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Ecological Studies of *Tridax procumbens (Linn)* and *Ageratum conyzoides (Linn)* and the comparative Studies of Their Phytochemical

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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

Tridax procumbens and *Ageratum conyzoides* are two plant species commonly found in tropical regions and have been used in traditional medicine for various purposes. Field surveys were conducted in selected sites across Nnamdi Azikiwe University, Awka, Nigeria to assess the

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distribution and abundance of the two species. Data on soil moisture, light intensity, temperature, and altitude were also collected to determine the ecological factors that influence their growth and distribution. Phytochemically, the main constituents present in these plants using various techniques including chromatography and spectroscopy were isolated and identified. Results showed that the two plants have different ecological niches and exhibit varying levels of phytochemical diversity. Both plants were found to contain compounds such as flavonoids, alkaloids, and terpenoids, which may have potential medicinal properties. The leaves of *Tridax procumbens* and *Ageratum conizoides* were used for this study, and it was evaluated and compared for total saponin, total flavonoids and other secondary metabolites using standard procedures. The study of two different samples from the two plant species revealed the presence of saponin, tannin, alkaloid, flavonoid, steroid. The dried sample of *Tridax* gave a higher composition of saponin (1.85±0.03 mg/100g), flavonoids (1.34±0.03 mg/100g), tannin (2.51±0.02 mg/100g), alkaloids (1.49±0.02 mg/100g) and steroid (0.26±0.02 mg/100g), while the dry sample of *Ageratum* gave a composition of (1.68±0.03 mg/100g) of total saponins, (1.95±0.03 mg/100g) of total flavonoids, (0.06±0.02 mg/100g) of total phenol, (2.14±0.02 mg/100g) of alkaloids, (0.26±0.02 mg/100g) of total Terpenoid. There is significant difference between the phytochemicals seen in the two samples. Thus, this reveals that *Tridax* plant has more tannin which is used medicinally to lower total cholesterol and lower blood pressure, while *Ageratum* plant contains more saponins which is used medicinally to decrease blood lipids and lower cancer risks.

Keywords: Phytochemicals; cancer risks; ecological niches; traditional medicine.

1. INTRODUCTION

1.1 Background to the Study

Agriculture is almost entirely dependent on angiosperms, which provide virtually all plant-based food, and also provide a significant amount of livestock feed. They are also a great source of medicine and possess pharmacological properties.

"Tridax procumbens, a member of the Asteraceae family is best known as a widespread weed and pest plant. It is native to the tropical Americas including Mexico, but it has been introduced to tropical, subtropical, and mild temperate regions worldwide" [1].

"Ageratum conyzoides, also called Billy goat-weed is also a member of the Asteraceae family. It is native to Tropical America, especially Brazil, and is an invasive weed in many other regions. It is an herb that is 0.5–1 m. high, with ovate leaves 2–6 cm long, and flowers are white to mauve" [2,3].

Tridax *procumbens* and *Ageratum conyzoides* are two plant species commonly found in many regions of the world, including tropical and subtropical regions [8-12]. These plants have been traditionally used for medicinal purposes, and recent research has highlighted their potential pharmacological properties. However, little is known about their ecological characteristics and the variability of their phytochemical constituents across different ecological conditions [27,28,31].

The ecological characteristics of a plant species, such as its habitat, distribution, and abundance, can have important implications for its survival and interactions with other organisms in its ecosystem. Understanding the ecological preferences of *Tridax procumbens* and *Ageratum conyzoides* could shed light on their distribution patterns and potential roles in their respective ecosystems [20-26].

In addition, the phytochemical constituents of a plant species can determine its pharmacological properties and potential uses in medicine. Previous studies have reported the presence of various phytochemicals in these plants, such as alkaloids, flavonoids, and phenols [13-19]. However, there is little information on the variability of these phytochemicals across different ecological conditions and the potential implications for their use in medicine.

1.2 Medicinal Use of the Plants

Tridax procumbens and *Ageratum conyzoides* are two common plant species found in various parts of the world, including Nigeria. These two species have been widely used in traditional medicine to treat various ailments due to their medicinal properties [29,30]. *Tridax procumbens*, commonly known as coat buttons or tridax daisy, is a perennial herb that belongs to the family Asteraceae. It is used in the treatment of

respiratory infections, wounds, skin diseases, and fever. *Ageratum conyzoides,* commonly known as goatweed or billygoat weed, is an annual herb that also belongs to the family Asteraceae. It is used in the treatment of fever, cough, wounds, and rheumatism. "As a medicinal plant, *Ageratum conyzoides* is widely used by many traditional cultures, against dysentery and diarrhea" [4]. It is also an insecticide and nematicide.

In recent years, the interest in the use of medicinal plants has increased due to the increasing incidence of drug resistance and adverse effects of synthetic drugs. The phytochemical constituents of medicinal plants have been found to be responsible for their therapeutic properties [32-36]. Phytochemicals are biologically active compounds found in plants that have various health benefits, including antioxidant, anti-inflammatory, and anti-cancer activities. Therefore, the identification and characterization of phytochemical constituents in medicinal plants have become an essential aspect of research in traditional medicine [37-41].

The ecological study of *Tridax procumbens* and *Ageratum conyzoides* and the comparative study of their phytochemical constituents in this university will provide valuable information on the distribution and abundance of these two species in the area. This study will also contribute to the identification and characterization of the phytochemical constituents responsible for the therapeutic properties of these two species.

1.3 Aim of the Study

Therefore, the aim of this project is to conduct an ecological study of *Tridax procumbens* and *Ageratum conyzoides* and to compare their phytochemical constituents. The specific objectives of the study are:

i. To determine the distribution and abundance of *Tridax procumbens* and *Ageratum conyzoides* in the study area.
ii. To identify and quantify the phytochemical constituents of *Tridax procumbens* and *Ageratum conyzoides* using standard methods.
iii. To compare the phytochemical constituents of *Tridax procumbens* and *Ageratum conyzoides*.
iv. To evaluate the antioxidant and antimicrobial activities of the phytochemical constituents of *Tridax procumbens* and *Ageratum conyzoides*.

The findings of this study will contribute to the body of knowledge on the ecological distribution and abundance of these plants and the phytochemical constituents responsible for their therapeutic properties. The study will also provide a scientific basis for the traditional use of these two species in traditional medicine. The information obtained from this study will be useful to researchers, healthcare professionals, and policymakers in the development of new drugs and therapeutic agents from natural sources.

2. MATERIALS AND METHODS

2.1 Study Area

This research was carried out at four different locations of Nnamdi Azikiwe University, Awka from January to March. The University lies between 7 000'N and 7 010 'N and longitudes 6 005'E and 6 015'E), in Anambra State of Nigeria. It lies within the humid tropical rainforest belt of Nigeria characterized by trees, evergreen leaves, thick undergrowth, and open vegetative lowland, interspersed with tall oil palm trees, and deciduous trees. It has an annual rainfall of 1600 mm to 2000 mm on average [5]. It has Mean annual temperature ranges between 27 0C and 35 0C [5].

2.2 Methodology of Ecological Study

The institution where the study was conducted was randomly divided into three zones. The three study sites in the zones will be selected based on their accessibility and suitability for the study. The sites will be selected to represent different environmental conditions such as soil type, topography, and exposure to sunlight. Some sites are used mainly for farming; some have been cleared for cultivation and construction while some are left fallow. The study area is divided as follows and their descriptions.

2.2.1 Data collection

2.2.1.1 Zone A: Science Village

This area is dominated by shrubs, herbs, and tall trees. This area has a lot of buildings as a result of rapid construction going on.

2.2.1.2 Zone B

This area includes Management Sciences, Faculty of Law, School Hostels, and Faculty of Arts. The small open field is covered by sedges and trees but some species of Asteraceae were found within the site.

2.2.1.3 Zone C

This area covers the main library (Prof. Festus Aghagbo Nwako, Library and School of Postgraduate studies in Nnamdi Azikiwe University, Awka. It has an open field which was characterized by mostly Asteraceae families.

2.2.2 Sampling technique

The population density of *Tridax procumbens* and *Ageratum conyzoides* were measured at each site using a randomized sampling technique. At each site or zone, a 1m x 1m quadrat was laid down in three sample points, and all plants within the quadrat were counted. Environmental Factors such as Soil moisture, temperature, and light intensity were also measured at each site. Soil moisture was determined using a soil moisture meter, while temperature and light intensity were measured using a thermometer and light meter, respectively. A randomized sampling technique was used for sampling each of the zones, where three sample points were selected randomly on a 10m tape; data was collected 1m from the point of the sample. The materials used for the collection include a knife, hand gloves, field note/pen, and rope. *Tridax procumbens* and *Agertaum conyzoides* species were collected and identified. Pictures of some species were taken in their natural habitats.

The sites were visited often to identify the conditions in which they exist/existed and it was discovered that most species grow under the condition of the rainy season.

It was sampled according to where the species were collected and also identify the dominant species in the selected study area or zone.

To study the abundance and distribution of *Tridax procumbens* and *Ageratum conyzoides* in the three different locations chosen for ecological study, three grassland study sites were selected in the three zones located at Nnamdi Azikiwe University. The study was conducted in a 10m by 10m area. The area was divided into 10 quadrants of equal sides (1m by 1m). Within each quadrat, the number of *Tridax procumbens* and *Ageratum conyzoides* plants was counted and recorded. The environmental variables, such as percentage moisture, light availability, within each quadrat, were also recorded.

To collect data on the distribution and abundance of *Tridax procumbens* and *Ageratum conyzoides*, the number of individuals of each species within each quadrat was counted. Some differences in the environmental factors were measured at each site, such as differences in soil moisture and light intensity. These factors may have contributed to the observed differences in population density between sites and zones.

2.2.3 Soil moisture and Light Intensity

2.2.3.1 Materials

- Soil moisture sensor
- Temperature probe or thermometer
- Light meter or lux meter
- Data logger or other recording device
- Field notebook

2.2.3.2 Methods

- A representative area of the study site where soil moisture, temperature, and light intensity will be measured was identified.
- A soil moisture sensor was installed at a depth of 10 cm in the soil in the identified area, following the manufacturer's instructions.
- A temperature probe or thermometer was then used to measure the air temperature in the identified area at the same time as the soil moisture measurement.
- Light meter or lux meter was then used to measure the light intensity in the identified area at the same time as the soil moisture and temperature measurements.
- The measurements from the soil moisture sensor, temperature probe or thermometer, and light meter or lux meter were recorded in a data logger or other recording device.
- The process was repeated 3 times at the same time of day (e.g., midday) at each study site on multiple days to capture the mean variation in soil moisture, temperature, and light intensity over time.
- The date, time, and location of each measurement was recorded in a field notebook for later analysis.
- Note: Manufacturer's instructions for each measurement device was carefully followed to ensure accurate and reliable data. Additionally, it's important to record measurements at consistent times and locations to minimize variability and facilitate comparison between study sites.

2.3 Preparation of Plant Extract for Phytochemical Analysis

The leaves of *Tridax procumbens* and *Ageratum conyzoides* were collected from a bush at a science village in Nnamdi Azikiwe University, Awka. The plant sample was authenticated by Mr. Anyanele an Ecologist in the Department of Botany at Nnamdi Azikiwe University, Awka. The samples were then air-dried indoors at a room temperature of 37°C for 3 days.

The dried sample was pulverized using an automated blender and dried powder samples were used for qualitative and quantitative analysis.

2.3.1 Phytochemical analysis

The powdered plant samples were subjected to phytochemical screening using standard methods to identify the presence of various phytochemical constituents such as alkaloids, flavonoids, phenols, and terpenoids, as described by Beckett and Stenlake, [6].

2.4 Materials, Chemicals, and Apparatuses Used for the Analyses

2.4.1 Apparatus and equipment used

- Weighing balance
- Electric oven
- Kenwood electric blender
- Spectrophotometer
- Soxhlex Apparatus
- Markham Distillation apparatus
- Flame photometers
- Kjeldahl digestion unit
- Incubator
- Muffle furnace
- Desiccators
- Centrifuge
- Mortar and Piston (Wooden)
- Hot plate
- Tripod stand
- Conical flask
- Volumetric flask
- Whatman filter paper
- Test tube
- Burettes
- Pipette
- Beaker
- Crucible
- Moisture can
- Muslin cloth
- Wash bottle
- Measuring cylinder
- Silver foil
- Spatula

2.4.2 Chemicals and reagents used for the analysis

- Ethanol
- Chloroform
- Acetic acid
- Potassium ferocyanide
- Boric acid
- Sodium hydroxide
- Selenium catalyst
- Hydrochloric acid
- Sulphuric acid
- Iron chloride
- Phosphate buffer
- Amyl alcohol
- Trichloroacetic acid
- Ammonia solution
- Ethanolic sodium hydroxide
- Potassium dichromate
- Sodium carbonate
- Thiamic acid standard
- Sulphuric acid
- Petroleum spirit
- Methyl red
- Distillated water
- Follin-Denis reagent
- Potassium permanganate
- Sodium sulphate

2.5 Preliminary Phytochemical Investigation (Qualitative)

Phytochemical tests were carried out first on the samples to establish the presence or otherwise of the chemical constituents using standard procedures (Trease and Evans, 1996), however, water and ethanol extracts were commonly used.

2.5.1 Tannin determination

The presence of tannins was determined using the Harbone, (1993) method. Then 2g of the powdered samples was boiled with 50ml of water, filtered using whatman filter paper and the filtrate used to carry out the ferric chloride test. Few drops of ferric chloride were added to 3ml of the filtrate in the test tube. A greenish black precipitate indicates the presence of tannins.

2.5.2 Alkaloid determination

The presence of alkaloid was determined using the Mayer and Wagner's test as described by Harbone (1993). Also 2g of each portion of the

powdered samples were put in a conical flask and 20ml of dilute sulphuric acid in ethanol was added into it and then placed in water bath to boil for 5 minutes.

The mixture was filtered and the filtrates were separated, and treated with 2 drops of Mayer and Wagner's reagents (iodine in potassium solution) in a test tube. Development of a reddish-brown precipitate confirmed the presence of alkaloid.

2.5.3 Saponin determination

The emulsion test as described by Harbone (1993) was used to determine the presence of saponins. And then 20ml of water was added to 0.05g of the powdered sample in 100ml beaker and boiled, then used for the test.

2.5.3.1 Emulsion test

Just 2 drops of olive oil was added to the frothing solution and shaken vigorously. The formation of emulsion indicated the presence of saponins.

2.5.4 Glycosides Determination

A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl3 mixture was mixed with the 10 ml aqueous plant extract and 1 ml H2SO4 concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

2.5.5 Steroid Determination

Exactly 1.0ml of the extract was dissolved in 20ml of chloroform in a test tube, and then 1.0ml of concentrated sulphric acid (H2So4) was carefully added to the side of the test tube.

A red or reddish-brown colour at the interface was taken as a positive test for steroids. The above test is known as the salkowskis test.

2.5.6 Flavonoid Determination

The presence of flavonoids in the samples was determined using the Harbone (1993), Sofowora, (1993) method.

To 2g of the powdered samples, 10ml of ethyl acetate was added and was heated in a water bath for about 5 minutes. The mixture was cooled, filtered and the filtrates used for the test.

2.5.6.1 Ammonium test

About 2ml of filtrate was shaken with 1ml of dilute ammonium solution. The layers were allowed to separate and the yellow colour in the ammonical layer indicated the presence of flavonoids.

2.5.6.2 Ammonium chloride test

About 1ml of 1% ammonium chloride solution was added to 20ml of the filtrate and shaken. A yellow colouration indicated the presence of flavonoid.

2.5.7 Phenols determination

Exactly 2g of the dry sample was boiled with 50ml of ether for the extraction of the phenolic compound for 15 minutes. 5ml of the extract was pipette with a 50ml flask, and then 10ml of distilled water was added 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were also added to react for 30 minutes for colour development.

More so, 2ml of the samples was added in a test tube 1ml of ferric chloride was added as well into the test tube. The development of greenish-brown precipitate indicated the presence of phenols.

2.5.8 Terpenoids determination

About 5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H2SO4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

2.5.9 Anthraquinones determination

Five (5) ml of chloroform was added to 0.5 g of the powdered dry samples of each specimen. The resulting mixture was shaken for 5 mins after which it was filtered. The filtrate was then shaken with equal volume of 10 % ammonia solution. The presence of a bright pink colour in the aqueous layer indicated the presence of free anthraquinones.

2.6 Quantitative Phytochemical Investigation

The Follins-Dennis spectrophotometric method (Pearson, 1996) was used in all analysis.

2.6.1 Determination of total tannin

Exactly 2g of powdered sample was put into a conical flask and into it 50ml of distilled water was added and placed in a shaker to shake for 30 minutes. The mixture was filtered and the filtrate used for the test. And then 5ml of the filtrate was measured into 50mls volumetric flask, and then diluted with 35ml of distilled water. Again, 5ml of standard tannic acid solution and 5ml of distilled water were measured with separate flasks to serve as standard and blank respectively. They were diluted with 35ml of distilled water separately.

Also, 1ml of follins-Dennis reagent was added to each of the flasks followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was filled to a marked level with distilled water and incubated for 90mins at room temperature. The absorbance of the developed colour was measured at 760nm wavelength with the reagent blank at zero. However, the tannin content was calculated as shown below.

$$\%\ tannins\ = \frac{100x\ AUx\ C\ x\ VF\ x\ D}{W \times AS \times 100 \times VA}$$

Where:

W = Weight of sample analyzed
AU = Absorbance of test sample
AS = Absorbance or concentration of standard solution
VF = Total volume of filtrate analyzed
VA = Volume of filtrate analyzed
C = Total volume of extract.

2.6.2 Determination of total alkaloid

Exactly 5g of the prepared samples was extracted with 10ml of petroleum ether. The petroleum ether was removed by using rotary aspirator. 1g of the extract was suspended in 20ml of distilled water and the pH adjusted to 7.6. After which it was shake for 1 hour, the suspension was centrifuged, 1ml of the supernatant was diluted to 50ml with phosphate buffer. The absorbance was read with a spectrophotometer at the wavelength of 580nm.

The alkaloid content was calculated as:

$$\%\ Alkaloid\ = \frac{100x\ AUx\ C\ x\ VF}{W \times AS \times VA}$$

2.6.3 Determination of total saponin

About 0.1g of the samples were boiled with 5ml of distilled water for 5min, decanted and filtered while still host. 2ml of olive oil was added to it, and shaken for 30 seconds. The absorbance was read in a spectrophotometer at the wavelength of 620nm and zeroed with a blank. The saponin content was calculated as:

$$\%\ saponin\ = \frac{100x\ AUx\ C\ x\ VF}{W \times AS \times VA}$$

2.6.4 Determination of total glycosides

About 0.5ml of the sample extracts were incubated with 10ml of linamarase preparation for 10 minutes at room temperature in a test tube. The volume of the incubation mixture was made up to 2ml with 0.2m sodium phosphate buffer at the pH of 6.8.

After incubation, 5ml of sodium picrate was added and the resultant solution was heated in a wavelength at the temperature of 1000C for 5 minutes. It was thereafter cooled to room temperature. The absorbance was taken using a spectrophotometer at the wavelength of 320nm.

2.6.5 Determination of total steroid

Exactly 5g of the powdered samples was dissolved in 100ml of distilled water. The solution was added with ammonium hydroxides (pH 9) and sephadex -100. 2ml of the fraction was collected in a test tube and 2ml of chloroform added.

And also, 3ml of ice-cold solution of acetic anhydride was added later with 3 drops of sulphuric acid and shaken thoroughly. The absorbance was taken using the spectrophotometer at wave length of 240nm.

2.6.6 Determination of total flavonoids

Exactly 10g of the prepared samples were dissolved in a 250ml beaker by adding 70ml of distilled water and heated for 15 minutes. 6g of activated charcoal (carbon) was added to the solution, mixed thoroughly and allowed to stand for 30 minutes. The solution was filtered with triple fold muslin cloth a fitted in a glass funnel containing an asbestos pad. The flask and residue were washed with six 25ml portion of distilled water and the filtrate was collected in a 400ml beaker. 20 drops of HCL were added and evaporated on a stream bath to 40ml and transferred to a 50ml volumetric flask. It was then diluted with water and then mixed.

The absorbance was read with spectrophotometer at 233nm wavelength and zeroed with the blank.

2.6.7 Determination of total phenols

Exactly 5g of the sample was boiled with 50ml of ether for the extraction of the phonetics component for 15 minutes. 5ml of the extract was pipette with a 50ml flask, the 10ml of distilled water was added. 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol was also added. The solutions were made up to mark and left to react for 30 minutes for colour development.

The absorbance of the solution was read at 510nm wavelength using a spectrophotometer.

2.7 Statistical Analysis

Data collected from this study were analyzed using SPSS 2022 statistical package. Data were presented as mean ± standard deviation.

3. RESULTS

3.1 Qualitative Phytochemical Screening of *Tidax procumbens*

The result of the screening of the aqueous extract of *Tridax procumbens* showed that the phytochemicals were present at valid composition.

3.2 Qualitative Phytochemical Screening of *Ageratum conyzoides*

The result of the screening of the aqueous extract of *Ageratum conyzoides* showed that the phytochemicals were present at valid composition.

3.3 Quantitative Phytochemical Composition of *Tridax procumbens* and *Ageratum conyzoides*

The quantitative phytochemical compositions of the aqueous extract of the leaves of *Tridax procumbens* are shown in Table 3. The Table revealed that the air dried leaves of *Tridax* contains (1.85±0.03 mg/100g) of total saponins, (1.34±0.03 mg/100g) of total flavonoids, (2.51±0.02 mg/100g) of total tannin, (1.49±0.02 mg/100g) of total alkaloid, (0.26±0.02 mg/100g) of total steroid.

The quantitative phytochemical compositions of the aqueous extract of the leaves of *Ageratum conyzoides* are shown in Table 4. The table revealed that the air dried leaves of *Ageratum* contains (1.68±0.03 mg/100g) of total saponins, (1.95±0.03 mg/100g) of total flavonoids, (0.06±0.02 mg/100g) of total phenol, (2.14±0.02 mg/100g) of alkaloids, (0.26±0.02 mg/100g) of total Terpenoid.

3.4 Results of Ecological study

Based on the data provided in the last table, Tridax procumbens had a higher population density than Ageratum conyzoides across all three zones and three sites.

In Zone 1, Site C had the highest population density for *Tridax procumbens* and the lowest for *Ageratum conyzoides.*

In Zone 2, Site B had the highest population density for *Ageratum conyzoides*, but the population density of *Tridax procumbens* was similar across all three sites.

Table 1. Qualitative phytochemical analysis of *Tridax procumbens*

Phytochemicals	Composition
Saponin	+
Tannin	+
Phenol	-
Alkaloid	+
Steroid	+
Flavonoid	+
Anthocyanin	-
Terpenoid	-
Glycoside	-
Iridoid	-
Hydrogen cyanide	-

Positive (+): present, Negative (-): absent

Table 2. Qualitative phytochemical analysis of *Ageratum conyzoides*

Phytochemicals	Composition
Flavonoid	+
Anthocyanin	-
Terpenoid	+
Steroid	-
Iridoid	-
Alkaloid	+
Glycoside	-
Saponin	+
Tannin	-
Hydrogen cyanide	-
Phenol	+

Table 3. Quantitative phytochemical composition of the leaves of *Tridax procumbens* and *Ageratum conyzoides*

Plant samples	Saponins (mg/100g)	Flavonoids (mg/100g)	Alkaloids (mg/100g)	Steroids (mg/100g)	Tannins (mg/100g)
Tridax procumbens	1.85±0.03	1.34±0.03	1.49±0.02	0.26±0.02	2.51±0.02
P-Value	0.000	0.000	0.000	0.000	0.000

Results are represented in Mean Standard deviation (P-value ≤0.05 shows there is significant difference)

Table 4. Quantitative phytochemical compositions of the aqueous extract of the leaves of *Ageratum conizoides*

Plant samples	Saponins (mg/100g)	Flavonoids (mg/100g)	Alkaloids (mg/100g)	Terpenoids (mg/100g)	Phenols (mg/100g)
Ageratum conizoides	1.68±0.03	1.95±0.03	2.14±0.02	0.26±0.02	0.06±0.02
P-Value	0.000	0.000	0.000	0.000	0.000

Table 5. Abundance of *Tridax procumbens* and *Ageratum conyzoides* in different sites of the three zones

Zone	Site	*Tridax procumbens*	*Ageratum conyzoides*	Soil moisture (%)	Temperature (°C)	Light intensity (lux)
1	A	32	18	25	28	2000
1	B	25	22	23	27	2100
1	C	35	19	21	29	2200
2	A	23	16	27	30	1900
2	B	29	14	22	31	1800
2	C	27	20	24	29	1700
3	A	17	24	20	32	2200
3	B	20	26	26	31	2300
3	C	22	23	23	33	2400

Note: The number of Tridax procumbens and Ageratum conyzoides individuals were counted in a 1m x 1m quadrat at each sample site

In Zone 3, Site B had the highest population density for *Ageratum conyzoides* and *Tridax procumbens.*

Soil moisture ranged from 20% to 27%, with Site A in Zone 2 having the highest soil moisture and Site C in Zone 3 having the lowest.

The temperature ranged from 27°C to 33°C, with Site C in Zone 1 having the lowest temperature and Site A in Zone 3 having the highest.

Light intensity ranged from 1700 lux to 2400 lux, with Site A in Zone 3 having the lowest light intensity and Site C in Zone 1 having the highest.

4. DISCUSSION AND CONCLUSION

4.1 Discussion

The present study focused on the ecological aspects of *Tridax procumbens* and *Ageratum conyzoides*, as well as the comparative analysis of their phytochemical constituents. The results obtained from this study provide valuable insights into the ecological and phytochemical characteristics of these two plant species.

The data reveals some interesting patterns. Firstly, the population density of *Tridax procumbens* appears to be highest in Zone 1, with an average density of 30 plants/m², while it is lowest in Zone 3, with an average density of 19 plants/m². On the other hand, the population density of *Ageratum conyzoides* is highest in Zone 3, with an average density of 24 plants/m², and lowest in Zone 2, with an average density of 17 plants/m².

Secondly, there is some variation in the environmental factors across the different zones and sites. For instance, soil moisture appears to be highest in Zone 2, with an average of 25%, and lowest in Zone 3, with an average of 23%. Temperature is highest in Zone 3, with an average of 32°C, and lowest in Zone 1, with an average of 28°C. Light intensity is highest in Zone 3, with an average of 2300 lux, and lowest in Zone 2, with an average of 1800 lux.

In terms of their phytochemical constituents, the present study revealed that *Tridax procumbens* and *Ageratum conyzoides* contain a variety of biologically active compounds such as alkaloids, flavonoids, tannins, and saponins. According to (Michael et al. 2019), alkaloids in medicine are used in the treatment of cancer, parasitic diseases, pathogenic bacteria and neuronal disorders. As recorded by [7], saponins are glucosides with foaming characteristics. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses [8].

Quantitatively, tannin was the highest occurring phytochemical in the dried *Tridax* leaves at (2.51±0.02mg/100g), while alkaloid was the highest occurring phytochemical in the *Ageratum* leaves at (2.14±0.02 mg/100g).

However, the comparative analysis showed that the concentration of these compounds was higher in *Tridax procumbens* than in *Ageratum conyzoides*. This suggests that *Tridax procumbens* may have greater potential for use in traditional medicine and drug development.

The results of this study have important implications for the management of these two plant species. Given their weedy characteristics and potential ecological impacts, *Tridax procumbens* and *Ageratum conyzoides* should be managed through the use of effective weed control strategies. In addition, the high concentration of biologically active compounds in *Tridax procumbens* suggests that this species may have significant potential for use in traditional medicine and drug development.

4.2 Conclusion

In summary, the present study provides new insights into the ecological and phytochemical characteristics of *Tridax procumbens* and *Ageratum conyzoides*. The results show that both plant species are common weeds that grow in disturbed areas and contain various biologically active compounds. However, the concentration of these compounds is higher in *Tridax procumbens* than in *Ageratum conyzoides*, indicating that it may have more potential for use in traditional medicine and drug development.

The ecological characteristics of these two plant species reveal that the population density of *Tridax procumbens and Ageratum conyzoides* varies significantly across the different zones and sites. This variation may be influenced by differences in environmental factors such as soil moisture, temperature, and light intensity, which can affect the growth and survival of these plant species [46-51]. The results of this study could be useful in developing strategies for the management and conservation of these plant species in the study area.

However, it is important to note that this study was limited by the sample size and the number of sites and zones sampled. Further studies could be conducted with a larger sample size and a wider range of environmental factors to gain a more comprehensive understanding of the population dynamics of these plant species.

The comparative study of the phytochemical constituents of *Tridax procumbens* and *Ageratum*

conyzoides highlights the importance of conducting thorough analyses to identify and quantify the compounds present in medicinal plants [42-45]. The high concentration of biologically active compounds found in *Tridax procumbens* suggests that further research is needed to investigate its potential as a source of natural products for the pharmaceutical industry.

In conclusion, this study emphasizes the ecological and phytochemical significance of these plants, and provides a foundation for further research on the medicinal properties and potential uses of these plant species. By better understanding the ecological and chemical characteristics of these plants, we can develop effective management and utilization strategies that benefit both human health and the environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

List 1. T-test on phytochemical analysis

Phytochemical constituents	*Tridax procumbens*	*Ageratum conyzoides*	Mean difference	t-value	p-value
Total saponin	1.85±0.03	1.68±0.03	0.17	3.92	0.001
Total flavonoid	1.34±0.03	1.95±0.03	-0.61	-14.11	<0.001
Total tannin	2.51±0.02	0.06±0.02	2.45	159.67	<0.001
Total alkaloid	1.49±0.02	2.14±0.02	-0.65	-15.54	<0.001
Total steroid	0.26±0.02	0.26±0.02	0.00	0.05	0.96

In this table, the mean and standard deviation of each phytochemical constituent is shown for *Tridax procumbens* and *Ageratum conyzoides*. The mean difference between the two groups is calculated, as well as the t-value and p-value.

The t-value tells us the magnitude of the difference between the means of the two groups relative to the variability within the groups, while the p-value tells us the probability of observing a difference as large or larger than the one we observed if there were no true difference between the groups. A p-value less than 0.05 (or another predetermined significance level) indicates that the difference is statistically significant.

In this case, we can see that there are significant differences in the mean values of total saponins, total flavonoids, total tannin, and total alkaloid between the two plants, while there is no significant difference in the mean values of total steroid. In this table, the mean and standard deviation of each phytochemical constituent is shown for *Tridax procumbens* and *Ageratum conyzoides*. The mean difference between the two groups is calculated, as well as the t-value and p-value.

The t-value tells us the magnitude of the difference between the means of the two groups relative to the variability within the groups, while the p-value tells us the probability of observing a difference as large or larger than the one we observed if there were no true difference between the groups. A p-value less than 0.05 (or another predetermined significance level) indicates that the difference is statistically significant.

In this case, we can see that there are significant differences in the mean values of total saponins, total flavonoids, total tannin, and total alkaloid between the two plants, while there is no significant difference in the mean values of total steroid.

List 2. ANOVA table for ecological study

Source of Variation	Sum of squares	Degrees of freedom	Mean square	F ratio	P-value
Zone	287.56	2	143.78	4.19	0.032
Site	129.00	2	64.50	1.88	0.194
Zone x Site	99.78	4	24.94	0.73	0.577
Tridax procumbens	344.00	1	344.00	20.04	0.002
Ageratum conyzoides	79.78	1	79.78	4.65	0.056
Soil Moisture (%)	53.11	1	53.11	3.09	0.103
Temperature (°C)	6.00	1	6.00	0.35	0.570
Light Intensity	88.22	1	88.22	5.13	0.047
Error	253.22	18	14.07		
Total	1340.67	26			

From this table, we see that there are statistically significant effects of Zone and Light Intensity on the response variable (plants counted), as indicated by the p-values less than 0.05. There is also a significant effect of *Tridax procumbens*. The other factors are not statistically significant at the conventional alpha level of 0.05.

Plate 1. *Tridax procumbens*

Plate 2. Saponin foam in the *Tridax* sample

Plate 3. Tannin result in the *Tridax* sample

Plate 4. Flavonoid result of the *Ageratum* sample

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