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COMPARATIVE EVALUATION OF PHYTOCHEMICAL COMPOSITION OF LEAF, ROOT AND STEM OF COPPER LEAF (*Acalypha wikesiana*) MULL. ARG. PLANT IN KADUNA NORTHERN GUINEA SAVANNA ECOLOGICAL ZONE, ZARIA – KADUNA, NIGERIA

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ABSTRACT

Comparative Analysis of Chemical composition of leaf, Root, and Stem of Copper leaf (Acalypha wilkesiana) plants in Zaria eco-zone of Kaduna state was studied. The roots, leaves, and stem samples were randomly collected from Ahmadu Bello University and her environs. They were identified at the herbarium of Savannah Forestry Research station and Department of Botany Ahmadu Bello University Zaria Kaduna. The sampled were air dried in the laboratory at a room temperature. The dried sampled was chopped into pieces and ground separately to powder using blender. The powdered samples were labelled and store in small plastic air tight container which were then taken for analysis. The data obtained from the laboratory was analyzed using two ways analysis of variance (ANOVA) using GLM procedure of SAS. The results show the presence of alkaloids, flavonoids, saponins, tannin, phytate and Oxalate in various concentrations as there were significant differences(P<0.05) between these phytochemicals and the plant parts. It is evident that the iconic Copper leaf (Acalypha wilkesiana) plant is an important medicinal resource used to treat and prevent a range of health challenges. The reasons been the presence of high level of active phytochemical compositions found in the plant's parts. In Nigeria, however, the plant gained little attention because of its ornamental nature. However, it is recommended that due to increase interest in the medicinal values and utilization of the copper plant, there is a need to create awareness about its conservation and further research should be carried out for its other uses.

Key Words: Phytochemical, Copper leaf, Ecological-zone, Root, Leaves, Stem, active.

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INTRODUCTION

Acalypha wilkesiana (muell. Arg.) is an evergreen shrub which grow in tropical and sub-tropical region, belonging to the family Euphorbiaceae, it is widely distributed throughout tropical Africa, South Africa, India and America .it grows up to about 3metets (9.8ft) in height and spread 2meters(6ft) across. The stem is erect with many fine hairy branches.it has closely arranged crown the leaves are large and broad with teeth around the edge They can grow up to 10 to 20cm (3.9 to 7.9 inches) long and 15cm (5 to 9 inch) wide heart shaped alternate elliptic to oval with combination of colours like red green purple yellow and pink or white depending on the cultivation. The flowers are reddish in spikes at the end of branches. They have male and female flowers on the catkin like racemes beneath the foliage on the same plant the flowers are in long spikes which hang down Wards while the female flowers are in short

spikes. It normally starts flowering between February to December (Sagun *et. al.,* 2010) it is planted around home, offices or parks for horticultural purposes and it is easily propagated by stem cutting or air layering at any time of the year.

Euphorbiaceae is one of the largest groups of flowering plant with 300 genera and7500 species that provides numerous plant use for the treatment of inflammatory condition, wounds and bacterial infections (Wiart, 2006) the leaves of *Acalypha wilkesiana* was reported to be highly medicinal when eating as vegetables in the management of hypertension (Ikewuchi *et. al.,* 2008) this plant is popularly used for the treatment of malaria and gastrointestinal disorder and skin infections course by pathogen such as *Candida intetrigo, Tinea pedis, Tenea versicolor and Pityriasis versicolor* (Akinde and Odeyemi 1987). This leaf can also be boiled in water to yield a dark red liquid which is added to a bathing water and the remaining portion of the boiled liquid is also giving to a baby to drink. The aqueous leaf extract of *Acalypha wilkesiana* is traditionally used to treat neonatal jaundice in western part of Nigeria on short term basis. Its ointment is widely used in Nigeria to treat fungal skin diseases. This plant has a variety of ethno medicinal uses such as treatment of skin rashes in babies, it also has antifungal and antimicrobial properties (Ogundaini,2005; Oladunmoye,2006, Awe and Eme,2014), Anti-obesity properties (Iyamu *et al.,* 2014), Anti-diabetic activity (Ikewuchi *et al*., 2011; Itankar *et al.*,2011), Anti- cholesterol and anti-arrhythmic properties (Kingsley, 2013) and so on. This knowledge of medicine was disappeared due to the Western culture that has been on us on the past and is reappearing again as their importance have been realized and lack of side effects are also an important aspect in these types of traditional medicine. Vishnu *et. al.,* (2019).

Phytochemicals are compound or chemicals that are produced by plant, these phytochemicals are important by plant to thrive or thwart other plants, animals, pathogen, insects and antimicrobial pest they also help plants and protect them from damage and diseases caused by environmental hazards such as stress and drought, UV, pollution. They are used as traditional medicine and as poison from ancient days. Vishnu *et. al.,* (2019). The phytochemicals are basically classified as primary metabolites and secondary metabolites.

The primary metabolites are responsible for the basic development of the plant which includes sugar, chlorophyll, nucleic acids protein and amino acids while the Secondary metabolites are those chemicals that are needed by the plant to survive in harsh environment, they form the colours, smells and teste of the plant. Interestingly, natural product research guided by ethno – pharmacological knowledge has made substantial contribution to drug innovation by providing novel chemical structures or mechanisms of action De Smet, (1997); Vishnu *et. al.,* (2019). Pharmaceutical industries use traditional medicines as a source of bioactive agents that can be used in preparation of medicines. Most medicinal plants are being formulated into pharmaceutical dosage forms like tablets, creams, ointment, syrups and lotions (Sodimu *et. al.,* 2020).

Phytochemicals do help to prevent pathogens by stimulating the immune system, the body's defense against viruses, bacteria and other disease-causing agents. It blocks the potential for carcinogens (cancer-causing substances) to be formed in the body from substances we eat, drink and absorb from the environment, prevent DNA damaged and help with DNA repair mechanism. Phytochemicals further helps to regulate hormones, such as estrogen and insulin, excess levels of these hormones are linked with increased risk for breast and colon cancer. Lastly, it reduces inflammation that provides a setting favorable for cancer growth.

MATERIALS AND METHODS

Study Area

The study was conducted in Ahmadu Bello University main campus and her environs in Samaru, Zaria – Kaduna, Nigeria. It is approximately bounded by latitude $11°09^{1}$ N and $11°10^{1}$ N longitude $7°38^{1}$ E and $7°39^{1}$ E (Figure 1). It has a total land area of 563km2 (217sqml) and a projected population of 408, 198 (NPC, 2006) Zaria has a tropical savanna climate with warm weather year-round a wet season lasting from April -September and drier

season from October to March. the vegetation in the local government area is northern Guinea savanna with annual rain fall of 100mm-1500mm, temperature of 25.6^{O}C (78.1^{0}f), precipitation of 1,117.6mm and humidity of 69% (Sodimu *et. al*, 2021)

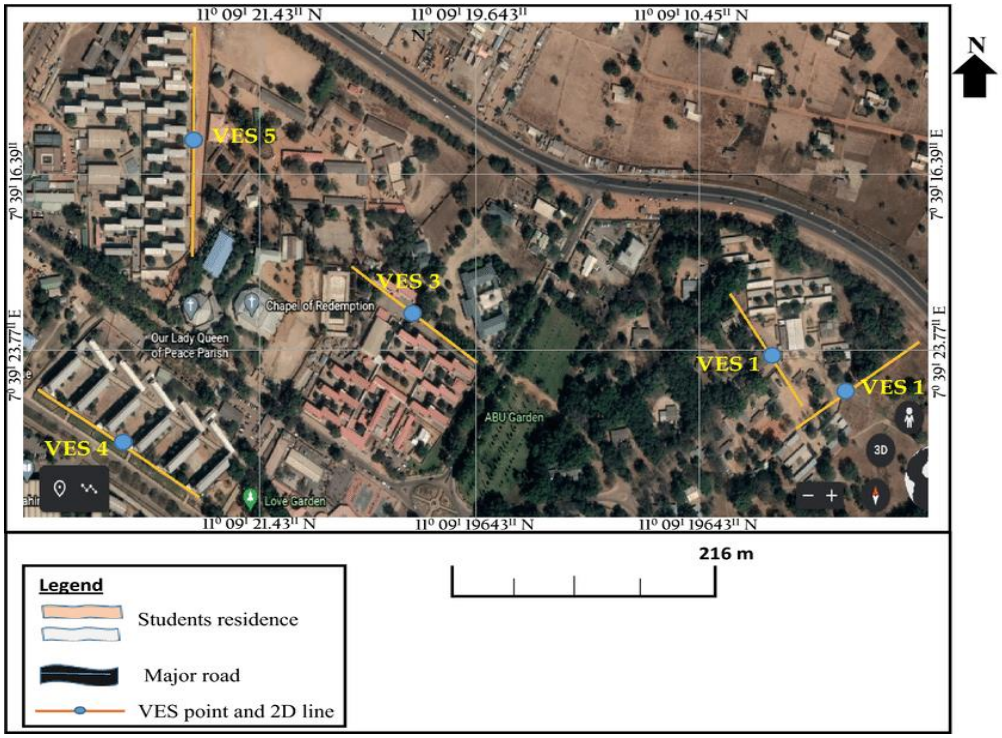

Figure 1: Map of Study Area Ahmadu Bello University (Samaru Campus A.B.U. Zaria)

Data Collection

Processing of the plant materials for phytochemical analysis

Acalypha wilkesiana leaves, stem and root were collected from Ahmadu Bello University and her environs. They were identified at the herbarium of Savannah Forestry Research station and Department of Botany Ahmadu Bello University Zaria Kaduna. The different materials were properly washed, air dried in the savannah forestry Research laboratory. The dried sample was chopped into pieces and ground separately to powdered form using blender. The powdered sample were store in small plastic Air -tight container which were later taken for Phytochemical analysis at Product Development Research Program in Institute for Agricultural Research (I.A.R) Ahmadu Bello University, Samaru - Zaria, Kaduna, Nigeria.

Phytochemical Screening

The extract was examined for the presence of the following phytochemicals; Flavonoids, Alkaloids, Phytate, Tannin, Saponin and Oxalate. The method described by Harbone (1998) was used to ascertain the presence of Alkaloids, Tannin was detected by adopting the method of Van-Burden and Robinson (1981). The presence of Saponins was detected using method adopted from Brunner (1984), the presence of Oxalate was detected using method adopted from Oke (1966). Phytate was determined using method adopted from Davies and Raid (1979) while flavonoids were screened using the method of Harbone (1973)

Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne (1998). Two (2)g of the sample was dispersed in 10%acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4hrs at 28^{0}c.It was later filtered using Whatman No 42 grade of filter paper. The filtrate was concentrated to one quarter of its original volume by evaporation and treated with drop wise addition of concentrated aqueous NH_4OH until the alkaloid was precipitated. The alkaloid precipitated was received in a weighed filter paper, washed

with 1% ammonia solution dried in the oven at 80^0c. Alkaloid content was calculated and expressed as percentage of weight of sample analyzed.

Determination of Saponins

The spectrophotometric method of Brunner (1984) was use for saponin analysis. 1g of the sample was weighed into a 250ml beaker and 100ml isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5h to ensure uniform mixing. Thereafter, the mixture was filtered through using a Whatman No. 1 filter paper into 100ml beaker and 20ml of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated $Mgc0_3$ was again filtered through a Whatman No. 1 filter paper to obtain a clear colourless solution. 1 ml of the colourless solution was pipetted into 50ml volumetric flask and 2 ml of 5% $FeCl_3$ solution as done for 1ml of the sample above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2ID Spectrophotometer at a wavelength of 380nm.

Percentage Saponin was calculated using the formula:

Saponin (%) = Absorbance of sample x Average gradient x Dilution factor/ Weight of sample x 10,00

Determination of Tannin

Tannin was quantified using the method adopted from Van-Burden and Robinson (1981). About 0.5g of the sample was weighed into a 50ml plastic bottle. Subsequently, 50ml of distilled water was added and final volume was shaken for 1 hour in a mechanical shaker. This was Filtered into a 50ml volumetric flask. Then, 5ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was read at 120nm after 10min of incubation

Determination of Flavonoids

This was determined according to the methods of Harborne (1973). 5g of the sample of the sample was boiled in 50ml of 2M HCl solution for 3omin under reflux, it was allowed to cool and then filtered through Whatman No 42 filtered paper. A measured volume of the extract was treated with equal volume of ethyl acetate starting with a drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference gave the weight of flavonoids in the sample.

Determination of Oxalate

This was determined by the method of Oke (1966). About 2g of the sample was weighed and digested with 1oml of 6M HCl for 1 hour. It was then filtered and made up to 250ml with H_2O in a volumetric flask. The pH was adjusted with concentrated NH4OH solution until the colour of the solution changed from salmo pink to a faint yellow colour. 10ml of 5% $CaCl_2$ solution was added to the precipitate, the insoluble oxalate. This was centrifuged at 250rpm and filtered. The residue or pellets was dissolved in 10ml of 20% (v/v) H_2SO_4 filtered and made up to 300ml. An aliquot of 125ml of the filtrate was taken and heated to near boiling point. This was treated against 0.05M of standardized $KMnO_4$ solution to give faint pink colour which persisted for 30 s.

Determination of Phytate

Davies and Reid (1979) described anion exchange methods for determining the amount of phytate in leaf samples. The filter (0.2-1.0 ml) was diluted to a final volume of 1.4 ml with distilled water, then 1.0ml ferric ammonium sulphate solution containing 50µg Fe was added and thoroughly mixed. After that, the test tubes were sealed and placed in a 20-minute boiling water bath. After the test tube had cooled to room temperature, 5 ml amyl alcohol were added, followed by 0-1 ml of a 100 g/l ammonium thiocyanate solution. Inversion and shaking were immediately used to mix the contents of the test tubes. Following brief centrifugation for 10 minutes at low speed, the colour intensity in the amyl layer was measured using a spectrophotometer at 465 nm against an amyl alcohol "blank"15 minutes after the HN4CNS was applied. The extinction at 465 nm in the amyl layer is inversely related to the phytate anion concentration because ferric ions complexed with phytate at pH 1-2 cannot interact with thiocyanate ion to create the characteristic pink complex

Analytical Techniques

Analysis of Variance (ANOVA)

Two-ways Analysis of Variance (ANOVA) using GLM procedure (prec. GLM) of SAS (Statistical Analysis System) was used to show the comparison between the photochemical composition of the leaf, stem and root of the plant. The data was expressed as mean standard deviation (mean of 3 determinations) and differences was considered significant at $P<0.05$.

RESULTS

Little or few studies have really mentioned the phytochemical constituents of Copper leaf (*Acalypha wilkesiana*) plants. However, the present study shows the presence of medicinally active compositions in various quantities in different parts of the plant. Oladunmoye (2006); Kingsley and Marshall (2014) reported the presence of alkaloids, tannins, and saponins in different composition within the plant (leaves, stems and roots). However, the quantitative estimation of the crude constituents in the plant's parts study are presented in table 1 below:

Table 1: Quantitative Phytochemical composition of copper leaf (*Acalypha wilkesiana*)

S/N	Parts	Flavonoids (Mg/100g)	Alkaloids (Mg/100g)	Phytate (Mg/100g)	Tannin (Mg/100g)	Saponin (Mg/100g)	Oxalate (Mg/100g)
1	Leaf	66.66 ± 6.1^{a}	3.92 ± 1.5^{b}	0.14 ± 0.00^{b}	4.05 ± 0.01^{c}	16.50 ± 0.01^{c}	0.10 ± 0.00^{c}
2	Stem	73.63 ± 1.5^{b}	3.80 ± 0.2^{c}	0.19 ± 0.01^{a}	3.75 ± 1.00^{a}	14.60 ± 3.2^{a}	0.13 ± 0.01^{b}
3	Root	79.221 ± 4.1^{c}	1.84 ± 2.0^{a}	0.21 ± 0.00^{b}	2.75 ± 0.20^{b}	14.30 ± 2.3^{b}	0.15 ± 0.02^{a}

Figures are expressed as mean ±SD; Figures bearing different alphabets differ significantly (P > 0.05); Figures bearing the same alphabets are not significantly different (P<0.05)

DISCUSSION

Result in table 1 above shows that significant differences ($P< 0.05$) exist between the plant parts. Leaves of *Acalypha wilkesiana* had the highest composition of alkaloids, tannins, and saponins while the root had the highest composition of flavonoids, phytate and oxalate. Flavonoids are mostly known for their anti-oxidant activity in vitro. The leaves may serve as a source of anti-oxidants which are useful in protecting against damage by free radicals. Furthermore, the beneficial effects of tea, fruits, vegetables or even red wine have sometimes been attributed to flavonoids compounds rather than to know micronutrients, include vitamins and dietary minerals (Felicien, 2008).

However, *A. wilkesiana* stem had the least composition of oxalate. Oxalic acid when combine with a divalent metallic cation such as calcium and iron to form a crystal of the corresponding oxalates which are then excreted in urine as minute crystals. Thus, 80% of kidney stones are from calcium oxalate (Coe et. al,2005). The low amount of oxalate in A. wikesiana stem may not pose a significant problem for those with kidney disorders, gout, rheumatoid arthritis or certain forms of chronic vulvar pain (Hossain et al., 2003 Morozumi et al.,2006). Thus, adding calcium to herbal preparation using stems of A. wikesiana may be beneficial, as it forms calcium oxalates which will precipitate out in the gut. Phytate found in the stems of the plant might be beneficial in small doses as it might have anticancer effects.

Kingsley and Marshall (2014), Aladejimokun *et. al.,* (2017) reported the presence of alkaloid, tannin, saponin, flavonoids, phytate and oxalate in the leaves of *Acalypha wilkesiana.* they thus, concluded that the presence of these chemicals could possibly explain the scientific basis of the plants in the medicinal utilization by the traditional healers in the treatment and prevention of various disease across West Africa countries. The results above are also in agreement with the work of Oyelami *et.al,* (2003) and Oladunmoye (2006) who documented that the presence of alkaloids, tannins, flavonoid and phytate in *A. wilkesiana* has contributed to the treatment of related skin infections, fungal diseases, allergies and ulcers.

CONCLUSION AND RECOMMENDATION

Conclusion

It is evident that the iconic Copper leaf (*Acalypha wilkesiana)* plant is an important medicinal resource used to treat and prevent a range of health hazards. The reason been the presence of high level of phytochemical compositions found in the plant's parts. In Nigeria, however, the plant gained little attention because of its ornamental nature.

Recommendation

Based on this study it is recommended that:

i. There is need to create awareness of the environmental conservation and protection of this medicinal plant biodiversity
ii. More research needs to be carried out to understand and tap the medicinal potential of this plant for the future use in modern drug industries.
iii. Government through relevance agency/ parastatals should stop individuals from indiscriminate destruction of the plant and encourage cultivation of Copper leaf (*Acalypha wilkesiana)* and medicinal plants in general. Similarly, sustainability of biodiversity and biological resources should be ensured so that individual plants do not go into extinction.

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