical compounds) and tables (for species diversity indices and
statistical comparisons) will significantly enhance the readability and accessibility of
your results.

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Given that the study involves both quantitative (phytochemical composition) and
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your results.

SAPONIN	**++**	**++**	**++**
FLAVONOID	**+++**	**+++**	**+++**
ALKALOID	**+++**	**+++**	**+++**
TANNIN	**+**	**+++**	**+**
STEROIDS	**-**	**-**	**-**
TERPENIODS	**+**	**+**	**-**
GLYCOSIDES	**+**	**+**	+
CARBOHYDRATES	+	+	+
PROTEIN	**-**	**-**	**-**
ANTHRAQUINONES	**++**	+	**-**
PHENOL	**+++**	+++	**+++**
OIL AND RESIN	**-**	**-**	**-**
REDUCING SUGAR	**-**	**-**	**-**

Key

+++ = Present in high concentration

++ = Present in moderate concentration

+ = Slightly or sparingly present

- = Absent.

Table 5 above shows the qualitative phytochemical (ethanol extract) of *Ageratum conyzoides* in Anambra, Enugu, and Imo state. From the table, saponin is present in moderate concentration in the three states. Flavonoid, alkaloid, and phenol were all present in high concentration in the three states. In Anambra an Imo, tannin is slightly or sparingly present while it is present in high concentration in Enugu. Steroids is absent in the three states while for terpeniods, it is slightly or sparingly present in both Anambra and Enugu, and absent in Imo. Glycosides and carbohydrates

were found to be slightly or sparingly present in the three states. Protein, oil and resin, and reducing sugar were all absent in the three states. For anthroquinones, it was found to be present in moderate concentration in Anambra, slightly or sparingly present in Enugu, and absent in Imo.

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TABLE 6: QUALITATIVE PHYTOCHEMICAL (WATER EXTRACT)

PHYTOCHEMICALS	Anambra	Enugu	Imo
SAPONIN	+	+	++
FLAVONOID	+++	+++	+++
ALKALOID	+	+++	+
TANNIN	++	++	+
STEROIDS	-	-	-
TERPENIODS	+	+	+
GLYCOSIDES	++	++	++
CARBOHYDRATES	+	+	+
PROTEIN	-	-	-
ANTHRAQUINONES	+	+	+
PHENOL	++	+++	++
OIL AND RESIN	-	-	-
REDUCING SUGAR	-	-	-

Key

+++ = Present in high concentration

++ = Present in moderate concentration

+ = Slightly or sparingly present

- = Absent.

Table 6 above shows the qualitative phytochemical (water extract) of *Ageratum conyzoides* in Anambra, Enugu, and Imo state. From the table, it can be seen that flavonoid is the only phytochemical that is present in high concentration in the three states. Steroids, protein, oil and resin, and reducing sugar were all absent in the three states. Saponin was found to be slightly or sparingly present in both Anambra and Enugu while in Imo, it is present in moderate concentration. In Anambra and Imo, alkaloid is slightly or sparingly present while it is present in high concentration in Enugu. Tannin is present in moderate concentration in both Anambra and Enugu, and is slightly or sparingly present in Imo. Terpeniods, carbohydrates and anthraquinones were slightly or sparingly present in the three states. Glycosides is the only phytochemical found to be present in moderate concentration in the three states. For phenol, it is present in high concentration in Enugu, and present in moderate concentration in both Anambra and Imo.

TABLE 7: QUANTITATIVE PHYTOCHEMICAL

PHYTOCHEMICAL	**Aqueous Extract**			**Ethanol Extract**		
LOCATION	**Anambra**	**Enugu**	**Imo**	**Anambra**	**Enugu**	**Imo**
SAPONIN	$7.90^{c}\pm 0.20$	$7.78^{d}\pm 0.05$	$7.93^{c}\pm 0.19$	$9.27^{ab}\pm 0.51$	$9.53^{a}\pm 0.38$	$10.90^{a}\pm 0.35$
FLAVONOID	$21.24^{d}\pm 0.52$	$21.17^{d}\pm 0.51$	$20.79^{d}\pm 1.00$	$27.05^{c}\pm 0.11$	$25.80^{b}\pm 0.45$	$27.35^{a}\pm 0.57$
ALKALOID	$26.80^{d}\pm 0.10$	$26.40^{d}\pm 0.20$	$27.13^{c}\pm 0.27$	$33.69^{b}\pm 0.60$	$38.63^{a}\pm 0.52$	$35.88^{b}\pm 0.10$
TANNIN	$0.93^{c}\pm 0.10$	$0.97^{c}\pm 0.35$	$0.91^{c}\pm 0.02$	$4.20^{b}\pm 0.30$	$4.78^{a}\pm 0.10$	$4.00^{ab}\pm 0.50$
STEROIDS	$1.72^{d}\pm 0.20$	$1.35^{d}\pm 0.10$	$1.35^{d}\pm 0.10$	$1.55^{c}\pm 0.07$	$1.79^{a}\pm 0.10$	$1.70^{b}\pm 0.50$
TERPENIODS	$0.78^{b}\pm 0.25$	$0.33^{c}\pm 0.10$	$0.70^{b}\pm 0.05$	$0.84^{b}\pm 0.11$	$1.00^{a}\pm 0.53$	$0.68^{b}\pm 0.10$
GLYCOSIDES	$3.05^{d}\pm 0.08$	$3.23^{c}\pm 0.13$	$3.37^{b}\pm 0.22$	$3.30^{b}\pm 0.02$	$3.50^{a}\pm 0.24$	$3.28^{b}\pm 0.07$

PHENOL	0.86^{c} ±1.27	1.12 ^{a}± 2.00	1.09^{a}±0.93	0.87^{c}±1.33	0.85^{c} ± 0.30	0.89^{b}± 1.10

Values are mean of three replicates. Mean in the same column with different superscripts are significantly different ($p<0.05$).

Table 7 above shows the quantitative phytochemical analysis of the aqueous and ethanol extract of *Ageratum conyzoides* in Anambra, Enugu, and Imo. The result showed that ethanol extract of samples from Imo state had the highest saponin content (10.90^{a}±0.35), although with a slight difference from ethanol extract in samples from Anambra and Enugu while that of aqueous extract showed that the samples from Imo state has the the highest saponin content (7.93^{c}± 0.19, followed by Anambra, then Imo. Flavonoid had an almost even composition in both aqueous and ethanol extract in samples from all states, with samples from Anambra having the highest flavonoid component in the aqueous extract (21.24^{d}± 0.52) and samples from Imo having the highest flavonoid component in the ethanol extract (27.35^{a}±0.57). For alkaloid, the highest composition was observed in ethanol extract from Enugu (38.63^{a}± 0.52). Tannin content showed an even composition in samples collected from all three states in both the aqueous and ethanol extract with the samples from Enugu in both extracts having the highest tannin component (0.97^{c}±0.35) and (4.78^{a}±0.10) respectively. Steroids was highest in ethanol extract of samples from Enugu (1.79^{a}± 0.10). Ethanol extract of samples collected from Enugu showed higher terpeniods composition (1.00^{a}± 0.53) while that of aqueous extract has the highest terpeniods composition in Anambra (0.78^{b}± 0.25). Glycosides was higher in ethanol extract of samples from Enugu (3.50^{a}± 0.24) while that of aqueous extract was from Imo (3.37^{b}± 0.22). Phenol has the highest composition from samples in Enugu (1.12 ^{a}± 2.00) for the aqueous extract while that of the ethanol extract has an even composition in all the three states with that of Imo (0.89^{b}± 1.10) having the highest composition. Alkaloid was the overall highest phytochemical constituent, with the ethanol extract of samples collected from Enugu state having a composition as high as (38.63^{a}± 0.52). Analysis of variance showed a significant difference in the percentage quantitative phytochemical composition between states and between extracts ($p<0.05$).

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DISCUSSION AND CONCLUSION

DISCUSSION

Ecologically, from table 4, it showed that Anambra state has the highest species diversity, followed by Enugu state, and Imo state. The species diversities are not significantly different from one another which show the homogeneity of the three states. Also, Anambra and Imo have the highest and the same species richness, followed by Enugu state. Also from the table, Anambra state has the highest species evenness, followed by Enugu state, then Imo state. No significant difference occurs between the species richness and evenness. In general, the mean value of the species diversity is 2.09.

The qualitative phytochemical screening results in table 5 and 6 showed that saponin, flavonoid, alkaloid, tannin, terpeniods, glycosides, carbohydrates, anthraquinones, and phenol are present in *Ageratum conyzoides* in the three states while steroids, protein, oil and resin, and reducing sugar are absent in *Ageratum conyzoides* in the three states.

The presence of saponins signifies that the plant could prevent cancer by preventing DNA damage (Ross, 2005). Flavonoids have inhibitory activity against organism that cause plant disease. E.g. Fusariumoxysporum (Galeotti *et al*., 2008). According to Khan *et al*., alkaloids have been reported to possess various biological activities, including: Antioxidant activity, anti-inflammatory activity, neuroprotective effects. etc. (Khan *et al*., 2011). The presence of tannin in *A. conyzoides* signifies that it plays a role in protection from predation and might help in regulating plant growth (Katie and Thorington, 2006). According to Specter, terpeniods contribute to the the scent of eucalyptus, the flavors of cinnamon, cloves and ginger, the yellow colour in sunflower, and the red colour in tomatoes (Specter, 2009). The presence of glycosides in *A. conyzoides* can signify its poisonous nature (Dutta, 2004). Carbohydrates and their derivatives play major roles in the way the immune system, fertilization, pathogenesis, blood clotting, and development in human takes place, while anthraquinonoes have been reported to possess various biological activities like: Antioxidant activity, anti-inflammatory activity, and anticancer activity (Lang *et al*., 2019). Presence of phenol in *A. conyzoides* can signify its ability to prevent heart ailment to an appreciable degree and sometimes an anti-inflammatory agent (Doughari, 2012).

Quantitatively, alkaloid was the overall highest phytochemical constituent, with the ethanol extract of samples collected from Enugu state having a composition as high as ($38.63^{a}\pm 0.52$),

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while terpeniods was the least occurring phytochemical constituent, with the aqueous extract of samples collected from Enugu state having a composition as low as ($0.33^{c}\pm 0.10$).

CONCLUSION

Ageratum conyzoides have shown to be a high diverse plant having high species diversity, species richness, and species evenness.

Ageratum conyzoides have also proven to possess various biological activities including: Antioxidant, anticancer, anti-inflammatory, and antimicrobial activity because of its qualitative phytochemical constituent. Quantitatively, alkaloid being the highest phytochemical constituent confirms this.

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