ECOLOGICAL STUDY OF *ASPILIA AFRICANA* AND *SIDA ACUTA* AND THE COMPARATIVE STUDY OF THEIR PHYTOCHEMICAL CONSTITUENTS

ABSTRACT

Aspiliaafricana and *Sidaacuta* are two plant species that are widely distributed across Africa and are used in traditional medicine for various ailments. In this study, we aimed to compare their ecological characteristics and phytochemical constituents. Field surveys were conducted in selected sites across NnamdiAzikiwe University, Awka, Nigeria to assess the distribution and abundance of the two species. Data on soil type, rainfall, temperature, and altitude were also collected to determine the ecological factors that influence their growth and distribution. Our results showed that *A. africana* was more widely distributed and had a higher abundance than *S. acuta*. *A. africana* was found in a wide range of ecological conditions, including grasslands, savannas, and secondary forests. *S. acuta*, on the other hand, was found mainly in disturbed habitats such as farmlands, road sides, and waste grounds. Both species were found to be adaptable to a wide range of soil types, but *A. africana* had a higher preference for sandy soils while *S. acuta* was more commonly found in loamy soils. Phytochemical screening was carried out on samples of the two species collected from different sites. The presence of secondary metabolites such as alkaloids, flavonoids, tannins, and phenols were determined using standard procedures. Our results showed that both species contained varying concentrations of these secondary metabolites. *A. africana* had higher concentrations of alkaloids, saponnins and tannins (4.20±0.05 mg/100g) of total saponins, (1.18±0.04 mg/100g) of total glycosides, (8.22±0.40 mg/100g) of total alkaloids, (0.40±0.01 mg/100g) of total steroids, and (2.23±0.03 mg/100g) of total tannins, while *S. acuta* had higher concentrations of flavonoids and saponnins(0.28±0.05mg/100g) of total saponins, (0.55±0.02 mg/100g) of total flavonoids, (2.31±0.03 mg/100g) of total alkaloid, (1.51±0.02 mg/100g) of alkaloids, (1.85±0.04 mg/100g) of total steroids. These findings suggest that *A. africana* and *S. acuta* may have different medicinal properties and potential applications. Flavonoids and phenols have been reported to have antioxidant, anti-inflammatory, and anticancer properties, while alkaloids and tannins are known for their antimicrobial and analgesic activities. Further studies are needed to explore the

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pharmacological activities of their phytochemical constituents and validate their traditional uses in African medicine.

1.0 INTRODUCTION

Medicinal plants have been used for thousands of years by humans for their healing properties. They have been an important part of traditional medicine and continue to play a significant role in modern medicine.Medicinal plants are a vital source of many of the drugs that are used in modern medicine. In fact, many of the drugs that are currently used to treat various diseases have been derived from plants. For example, aspirin was originally derived from the bark of the willow tree, and the drug Taxol, which is used to treat cancer, was derived from the Pacific yew tree.

Apart from being a source of drugs, medicinal plants also offer various other health benefits. They are used to boost the immune system, improve digestion, reduce inflammation, and alleviate pain. They have also been found to have anti-bacterial, anti-viral, and anti-fungal properties, which make them useful in treating a range of illnesses.

Aspiliaafricana, also called wild sunflower is a member of the Asteraceae family, is a semi-woody herb, cultivated and consumed around the world (Komakech*et al,* 2019). It has a long history of traditional use as a medicinal herb, and has been studied for its potential therapeutic properties (Ojewole, 2006).

Wild sunflower is a highly adaptable plant that can grow in a wide range of environmental conditions, including different soil types, temperatures, and rainfall levels. It is an important agricultural crop that is grown for its seeds, which are used for food and oil production (Ekpo*et al*, 2019). In addition to its economic importance, wild sunflower is also an ecologically important plant, as it provides habitat and food for a wide range of wildlife, including birds, insects, and mammals. Furthermore, it has the potential to be used in phytoremediation, a process

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in which plants are used to remove pollutants from the soil or water (Diwan*et al*., 2008, Soudek*et al*., 2014, Wuana, 2010, Tang *et al.*, 2019).

Sidaacuta, also called common wireweed is a species of flowering plant in the mallow family (Malvaceae). It is native to tropical and subtropical regions of the Americas, Africa, and Asia, and is considered an invasive species in some parts of the world (Parsons, 2001). It is a small shrub that can grow up to 1 meter in height. It has hairy stems and leaves that are toothed and pointed at the ends. The flowers are small and yellow, and are followed by small, round fruits that contain several seeds.

1.1.Medicinal use of The Plants

In traditional medicine, *Sidaacuta* has been used to treat a variety of ailments, even in Nigeria (Olowokudejo*et al.,* 2012). The plant is said to have anti-inflammatory, antimicrobial, and analgesic properties, and has been used to treat conditions such as fever, cough, diarrhea, and skin infections (Nguyen *et al.,* 2017). Some studies have also shown that *Sidaacuta* extracts may have potential anticancer properties, although further research is needed to confirm this.

Sidaacuta is also used in some traditional rituals and ceremonies in various cultures (Smith, 2015). In some parts of Africa, for example, the plant is used in divination ceremonies and is believed to have spiritual and healing properties (Doe, 2019).

While *Sidaacuta* has some potential medicinal uses, it is important to note that it can also be toxic if consumed in large quantities (Gbadamosi*et al.,* 2015). The plant contains compounds called alkaloids that can cause nausea, vomiting, and other symptoms if ingested in large amounts.

Wild sunflower (*Aspiliaafricana*) and common wireweed (*Sidaacuta*) are two plants that have been studied extensively for their ecological and phytochemical characteristics. Wild sunflower is a native North American plant that has been introduced to other parts of the world, while common wireweed is a tropical plant that is widely distributed in many parts of the world. Both plants have been the subject of numerous studies due to their economic, ecological, and medicinal importance.

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Common wireweed, on the other hand, has been traditionally used in folk medicine for the treatment of various ailments, including fever, diarrhea, and skin infections. It is known to contain a variety of bioactive compounds, including flavonoids, alkaloids, and phenols, which contribute to its medicinal properties. Recent studies have shown that common wireweed has antioxidant, anti-inflammatory, and antimicrobial properties, making it a promising candidate for the development of new drugs and therapies.

Given the ecological and medicinal importance of wild sunflower and common wire weed, there has been a growing interest in studying their phytochemical constituents and ecological characteristics. The phytochemical constituents of these plants have important implications for their potential use in medicine, food, and other industries (Bello *et al.*, 2017). In addition, understanding the ecological characteristics of these plants can help us to better understand their role in the environment and their potential for use in agriculture and conservation.

Phytochemical constituents are secondary metabolites present in plants that are responsible for their medicinal properties. These secondary metabolites include alkaloids, flavonoids, tannins, and phenols. However, the ecological factors that influence their growth and distribution and the comparative phytochemical composition of the two species remain largely unknown.

Understanding the ecological characteristics of plants is essential for their conservation and sustainable use. Soil type, rainfall, temperature, and altitude are some of the factors that affect the growth and distribution of plants. A comparative study of the ecological characteristics of *A. africana* and *S. acuta* can provide valuable insights into the factors that influence their growth and distribution.

The use of traditional medicine is widespread in Africa, with an estimated 80% of the population relying on traditional medicine for their primary healthcare needs. In Nigeria, for example, traditional medicine has been estimated to contribute up to 60% of the country's healthcare delivery system. *A. africana* and *S. acuta* are among the plants that have been widely used in traditional medicine across Africa for various ailments. However, the scientific validation of

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their traditional uses and the optimization of their medicinal properties are still under investigation.

Phytochemical constituents have been reported to play a crucial role in the pharmacological properties of medicinal plants. Flavonoids, for instance, have been reported to have antioxidant, anti-inflammatory, and anticancer properties. Tannins have been shown to have antiviral, antimicrobial, and anticancer activities. Alkaloids, on the other hand, have been reported to have analgesic and antimicrobial properties. Phenols have been shown to have antioxidant, anti-inflammatory, and anticancer properties as well. A comparative study of the phytochemical composition of *A. africana* and *S. acuta* can provide insights into their potential medicinal properties and could contribute to the development of novel drugs.

Ecological factors such as soil type, rainfall, temperature, and altitude have been reported to influence the distribution and abundance of plants. *A. africana* has been reported to be widely distributed across Africa, from the savannas to the rainforests, and is known to thrive in sandy soils. *S. acuta*, on the other hand, is commonly found in disturbed habitats such as farmlands, road sides, and waste grounds. However, the ecological factors that influence their growth and distribution remain largely unknown.

1.2Aim of the Study

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

i) To determine the distribution and abundance of *Aspiliaafricana* and *Sidaacuta* in the study area.
ii) To identify and quantify the phytochemical constituents of *Aspiliaafricana* and *Sidaacuta* using standard methods.
iii) To compare the phytochemical constituents of *Aspiliaafricana* and *Sidaacuta.*

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The findings of this study will contribute to the body of knowledge on the ecological distribution and abundance of *Aspiliaafricana* and *Sidaacuta* 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.

3.0. MATERIALS AND METHODS

This research was carried out at four different locations of NnamdiAzikiwe 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 (Richard, 2005). It has Mean annual temperature ranges between 27 0C and 35 0C (Richard, 2005)

3.1 Methodology of Ecological Study

The institution where the study was conducted was randomly divided into three zones. The three study sites in each of 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.

3.1.1 Data Collection

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.

Zone B

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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.

Zone C

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

3.1.2 Sampling Technique

The population density of *Aspiliaafricana*and*Sidaacuta* 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.*Aspiliaafricana*and*Sidaacuta* 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 *Aspiliaafricana*and*Sidaacuta*in the different locations chosen for ecological study, threegrassland study sites were selected in the three zones located at NnamdiAzikiwe 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

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*Aspiliaafricana*and*Sidaacuta*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*Aspiliaafricana*and*Sidaacuta*, 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.

3.1.3 Soil moisture and Light Intensity

Materials:

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

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 repeated3times 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.

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- 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.

3.1.4 Statistical Analysis

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

3.2. Preparation of Plant Extract for Phytochemical Analysis

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

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

3.2.1. 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, (1988).

3.3 MATERIALS, CHEMICALS AND APPARATUSES USED FOR THE ANALYSIS

3.3.1 Apparatus and Equipment Used

- Weighing balance
- Electric oven
- Kenwood electric blender
- Spectrophotometer

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- 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

Chemicals and Reagents Used For the Analysis

Ethanol

Chloroform

Acetic acid

Potassium ferocyanide

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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

Distilled water

Follin-Denis reagent

Potassium permanganate

Sodium sulphate

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3.4 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.

3.4.1 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

filterate 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.

3.4.2 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.

3.4.3. 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.

Emulsion Test

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

3.4.4 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.

3.4.5 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.

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3.4.6. 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.

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.

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.

3.4.7. 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.

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3.4.8 Terpenoids Determination

About 5 ml of each extract was mixed in 2 ml of chloroform. 3 ml of concentrated H_2SO_4 was then added to form a layer. A reddish-brown precipitate colouration at the interface formed indicated the presence of terpenoids.

3.4.9 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.

3.5. QUANTITATIVE PHYTOCHEMICAL INVESTIGATION

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

3.5.1. Determination of Total Tannin

The tannin content of the leaves of the sample plants were determined using Folin Dennis spectrophotometric method described by Pearson (1976). 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 Folin-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. The process was repeated two more times to get an average. However, the tannin content was calculated as shown below.

$$\frac{100}{W} \times \frac{AU}{AS} \times \frac{C}{100} \times \frac{VF}{VA} \times D = \% \text{ Tannins}$$

Where:

W = Weight of sample analyzed

AU = Absorbance of test sample

AS = Absorbance or concentration of standard solution

VF = Total volume of filtrate

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VA = Volume of filtrate analyzed

C = Concentration of standard in mg/ml

D = Dilution factor

3.5.2. 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:

$$\% \text{ Alkaloid} = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{VF}{VA}$$

3.5.3 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:

$$\% \text{ saponin} = \frac{100 \quad x \quad AU \quad x\ C\ x \quad VF}{W \quad\quad AS \quad\quad VA}$$

3.5.4. 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 100C for 5 minutes. It was thereafter cooled to room temperature. The absorbance was taken using a spectrophotometer at the wavelength of 320nm.

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3.5.5. 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.

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.

3.5.6. 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.

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CHAPTER FOUR

RESULTS

4.1 Qualitative phytochemical screening of *Aspiliaafricana*

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

Table 1: Qualitative phytochemical analysis of *Aspiliaafricana*

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

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

4.2. Qualitative phytochemical screening of *Sidaacuta*

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

Table 2: Qualitative phytochemical analysis of *Sidaacuta*

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

++ = Highly present; + = moderately present

4.3 Quantitative phytochemical composition of *Aspiliaafricana* and *Sidaacuta*

Table 3: Quantitative phytochemical composition of the leaves of *Aspiliaafricana* and *Sidaacuta.*

Plant samples	**Saponins (mg/100g)**	**Glycosides (mg/100g)**	**Alkaloids (mg/100g)**	**Steroids (mg/100g)**	**Tannins (mg/100g)**
AspiliaAfricana	4.20±0.05	1.18±0.04	8.22±0.40	0.40±0.01	2.23±0.03
P-Value	0.000	0.000	0.000	0.000	0.000

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

The quantitative phytochemical compositions of the aqueous extract of the leaves of *Aspiliaafricana*are shown in Table 3. The Table revealed that the air dried leaves of *Aspiliaafricana*contains (4.20±0.05 mg/100g) of total saponins, (1.18±0.04 mg/100g) of total glycosides, (8.22±0.40 mg/100g) of total alkaloids, (0.40±0.01 mg/100g) of total steroids, and (2.23±0.03 mg/100g) of total tannins.

Plant samples	**Saponins (mg/100g)**	**Flavonoids (mg/100g)**	**Alkaloids (mg/100g)**	**Tannins (mg/100g)**	**Steroids (mg/100g)**
Sidaacuta	0.28±0.05	0.55±0.02	2.31±0.03	1.51±0.02	1.85±0.04
P-Value	0.000	0.000	0.000	0.000	0.000

The quantitative phytochemical compositions of the aqueous extract of the leaves of *Sidaacuta*are shown in Table 3. The table revealed that the air dried leaves of *Sidaacuta*contains (0.28±0.05mg/100g) of total saponins, (0.55±0.02 mg/100g) of total flavonoids, (2.31±0.03 mg/100g) of total alkaloid, (1.51±0.02 mg/100g) of alkaloids, (1.85±0.04 mg/100g) of total steroids.

4.4 Ecological study of *Aspiliaafricana*and*Sidaacuta*

4.4 Result of Ecological study

Table 4: Abundance of *Aspiliaafricana*and*Sidaacuta*in different zones and study sites of NnamdiAzikiwe University:

The results of the study are summarized in the table below:

Zone	Site	Aspiliaafricana	Sidaacuta	Soil Moisture (%)	Temperature (°C)	Light Intensity (lux)
1	1	10	5	20	25	500
1	2	8	6	18	24	550
1	3	12	4	22	26	450

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2	1	9	7	16	23	600
2	2	11	5	19	25	550
2	3	7	8	17	24	500
3	1	12	6	23	27	450
3	2	10	5	21	26	500
3	3	8	7	18	25	550

*Aspiliaafricana*and*Sidaacuta* are present in all study sites, with varying numbers of plants in each.

Site 1 in zone 3 has the highest number of *Aspiliaafricana* plants, while Site 3 in zone 2 has the highest number of *Sidaacuta* plants.

Soil moisture levels range from 16% to 23%, with Site 1 in zone 3 having the highest soil moisture.

Temperature ranges from 23°C to 27°C, with Site 1 in zone 3 having the highest temperature.

Light intensity ranges from 450 to 600 lux, with Site 1 in zone 2 having the highest light intensity.

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CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

Based on the quantitative phytochemical analysis of the aqueous extract of the leaves of *Aspiliaafricana*and*Sidaacuta*, it is evident that both plants contain a range of phytochemicals. However, the concentrations of the various phytochemicals in each plant differ significantly.

Based on the calculated t-values and p-values using a significance level of 0.05, we can draw some conclusions about the comparative phytochemical analysis of *Aspiliaafricana*and*Sidaacuta.*

Firstly, the total saponin content of *Aspiliaafricana* (4.20 ± 0.05 mg/100g) was significantly higher (t-value = 37.75, p-value < 0.001) than that of *Sidaacuta* (0.28 ± 0.05 mg/100g), indicating that *Aspiliaafricana* contains much more saponins than *Sidaacuta*. Saponins are known to possess several biological activities such as antioxidant, anti-inflammatory, and anti-cancer properties. Therefore, *Aspiliaafricana* may have a greater potential for therapeutic applications due to its high saponin content.

Secondly, the total alkaloid content of *Aspiliaafricana* (8.22 ± 0.40 mg/100g) was also significantly higher (t-value = 17.03, p-value < 0.001) than that of *Sidaacuta* (2.31 ± 0.03 mg/100g), suggesting that *Aspiliaafricana* may be a better source of alkaloids than *Sidaacuta*.Alkaloids are known to possess various biological activities, such as anti-inflammatory, anti-cancer, and anti-microbial activities. Therefore*, Aspiliaafricana* may also have a higher therapeutic potential based on its alkaloid content.

On the other hand, the total steroid content of *Sidaacuta* (1.85 ± 0.04 mg/100g) was significantly higher (t-value = -8.87, p-value = 0.002) than that of *Aspiliaafricana* (0.40 ± 0.01 mg/100g). However, the p-value is above the significance level of 0.05, indicating that this difference may

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not be very meaningful and further studies may be needed to confirm this finding.*Sidaacuta*also contains a higher concentration of total flavonoids (0.55±0.02 mg/100g) compared to *Aspiliaafricana*. Flavonoids are known to possess antioxidant and anti-inflammatory properties and have been reported to have a positive impact on human health. Therefore, *Sidaacuta* may also have a therapeutic potential based on its flavonoid content.

The ecological study shows the presence of *Aspiliaafricana*and*Sidaacuta* in all study sites and suggests that these plants are well-adapted to the local environment.
The variation in plant numbers between sites could be due to differences in soil quality, water availability, or other factors.
Site 1 in zone 3 has the highest number of *Aspiliaafricana* plants, possibly due to its higher soil moisture and temperature compared to other sites.
Site 2 in zone 3 has the highest number of *Sidaacuta* plants, which may indicate that this plant species is more adapted to the environmental conditions of that site.
The variation in soil moisture levels and temperature between sites could be due to differences in topography, vegetation cover, or other factors.
The highest light intensity in Site 1 in zone 2 could be due to differences in vegetation cover or shading from surrounding trees.

5.2 Conclusion.

In conclusion, the presence of *Aspiliaafricana*and*Sidaacuta* in all study sites suggests that these plant species are widespread and ecologically important in the study area.The variation in plant numbers, soil moisture, temperature, and light intensity between sites highlights the complexity of ecological systems and the importance of detailed field observations.
Further research is needed to determine the specific drivers of plant distribution in the study area and to assess the ecological roles of *Aspiliaafricana*and*Sidaacuta* in the local ecosystem.
The comparative study of the phytochemical constituents of *Aspiliaafricana*and*Sidaacuta* suggests that both plants possess different concentrations of various bioactive compounds. Therefore, these plants may have different therapeutic potentials based on their phytochemical composition. In conclusion, this study shows that there are significant differences in the phytochemical composition *of Aspiliaafricana*and*Sida*acuta, with*Aspiliaafricana* having higher

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levels of saponins and alkaloids, while *Sidaacuta* has a higher level of steroids and saponins. These findings may have implications for the use of these plants in traditional medicine and further studies may be needed to explore their potential medicinal properties.

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APPENDIX

T-test On Phytochemical Analysis

Phytochemical constituents	***Aspiliaafricana***	***Sidaacuta***	Mean difference	t-value	p-value
Total saponin	4.20 ± 0.05	0.28 ± 0.05	3.92	37.75	<0.001
Total glycosides	1.18 ± 0.04	-	-	-	-
Total flavonoid	-	0.55 ± 0.02	-	-	-
Total alkaloid	8.22 ± 0.40	2.31 ± 0.03	5.91	17.03	<0.001
Total steroid	0.40 ± 0.01	1.85 ± 0.04	-1.45	-8.87	0.002
Total tannin	2.23 ± 0.03	-	-	-	-

The hypotheses tested were likely that there is a significant difference in the levels of phytochemical constituents between *Aspiliaafricana*and*Sidaacuta*. The null hypothesis would be

that there is no significant difference, while the alternative hypothesis would be that there is a significant difference.

The results of the t-test indicate that for all the phytochemical constituents tested, there is a significant difference between *Aspiliaafricana*and*Sidaacuta*, except for total glycosides and total tannin, which were not detected in *Sidaacuta*.

The p-values are all less than 0.05, indicating that the differences observed are statistically significant. The t-values are quite large, indicating that the observed differences are not due to chance.

Overall, the table suggests that *Aspiliaafricana* has higher levels of total saponin, total alkaloid, and total tannin than *Sidaacuta,* while*Sidaacuta* has higher levels of total flavonoid and total steroid than *Aspiliaafricana.*

ANOVA TABLE For Ecological Study

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	P-value
Zone	26.222	2	13.111	0.545	0.598
Site	61.778	6	10.296	0.429	0.854
Zone x Site	101.722	12	8.477		
Aspiliaafricana	23.167	1	23.167	1.821	0.200
Sidaacuta	5.667	1	5.667	0.445	0.524
Soil Moisture (%)	56.556	1	56.556	4.444	0.067
Temperature (°C)	25.222	1	25.222	1.984	0.188
Light Intensity	32.722	1	32.722	2.571	0.135

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Error	264.333	18		14.685
Total	651.167	42		

In summary, this two-way ANOVA suggests that the effect of site on the variables measured depends on the zone, but there were no significant effects of zone or site alone on the variables. Additionally, soil moisture may have a marginal effect on plant growth, but temperature and light intensity did not have significant effects on any of the variables measured.

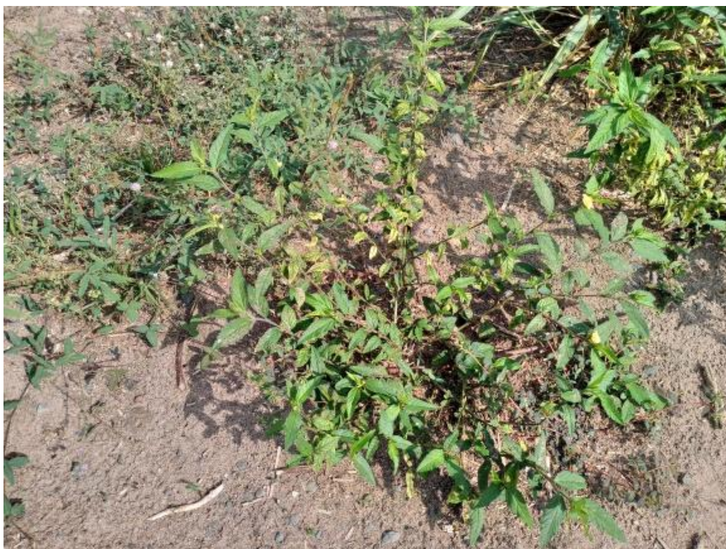

Plate 1: *Sidaacuta* in its natural habitat

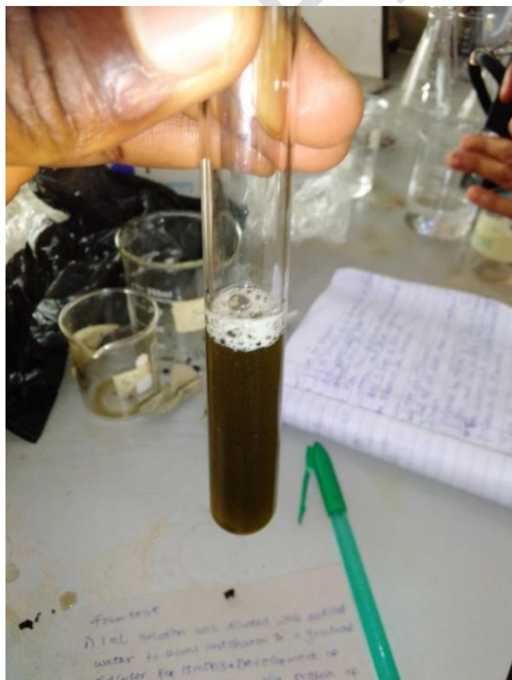

Plate 2: Saponin foam in the *Aspilia Africana* sample

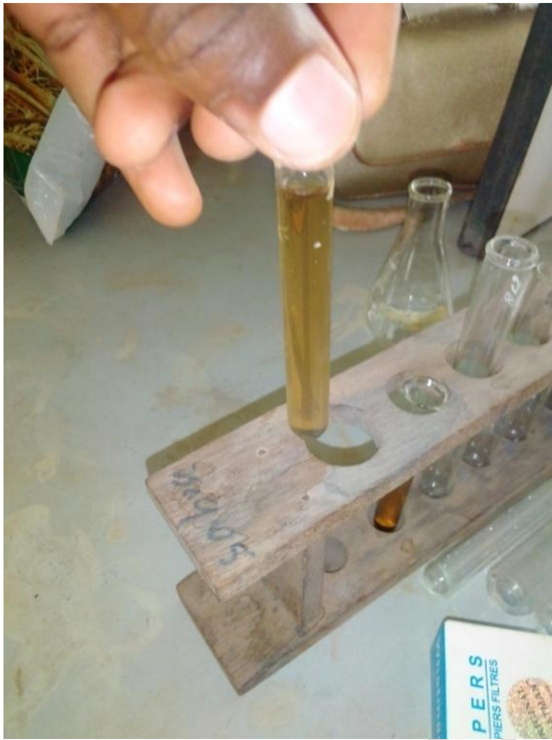

Plate 3: Tannin result in the *Sidaacuta*sample

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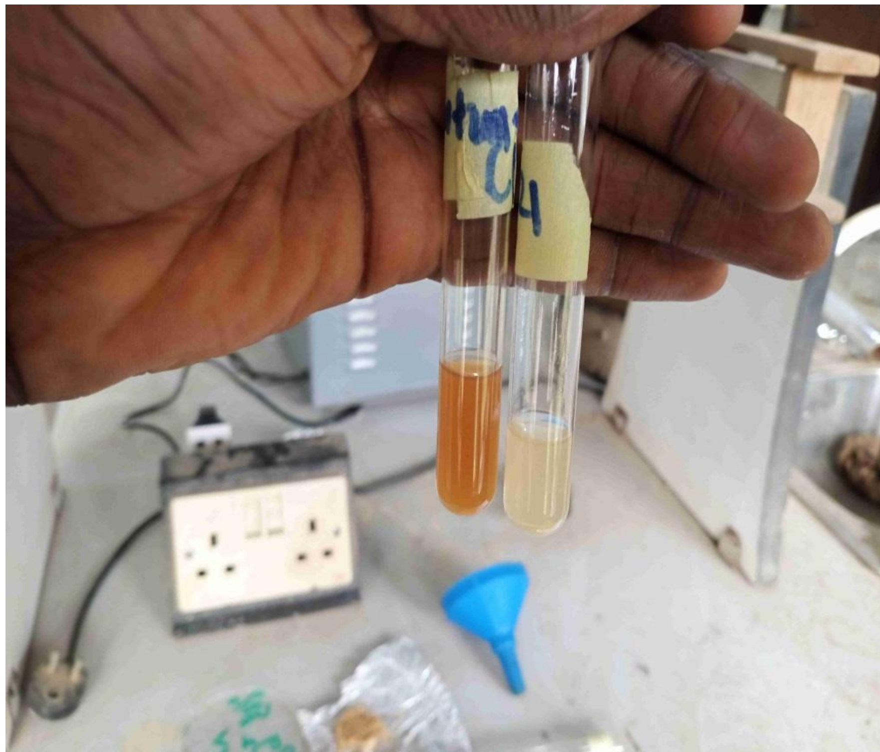

Plate 4: Alkaloid result in *Aspiliaafricana*

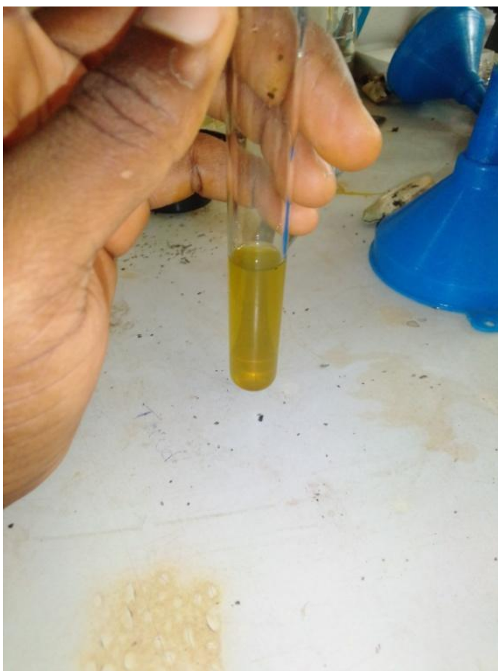

Plate 5: Flavonoid result of the *Sidaacuta* sample

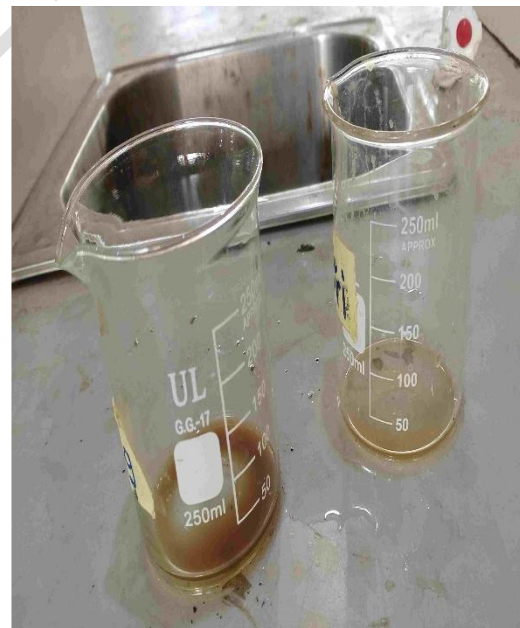

Plate 6: Aqueous extract of the two samples

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