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Comparative Study on the Phytochemical Analysis of the Leaves and Stem of *Ageratum houstonianum. mill*

W. C. Anyanele [a*], S. I. Anyanele [b] and C. S. Ejidike [a]

[a] Department of Botany, Nnamdi Azikiwe University, Awka, Anambra, Nigeria.
[b] Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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

Ageratum houstonianum is an annual herbaceous weed which is widely used as an ornamental plant for summer borders and balcony boxes. It is considered a safe weed with several medicinal properties. This Study was carried out to know the Phytochemicals constituent of *Ageratum houstonianum* leaves and stem. *Ageratum houstonianum* was evaluated for total Saponins, total Phenol and other Secondary metabolites contents using standard procedures. The study of the leaves and stem revealed the presence of Saponins, Flavonoids, Alkaloids, tannins, Phenol and Steroids. The Leaves of *Ageratum houstonianum* gave higher composition of tannins, alkaloid, saponin, flavonoid and phenols at (3.41±0.015 mg/100g), (2.40±0.015 mg/100g), (1.16±0.021 mg/100g), (1.55±0.031 mg/100g) and (1.22±0.015 mg/100g) respectively than the stem. And there is significant relationship between the phytochemicals present in the two plant parts.

Keywords: Ageratum houstonianum; asteraceae; phytochemical; composition; plants; extraction; herbal.

**Corresponding author: E-mail: c.anyanele@unizik.edu.ng;*

1. INTRODUCTION

"Phytochemicals are chemical compounds produced by plants, generally to help them resist fungi, bacteria and plant virus infections and also consumption by insects and other animals" (Breslin and Andrew, [1]. According to Liu [2] Phytochemicals can also be defined as bioactive nutrient, plant chemical in fruits, vegetables, grains and other plant foods that may provide desirable health benefits beyond basic nutrition to reduce the risk of major chronic disease. Phytochemical analysis refers to the extraction, screening and Identification of the medicinally active substance found in plants as defined by Letourneau [3]. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, cartenoids, tannin, antioxidant and phenolic compounds. *Ageratum houstonianum* belongs to the family Asteraceae. It is commonly known as floss flower, blue mink, blueweed or Mexican paintbrush, it is a cool-season annual plant often grown as bedding in gardens. This herbaceous annual or dwarf shrub grows to 0.3–1 m (1 ft 0 inch–3 ft 3 inch) high, with ovate to triangular leaves 2–7 cm (0.79–2.76) long, and blue flower heads (sometimes white, pink, or purple). The flower heads are borne in dense corymbs. The ray flowers are threadlike and fluff-haired, leading to the common name. The narrow lanceolate bracts are pointed, denticulate only at the top and glandular hairy. The flowering period is from May to November in the northern hemisphere. The plant attracts butterflies due to its pleasant fragrance.

"The plant is native to Central America in Guatemala and Belize, and adjacent parts of Mexico, but has become an invasive weed in other areas. It was also naturalized in large parts of the tropics and in the southern United States. Their habitat is pastures, moist forest clearings and bushes up to altitudes of 1,000 metres (3,300 ft).

Today, it is widely used as an ornamental plant for summer borders and balcony boxes, high varieties also as cut flowers. The species is cultivated once a year, having numerous varieties whose crowns may be dark blue, purple, pink and white. Preferring cool soils and exposure in full sun, high varieties reach stature heights up to 60 centimeters (24 inches)", [4].

Ageratum houstonianum is prone to becoming a rampant environmental weed when grown outside of its natural range. It has become an invasive weed in the United States, Australia, Europe, Africa, China, Japan, New Zealand, and the Philippines.

"Ageratum houstonianum is an annual herb native to Mexico and Central America. It was brought to Europe shortly after its discovery, where its use as an ornamental started [5]. The species is reported to be a weed and invasive outside its native range, due to its high production of small seeds with easy dispersal by wind or water [6]. It is also dispersed by animals, clothing, vehicles, contaminated soils and agricultural produce [5,6]. Mainly as a garden escape, it has naturalized on farmlands, wastelands, roadsides, forest trails, crops, riverbanks and wetlands [6]. The species is reported as invasive in China, Taiwan [7], Mozambique, Swaziland, Tanzania, Zimbabwe, USA (Hawaii), Cuba, Peru, Australia, Fiji, French Polynesia, New Zealand, and as a declared weed and alien invader plant with the highest category of invasiveness in South Africa [8,6,7,9]. It is also reported as invasive in Kenya, Malawi and Rwanda [10]. report the species as an invasive which has affected ecosystems, causing the decline of native species in Assam, India. It can be particularly invasive along waterways and in riparian vegetation" [11].

2. MATERIALS AND METHODS

2.1 Experimental Site

This research was carried out at the department of botany laboratory, Nnamdi azikiwe, University Awka. Anambra State.

2.2 Instruments/ Equipments Used

Petri Dish, Oven, Weighing Balance, Blender, Knife, Spectrophotometer, Water bath, Volumetric Flask.

2.3 Chemicals/ Reagents Used

Ether, Methanol, Distilled water, Ammonia, Sulphuric acid, Mayer's Reagents, ferric chloride, sodium chloride, Chloroform, ethanol.

2.4 Source of Plant Material

2.4.1 Sample collection

Ageratum houstonianum leaves and stem were collected from the surrounding of Nnamdi Azikiwe University, Awka in Awka South L.G.A. Anambra State, Nigeria.

2.4.2 Sample preparation

The plant was examined to be free from any disease. Only healthy leaves and stem were used. Extraneous materials were also removed from the plant material. They were cut into pieces using kitchen knife. The leaves and stem was Oven dried for three days.

3. METHODOLOGY

3.1 Phytochemical Screening

The phytochemical screening was carried out according to the method of (Enemor et al., 2014).

3.1.1 Screening for saponin

Two grams of the powdered sample was boiled in twenty milliliters of distilled water in a water bath for five minutes. The filtrate was mixed with five milliliters of distilled water and shaked vigorously and formation of foam was observed.

3.1.2 Screening for flavonoids

Five milliliters of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant extract followed by the addition of concentrated H2SO4. A Yellow colouration which indicates the presence of flavonoids was observed.

3.1.3 Screening for phenols

Two milliliters of distilled water was added to two milliliters of plant extract followed by the addition of10% FeCl2. Bluish black colour indicates the presence of phenol.

3.1.4 Screening for alkaloids

0.5g of the sample was defatted with 5% ethyl ether for fifteen minutes. The defatted samples were boiled for 20minutes with five milliliters of HCl in a water bath. The resulting mixture was centrifuged for ten minutes at 3000rpm. The filtrate was then treated with few drops of Meyer's reagent and turbidity observed.

3.1.5 Screening of steroids

Two milliliters of acetyl anhydride was added to 0.5g of the ethanolic extract with two milliliters of H2SO4. Blue-green coloration was observed which indicated the presence of steroid.

3.1.6 Screening for tannins

0.5g of the powdered sample was boiled in twenty milliliters of distilled water and filtered. A few drop of 0.1% of ferric chloride was added, and brownish-green or blue-black coloration was observed.

3.1.7 Screening for Glycoside

Five milliliters of extract was treated with two milliliters of glacial acetic acid, Ferric chloride acid was added in drops than addition of 1ml of suphuric acid. Formation of brown ring was observed.

3.2 Quantitative Determination of the Phytochemical Constituent of the Plant Parts Studied

3.2.1 Alkaloid determination

"The determination of the concentration of alkaloid in the sun dried and air dried leaves of *Tridax procumbens* was carried out using the alkaline precipitation gravimetric method described" by Harbone [12]. Five grams of the powdered samples was soaked in twenty milliliters of 10% ethanolic acetic acid. The mixture was stood for hours at room temperature. Therefore, the mixture was filtered through whatman filter paper, (NON2). The filtration was concentrated by evaporation over a steam bath to one quarter of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was excess. The resulting alkaloid precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for thirty minutes, cooled in a desicator and reweighed. The process was repeated two more times and the average was taken. The weight of the alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown:

$$\%\ \text{Alkaloid} = \frac{\text{W2} - \text{W1}}{\text{Weight of sample}} \times \frac{100}{1}$$

W1= weight of filter paper

W2= weight of filter paper + alkaloid precipitate.

3.2.2 Saponin determination

20g of extract was put into a conical flask and one hundred meters of 20% ethanol was added to the sample. The sample was heated over a hot water bath for four hours with continuous stirring at about 55°C. The mixture was then filtered and residue re-extracted with another two hundred milliliters of 20% ethyl alcohol. The

combined extracts were reduced to forty milliliters over a water bath of about 90°C, the concentrate was then transferred into a two hundred and fifty milliliters conical flask and twenty milliliters of diethyl ether was added to the extract and vigorously shaken. The aqueous layer was recovered while the diethyl ether was discarded and purification process i.e. washing twice with ten milliliters of 5% sodium chloride was carried out. The remaining solution was then heated in a water bath and allowed to evaporate, the sample was dried in the oven to a constant weight and values expressed as mg/g of extract.

$$\% \text{ Saponin } = \frac{\text{W2 } - \text{ W1}}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W2-W1 = Weight of Saponins
W1= weight of conical flask
W2= Weight of conical flask+ Saponins

3.2.3 Flavonoids determination

"The Flavonoids content was determined by the gravimetric method as was described by" Harbone [12]. 5g of the powdered sample was placed into a conical flask and fifty milliliters of water and two milliliters of HCl solution was added. The solution was allowed to cool before it was filtered through whatman filter paper. The filtrate was treated with ethyl acetate starting with drops of it, until in excess. The precipitated flavonoids were recovered using a weighed filter paper, and then placed in an oven to dry at 80°C. It was cooled in a desiccator and reweighed. The difference in weight gave the weight of the flavonoids which was expressed in percentage of the sample weight analyzed. Given by the formula.

$$\% \text{ Flavonoids } = \frac{\text{W2 } - \text{ W1}}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W= weight of sample
W1= weight of empty filter paper
W2= weight of paper + flavonoids extract.

3.2.4 Phenols determination

This was carried out using the salkowki test as described by Harbone [13], the sample was agitated in a vibrator at relatively low speed for three hours, after which it was allowed to stand overnight. The mixture was then into a beaker with the aid of whatman grade filter paper. The filtrate was eludated with ten milliliters of NH2OH and two milliliters of the filtrate was mixed with two milliliters of chloroform. This was followed by the addition of three milliliters of acetic anhydride and two milliliters of concentrated sulphuric acid in drop. The combined extract was diluted with one hundred milliliters of distilled water and we're dispensed into two hundred and fifty milliliters conical flask containing 10g of the sample. Then the absorbance was read in UV/ visible spectrophotometer at the wavelength of 420nm. The steroid content was calculated as show below:

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

Where:

W= weight of sample analyzed
AU= absorbance of test sample
AS= Absorbance of standard solution
C= Concentration of standard in mg/ml
VF= total filtrate volume
VA= Volume of filtrate analyzed
D= Dilution factor

3.2.5 Tannin determination

"The tannin content of the plants was determined using Folìn-Dennis spectrophotometric method described" by [14]. 2g of the powdered sample was mixed with fifty milliliters of distilled for fifty minutes in a shaker. The mixture was filtered and the filtrate used for the experiment. Five milliliters of the filtrate was measured into fifty milliliters volumetric flask and diluted with three milliliters of distilled water. Similarly, five milliliters of standard tanuric acid solution and five milliliters of distilled water were added separately. One milliliters of Folìn-Dennis reagent was added to each of the flask, followed by 2.5ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for ninety minutes at room temperature. The abundance of the developed colours was measured at 760nm wavelength with reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as follows:

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

Where:

W= weight of sample analyzed
AU= absorbance of test sample

Table 1. Qualitative phytochemical composition of the leaves and stem of *Ageratum houstonianum*

Phytochemicals	*Ageratum houstonianum* plant parts	
	Leaf	Stem
Saponin	+	+
Flavonoid	++	+
Alkaloid	++	++
Steriod	-	-
Tannin	+++	++
Phenol	++	++
Glycoside	-	-

Key: +++: Deeply present; ++: Present; +: Trace; -: Absent

Table 2. Quantitative phytochemical composition of the leaves and stem of *Ageratum houstonianum*

Plant samples	Saponin	Alkaloid	Flavonoid	Tannin	phenol
Leaves	1.16±0.021	2.40±0.015	1.55±0.031	3.41±0.015	2.24±0.021
Stem	0.97±0.01	1.96±0.021	1.22±0.015	3.03±0.015	1.99±0.015
LSD	0.000	0.114	0.000	0.000	0.46
P-Value	0.000	0.036	0.000	0.000	0.036

Results are represented in Mean±Standard deviation (P-value >0.05 there is no significant difference, <0.05 there is significant difference)

AS= Absorbance of standard solution
C= Concentration of standard in mg/ml
VF= total filtrate volume
VA= Volume of filtrate analyzed
D= Dilution factor

3.2.6 Statistical analysis

"The data generated was subjected to analysis of variance (ANOVA) to determine the level of significance. Significant difference (LSD) technique as described by" Gomez (1984) and Gomez (1980).

4. RESULTS

4.1 Qualitative Phytochemicals Composition of *Ageratum houstonianum* Leaves and Stem

Results of qualitative Phytochemical composition of *Ageratum houstonianum* are present in Table 1. The result revealed the presence of saponins, flavonoids, alkaloids, tannins, and phenols in both the leaves and stem of *Ageratum houstonianum* while glycoside and steroid were absent in both extracts.

The quantitative phytochemical compositions of the leaves and stem of *Ageratum houstonianum* are shown in Table 2. The Table revealed that the leaves of *Ageratum houstonianum* contains (1.16±0.021 mg/100g) of total saponins, (1.55±0.031 mg/100g) of total flavonoids, (3.41±0.015 mg/100g) of total tannin, (2.40±0.015 mg/100g) of total alkaloid, (2.24±0.021 mg/100g) of total phenol. The stem then contains (0.97±0.01 mg/100g) of total saponin, (1.22±0.015 mg/100g) of total flavonoids, (3.03±0.015 mg/100g) of total tannin, (1.96±0.021 mg/100g) of alkaloids, (1.99±0.015 mg/100g) of total phenol. The result shows that there is significant difference between the phytochemicals present in the leaves and stem of the plant.

5. DISCUSSION AND CONCLUSION

The Phytochemical screening results in the Table 1 showed that saponins, flavonoids, alkaloids, tannins and Phenols are present in both the leaves and stem of *Ageratum houstonianum,* while glycoside and steroids were absent in both the leaves and the stem. These results are in support with the observation made by James et al*.,* [15] and Illoh et al*.,* [16]. "The presence of these Phytochemicals in the leaves and the stem of *Ageratum houstonianum* confer its medicinal value" [17].

The presence of Saponins signifies that the plant could be an important source for the treatment of tumour effects. Flavonoids have antioxidant as recorded by Illoh et al*.,* [16], Sharma, K.R. [18]. According to Michael [19] "alkaloids in medicine

are used in the treatment of cancer, parasitic diseases, pathogenic bacteria and neuronal disorders. As recorded" by Nnam et al*.,* [20]," Saponins are glucosides with foaming Characteristics, saponins consist of polycyclicaglycones attached to one or more sugar side chains, the aglycone, a part which is also called Sapogenin. The foaming ability of saponins is caused by the combination of hydrophobic (fat-soluble) Sapogenin and a hydrophobic (water-soluble) sugar part called Saponins which is characterized by their bitter taste. It has antifungal properties and also is an emulsifying agent. Phenols are known to have antimicrobial effect" [21].

Quantitatively, the highest occurring phytochemical in the plant is Tannins (3.41± 0.015) in the leaves as shown in Table 2, while saponins in the stem is the least (0.97 ±0**.**01). The variation in Phytochemicals compositions of medicinal plants varies with different plants and parts of plant (root, stem or leave) and extraction solvents [22]. "Similar, variation in the concentration of Phytochemicals in plants were also observed by other researchers" [23,24]. According to Pietta, [25] the qualitative results shows the relevance of the leaf and stem of the plant in ethnomedicine and pharmacognosy, Sharma, [18], as these compounds cures sicknesses such as leukemia, fever, cancer, diabetes, cough and catarrh, acute and chronic headache amongst others [26]. The high level of Tannin and Phenol composition in this plant shows that it highly medicinal and is needed in pharmacology. This is as a result of its antioxidant nature which is a substance that can prevent or slow damage to cells caused by free radicals.

In conclusion, the bioassay guided for isolation and identification of the bioactive components are still needed and detailed researches are also required to reveal the structure activity relationship of these active constituents. Outcome of the future research in the aforementioned areas will provide a convincing support for the future clinical uses of *A. houstonianum* in modern medicine.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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