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Comparative Phytochemical Analyses of *Aspilia africana* (Pers) C.D. Adams, *Chromolaena odorata* (L) King and H.E. Robinson and *Gongronema latifolium* (Benth)

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ABSTRACT

Phytochemical analyses of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* were investigated for basic bioactive components of medicinal values. Standard methods were adopted for the analysis. The results revealed the presence of alkaloid, saponins, flavonoids, phenols, tannins and glycoside in the plant stems and leaves samples. The qualitative analysis showed that *Aspilia africana* has the highest alkaloid and glycosides values (15.16 ± 0.07 and 2.24±0.01). *Chromolaena odorata* recorded the highest flavonoid and saponin of 6.06 ± 0.01 and 0.36±0.01 respectively. The highest values of Tannins and phenol were observed in *Gongronema latifolium*. The rich phytochemical content of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* observed in this study suggests their use in ethnomedicine and their importance as industrial raw materials. These plant materials, if properly exploited can be a great source of potentially important compounds that can be useful for drug production in pharmaceutical industries.

Keywords: Medicinal plants, phytochemicals, *Aspilia africana*, *Chromolaena odorata*, *G. latifolium*, saponins, flavonoids.

Introduction

Phytochemicals are by-products of primary metabolism that are less widespread in plants (Audu *et al.*, 2018; Okigbo *et al.*, 2020) and it is of course this restricted occurrence among plants that renders them valuable and useful in taxonomic delimitation of species (Nwokocha *et al.*, 2011). In recent years, chemical analysis and biological assays have begun to play an important role in ethnobotanical studies (Jana *et al.*, 2009). In several cases, such analyses have led to the discovery of novel bioactive phytochemicals. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Okigbo and Anyaegbu, 2021; Arif *et al.*, 2022), hence the medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body (Mir *et al.*, 2013). Medicinal plants besides therapeutic agents are also a big source of information for a wide variety of chemical constituents which could be developed as drugs with precise selectivity. These plants are the reservoirs of potentially useful chemical compounds which could serve as newer leads and clues for modern drug design (Vijyalakshmi and Ravindran, 2012). The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds (Rodon *et al.*, 2018). Correlation between the phytoconstituents and the bioactivity of plant is desirable to know for the synthesis of compounds with specific activities to treat various health ailments and chronic diseases as well (Pandey *et al.*, 2013). Phytochemical studies have attracted the attention of plant scientist due to development of new and sophisticated techniques. These techniques played a significant role in giving the solution to systematic problems on the one hand and in the search for additional resources of raw material for pharmaceutical industry on the other hand (Resmi *et al.*, 2013, Okigbo *et al.*, 2023). In human history, indigenous people develop a deep knowledge of the plant that grew in their country (Cowan, 1999).

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This knowledge has provided the world with substances such as aspirin, quinine to treat malaria, drugs for cancer treatment and many other drugs that are relied upon in western medicine.

For a long period, plants have been a valuable source of natural products for maintaining human health and according to the World Health Organization (WHO); medicinal plants would be the best source to obtain a variety of drugs. Plants with their wide variety of chemical constituents offer a promising source of new antimicrobial agent with general as well as specific activity (Gowthami, 2012; Okigbo *et al.,* 2023). A large number of plants are used to combat different diseases and are known to possess antimicrobial activity (Ashraf *et al.,* 2018). The medicinal flora in the tropical eco-region has enormous plants that provide raw material for addressing a range of medical disorders and pharmaceutical requirements. Nigeria is long recognized for her grass land savanna vegetation with a very rich botanical diversity (Jones *et al*., 2001; Hepper and Keay, 2000). Nigeria is enriched with different types of useful plants whose fruits, seeds, stems, roots and leaves serve various important role in medicine and nutrition. These plants include but not limited to *Chromolaena odorata, Aspilia africana, Gongronema latifoliume, etc*. Bioactive ingredient made from these plants has proven medicinal properties.

Consequent upon the background established, it is pertinent to carry out preliminary phytochemical screening and pharmacological evaluations of these plants to ascertain their possible therapeutic potential and determine their basic bioactive component of medicinal values since there is a dearth of information on the phytochemical constituents of these plants. Therefore, the objective of this work is to identify the phytochemical components of *Aspilia africana, Chromolaena odorata* and *Gongronema latifolium* which will be useful in providing information that will lead to increase utilization of these plant species for medicinal purposes in Nigeria in the treatment of infections and healing of wounds.

Materials and Methods

Source of Plant Material

Leaf samples of *Aspilia africana* (Pers) C.D Adams (Azuzo) and *Chromolaena odorata* (L) King H.E. Robins (Obiara Ohuru) were collected in the bush at the Science Village, Nnamdi Azikiwe University, Awka, while the leaves of *Gongronema latifolium* Benth (Utazi) was purchased from Kenyatta market, Enugu, Nigeria. The plant samples were authenticated by Mr. Chisom Iroka, a curator in the Department of Botany, Nnamdi Azikiwe University, Awka. The plants samples were given number NAU/BOT/ 0014, NAU/BOT/ 0018 and NAU/BOT/ 0028. The samples were stored in good condition, stored in the refrigerator before taking them to the laboratory for the analysis.

Plate 1: *Aspilia africana* (Pers) C.D Adams

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Plate 2: *Chromolaena odorata* (L) King H.E. Robins

Plate 3: *Gongronema latifolium* Benth

Preparation of Plant Extract

The *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* were examined. The fresh plants were pounded with mortar and pestle. Twenty grams of each plant sample were weighed with YP-1002N electrical sensitive weighing balance, India and soaked with 100ml of Ethanol over night. GG-17 SHUNIU Plastic funnel and Whatman No. 1 filter paper were used to decant separately the plant extracts of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* into 250 ml beakers (Simax, Zechoslovakia).

Qualitative Phytochemical Analysis

Qualitative tests were conducted to evaluate the presence or absence of phytochemicals of interest. It was conducted using different standard method describe below.

Test for Alkaloids

The method used was that of Association of officials of analytical chemistry (A.O.A.C, 1990). One millilitre of 1% HCl was added to 3ml of each of the plant extract in a test tube with a syringe. The mixture was heated with sunbin heating mantle, India for 20 mins in a water bath and was shaken continuously. After heating, it was allowed to cool before filtering it with Whatman No. 1 filter paper. This procedure was repeated with each plant extracts.

One millilitre of each filtrate from different plant sample and different solvent was added to 0.5ml of Wagners reagent. A brown colour precipitate indicated the presence of alkaloids.

Test for Saponins

Frothing Test: Three milliliter of each extract was diluted with 2ml of distilled water in a test tube. The mixture was shaken vigorously. A persistent frothing shows the presence of saponins (A.O.A.C, 1990).

Test for Flavonoids

Three millilitre of each plant extract was added to 10ml of distilled water. The solution was shaken. One millilitre of 10% NaOH solution was added to the mixture, development of yellow colouration indicated the presence of flavonoids (A.O.A.C, 1990).

Test for Steroids

Salkowski Test: Five drops of conc. H_2SO_4 was added to 1ml of each extract in a separate test tube. A red colouration indicated the presence of steroids.

Test for Phenols

One millilitre of dissolved sample (extract) was added to 1ml of water and a few drops of 5% NaOH in a test tube and it was shaken. Orange colouration confirmed the presence of phenol.

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Test for Tannins

Two millilitre of each extract in separate test tube were boiled gently for 2 mins and was allowed to cool. Three drops of ferrous chloride solution was added to it. A greenish colouration showed the presence of tannins (A.O.A.C, 1990).

Test for Glycosides

One milliliter of aqueous extract was mixed with 1ml of 2% solution of 3,5 dinitrosalicilic acid in methanol and 1ml of a 5% aqueous NaOH. An immediate bright orange colour was observed indicating the presence of cardenolides in the extract. The colour fades gradually through reddish brown to brownish yellow. This indicated the presence of glycosides.

Quantitative Analysis of Phytochemicals

Determination of Alkaloid

This was determined by using Harbone (1973). Five gram of the sample was weighed with YP-1002N electronic sensitive weighing balance into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hrs. This was filtered with Whatman No. 1 filter paper and the extract was concentrated on a water bath to one-quarter of the original volume. Twenty millilitre of Conc. ammonium hydroxide was added dropwise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute (1%) ammonium hydroxide, then filtered. The residue was the alkaloid which was dried and weighed. Alkaloid content was calculated and expressed as a percentage weight of samples analyzed as shown below. Each treatment was repeated three times.

Calculation

Weight of filter paper = x
Weight of filter paper + sample after drying = Y
Weight of residue (precipitate) = Y- X = Z

$$\% \text{ alkaloid} = \frac{Z \times 100}{\text{Weight of sample}}$$

Determination of Saponin

The method used was that of Obadoni and Ochuko (2001). The samples were pounded with wooden mortar and pestle and 20g of each were put into a conical flask and 20% aqueous ethanol was added. The samples were heated over a hot water bath for 4hrs with continuous stirring at about 55ºC. The mixture was filtered, and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90ºC. The concentrate was transferred into a 250ml separating funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the other layer was discarded. The purification process was repeated. Fifty milliliter of n-butanol was added. The combined n-butanol extract were washed twice with 10ml of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated as percentage. Each treatment was repeated three times.

Calculation

Wt. of empty beaker = X
Wt. of beaker + sample = Y
Wt. of sample = 20g

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

Determination of Flavonoids

This was determined by the method of Bohm and Kocipal Abyazan (1994). Ten gram of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman No. 1 filter paper (125mm). The filtrate was later transferred into a crucible and evapourated into dryness over a water bath and weighed to a constant weight. Each treatment was repeated three times and calculated as percentage.

Calculation

Wt. of empty beaker = X
Wt. of empty beaker + sample = Y
Wt. of sample = 10g

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

Determination of Phenols

The method used was that of Association of officials of analytical chemistry (AOAC), (1990). This was done by spectrophotometric method. The sample was boiled with 50ml of diethyether for the extraction of the phenolic component for 15 mins. Five milliliter of the extract was pipette into a 50ml flask. Then 10ml of distilled water was added 2ml of ammonium hydroxide solution and 5ml of conc. Amylalcohol

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were also added. The samples were made up to mark and left to react for 30 mins for colour development. This was measured at 505nm.

Calculation

The absorbance obtained with the aid of spectronic 20 machine and the concentration was traced using phenol standard graph. The dilution factor was determined by dividing 5ml of the extract which was used with the volume of the flask. Each treatment was repeated three times and was calculated as percentage.

Determination of Tannins

Half gram of the sample was weighed into 250ml conical flask. Fifty milliliter of distilled water was added to it and shaken on a rotating shaker for 1 hr. It was filtered into a volumetric flask. Five millilitre of the filtrate was pipette into 50ml volumetric flask. Half gram of tannic acid was dissolved in 100ml of water to form tannic acid solution and 5ml was pipette to another 50 ml volumetric flask. A blank sample was prepared using 5ml distilled water. The three samples were made up with water. The three samples were put in an incubator for 1½ hrs at 20-30ºC. After 1½hrs has elapsed, the three samples were made up with water up to the 50cm^3 mark. Abundance of the samples was measured at 760nm using spectronic 20 (AOAC, 1990).

Each experiment was repeated three times.

Calculation

Let the absorbance of 5ml of extract be x

Let the absorbance of tannic solution be Y

Let the absorbance of blank be Z

$$\% \text{ Tannin} = \frac{X - Z}{Y - Z} \times \frac{100}{1000} \frac{(\text{Extraction} - \text{blanks})}{\text{Standard} - \text{blank})}$$

Determination of Glycoside

This was determined by the method of Association of Officials of Analytical Chemistry (A.O.A.C, 1990). Here, 5g of the samples were weighed into a beaker and 100ml of distilled water added to them. They were soaked for 3 hrs and filtered to get the filtrate. One milliliter of the filtrate was pipette into a test tube and 2ml of 2,5-DNS was added to it. After this, they were boiled in a water bath for 10-15mins. The test tubes were cooled and 10ml of distilled water was added to them. The absorbance was read at 540nm for glycoside. Two milliliter of DNS was used as blank to standardize. Each treatment was repeated three times and was calculated as percentage.

Calculation

$$\% \text{ Glycoside} = \frac{\text{Conc. X Vol of extract x 100}}{1000 \text{ x wt. of sample}}$$

Results

The result of the qualitative phytochemical analysis of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* showed that Alkaloid is highly present in *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium;* saponin is slightly present in *Aspilia africana,* and present in *Gongronema latifolium* and in *chromolaena odorata;* flavonoid is highly present in all the plant leaves screened; Phenol is present in *Aspilia africana,* and in *Gongronema latifolium* but slightly present in *Chromolaena odorata;* Tannin is present in all the plant leaves and Glycoside is highly present in *Aspilia africana*, present in *Chromolaena adorata* slightly present in *Gongronema latifolium* (Table 1).

Table 1: Phytochemicals present in *Aspilia Africana, Chromolaena odorata* and *Gongronema latifolium*

Phytochemical (%)	**Plant Leaf Extract**		
	Aspilia africana	***Chromolaena odorata***	***Gongronema latifolium***
Alkaloid	+++	+++	+++
Saponins	+	++	++
Flavonoids	+++	+++	+++
Tannins	++	++	++
Glycosides	+++	++	+
Phenols	++	+	++

Key: - = Absent, **+** = Slightly present**,** ++ = Present**,** +++ = Highly present

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Table 2: Quantity of Phytochemicals in *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium*

Phytochemical (%)	Plant Leaf Extract		
	Aspilia africana	***Chromolaena odorata***	***Gongronema latifolium***
Alkaloid	15.16 ± 0.07	5.42 ± 0.02	3.91 ± 0.17
Saponins	0.15 ± 0.01	0.36 ± 0.01	0.30 ± 0.01
Flavonoids	2.23 ± 0.01	6.06 ± 0.01	5.05 ± 0.03
Tannins	0.27± 0.04	0.32 ± 0.04	0.90 ± 0.99
Glycosides	2.24 ± 0.01	0.25 ± 0.01	0.16 ± 0.01
Phenols	0.30± 0.01	0.09 ± 0.01	0.67 ± 0.01

The result of quantitative value of phytochemical present in *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* shows that Alkaloid content of *Aspilia africana,* is the highest (15.16 ± 0.07), followed by *Chromolaena odorata* (5.42 ± 0.02) and lowest in *Gongronema latifolium.* The flavonoid content of *Chromolaena odorata* is the highest (6.06 ± 0.01), followed by *Gongronema latifolium* (5.06± 0.03) and lowest in *Aspilia africana* (2.23 ± 0.01). The percentage quantity of Glycoside and Phenol is insignificant in the three samples. The difference in the percentage quantity of Saponin and Tannin in *Aspilia africana*, and *Chromolaena odorata* and *Gongronema latifolium* is insignificant. The values of saponin and tannins across the three plants are relatively very low compared to other phytochemicals (Table 2).

Discussion

Quantitative phytochemical screening revealed that all the plant materials tested contain varying degrees of phytochemicals. This actually confirms their medicinal properties which can be attributed to the various physiological and biosynthetic reactions taking place inside the plant; the effect of environment cannot be neglected, because the environment always modifies the chemical constituents of the plant parts (Farhat *et al.*, 2011; Okigbo *et al*., 2020).

It was found that *Chromolaena odorata* and *Gongronema latifolium* are rich in flavonoid and alkaloid, this result agrees with the report of Akinmoladin *et al.*, (2007). The slight presence of saponin and tannins in *Gongronema latifolium and Chromolaena odorata* also agrees with the reports of Okoli *et al.*, (2007) and Okwu and Josiah (2006).

The highest amount of Phenol was detected in *Gongronema latifolium*, however phenol is widely used as an antiseptic especially as carbolic soap and also used in preparation of cosmetics including sun screens, hair dyes and skin lightening preparation (Svobodova *et al.*, 2003; Deselm, 2008). Furthermore, the phytochemicals detected were known to show medicinal as well as physiological activities (Harborne, 1973, Okigbo *et al*., 2023).

The result also revealed that alkaloid, flavonoid and glycosides are found abundant in *Aspilia africana*. It should be noted that alkaloid and glycosides components are of great importance in pharmacy due to their relationship with cancer activity (Sunity, 2012), hence Glycosides are effective in the treatment of heart disease (Trease and Evans, 1996), cancer and sickle cell anemia (Alamgir 2017, Okigbo *et al*., 2023). Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects (Stary, 1998). Flavonoid in intestinal tract lowers the risk of heart disease. They prevent oxidative cell damages; have strong anticancer activity (Okwu, 2004). They possess anti-inflammatory properties and acts as modulators of the immune system in a number of biological system (Okolie *et al.,* 2008). Furthermore, the difference in the percentage quantity of Saponin, Tannin, Glycoside and Phenol in *Aspilia africana, Chromolaena odorata* and *Gongronema latifolium* is insignificant.

Saponins have a characteristic haemolytic activity; cholesterol binding properties, bitterness and ability to form foams in aqueous solutions (Sadipo *et al.*, 2000; Okwu, 2004), have the property of precipitating and coagulating red blood cells (Okwu and Josiah, 2006).

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Tannins have astringent properties that hasten the healing of wounds and inflamed mucous membrane (Okwu and Josiah, 2006, Okigbo *et al*., 2018). This suggests that the leaves of *Chromolaena odorata* and *Gongronema latifolium* can be exploited for these purposes.

This study has therefore provided a biochemical basis for the ethno medicinal use of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* leaf extracts. Also as a rich source of phytochemicals components, they have potentials as source of drugs and with appropriate extraction and processing methods for human consumption and preparation of industrial raw materials such as saponins in soap making and phenol in preparation of cosmetics.

In conclusion, the leaf extracts of *Aspilia africana*, *Chromolaena odorata* and *Gongronema latifolium* studied showed an abundant production of Phytochemicals. The rich active biological components of these plants provide a basis for their ethno medicinal uses.

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