PRELIMINARY PHYTOCHEMICAL ANALYSIS OF KANE PLANT (*Anogeissus leiocarpus* Guill and Perr) IN BAUCHI, NIGERIA.

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ABSTRACT

Laboratory experiments were conducted on Kane plants (Anogeissus leiocarpus Guill and Perr.) during the 2011 cropping season. Kane plant (A. leiocarpus) is a graceful tree of the Sahel and Forest zones, belonging to the family Combretaceae. The objectives of the Laboratory experiments were to extract and identify the chemical compounds present in the various parts (leaves, stem and root barks) of Kane plant Anogeissus leiocarpus Guill and Perr. This research was therefore conducted with the aims of extracting and identifying the secondary metabolites and quantity present in the leaves, stem and root barks of Kane plant. Fresh leaves, stems and root barks of Kane plant were collected, prepared and subjected to extraction process at the National Research Institute for Chemical Technology (NARICT, Zaria. The extracts were subjected to photochemical screening. The results of the analysis indicated that there were nine chemical compounds present in the various parts of the Kane plant. These includes, tannins, saponins, flavonoids, steroids, terpiniods, cardiac glycosides, alkaloids, and cynogenic glycosides . All the chemicals except Phlobotannins were contained in the leaves extracts. The root extract also contained all the compound extracts except Steroids, Terpinoids, and Cynogenic glycosides while the stem extract contained only three of the chemicals (Tannins, Phlobotannins and Saponnins.).The leaves extract contained more compounds than the remaining extracts.

Keywords: *Anogeissus leiocarpus,* screening, extracts, chemical compounds, analysis

INTRODUCTION

Kane, *Anogeissus leiocarpus* (Guill and Perr.) is a graceful tree of the Sahel and Forest zones, belonging to the family Combretaceae. The leaves serve as fodder for livestock (Burkill, 1985). It is also used in traditional medicine as a remedy for many ailments of livestock and man which include, Helminthosis, Schistosomiasis, leprosy, and Psoriasis (Burkill, 1985; Ibrahim, 1987; Onyeyili, 2000). Similarly, Sanongo*et al*., (1998) reported that *A. leiocarpus* has a significant activity against some strains of Bacteria which include, *Haemophilus influenzae* (6 strains); *Staphylococcus aureus* (5 strains); *Streptococcus pneumoniae* (3 (strains) *Streptococcus pyogenes* (8 strains) and *Moraxella catarrhalis* (5 strains) responsible for respiratory infections. Investigations carried out also revealed the antimicrobial activity of *A. leiocarpus* posses against oral microbial flora such as *S. aureus;* and *S. auricularis*, Candida albicans, *Aspagillus flavus,Microsporium gypscum* and *Trychophyton metagrophyte* (Adekunle and Odukoya, 2006).

Agaie *et al.* (2007) also revealed that aqueous leaf extract of *A. leiocarpus* showed acute toxicity to rats which showed signs of depression. The manifestation of depression and inappetance observed in the rats may however, be linked to some chemical constituents present in the extract such as tannin. The result of the intraperitoneal acute toxicity study showed the LD_{50} of the extract as 1400 mg/kg, indicating that the extract is of low toxicity. Clarke and Clarke (1975) reported that any substance with an i/p LD_{50} of above 1000 mg/kg should be regarded as safe. However, no research was carried out to test the insecticidal nature of these plant extracts. This research was therefore conducted with the objectives of extracting and identifying the secondary metabolites and quantity present in the leaves, stem and root barks of Kane plant.

Chemical insecticides, in practice were the major control measures of insect pests Singh *et al*., 1983, Singh and Jackai 1985; Jackai and Adalla, 1997. However, excessive or increased use of chemical insecticides can lead to general environmental contamination, elimination of economically beneficial insects and development of resistance to the chemicals by the pests (Royer *et al*., 1986; Immaraju *etal*., 1992; Broadsguaard, 1994). Besides, chemical insecticides are toxic to human health and are not affordable by majority of the resource- poor farmers whose population constitutes 80% of the Nigerian farmers (Oparaeke *et al*., 2003).

The deleterious effect of the use of chemical insecticides especially on the human and the environment is now a global concern. Consequently, the demand for pesticide-free food has triggered

efforts for finding alternative and effective suitable pest management strategies for dissemination and implementation. Presently, considerable interest has been focused on the development of more benign strategies for controlling insect pests using plant extracts (Oparaeke *et al.*, 2003 and Rahman *et al.*, 2007).

MATERIALS AND METHOD

Fresh leaves, stem bark and root bark of Kane plant (*Anogeissus leiocarpus* Guill and Perr.) were obtained from old trees around the Abubakar Tafawa Balewa university (ATBU) Bauchi where they grow in wild and taken to the Chemistry Department of the Ahmadu Bello University, Zaria where the extraction were carried out. Prior to extraction, the samples were dried at $55\pm1\ ^0C$, ground and passed through a sieve of 1mm diameter. The extraction was done using 400 mg ground sample in conical flask with 40 ml diethyl ether containing 1% acetic acid (v/v) and mixed to remove the pigment material. The supernatants were carefully discarded after five minutes and 20 ml of 70% aqueous acetone was added and the flask was sealed with cotton plug covered with aluminum foil and kept on electric shaker for two hours before the extraction was carried out. It was then filtered through Whatman filter paper No. 1 and kept in a refrigerator at 4^oC. The extracts from the three parts of *A. leiocarpus* were then taken to the National Research Institute for Chemical Technology (NARICT), Zaria where the phytochemical and quantitative analysis were conducted in order to determine the compounds, their quantities in each of the three parts.

Test or Cyanogenic Glycosides

About 0.5 g of the powdered root bark sample of each plant was used for the test. They were placed in separate test tubes and sufficient water to cover the bark was added. A moist sodium picrate paper (prepared by dipping picric acid paper in sodium carbonate solution) was suspended in the neck of the tube and trapped by means of a cork. The closed tube was placed in an oven at 45^oC for one hour. A brick-red colour observed on the paper strip indicated release of hydrocyanin (HCN) from the crude plant sample.

Extraction of Cyanide sample:

Five grammes of each sample was ground, weighed and dissolved in 50 ml distilled water in a cork conical flask. Cyanide extract was allowed to stay overnight and then filtered.

Test for Saponin Glycosides

The Frothing Test

A small quantity of each extract was shaken with distilled water in a small test tube. Frothing which persisted on **Determination of quantity of Saponin** warming was taken as evidence for the presence of saponins.

A gravimetric method of AOAC (1984) employing the use of a soxhlet extractor and two different organic solvents was used. The first solvent extracted lipids and interfering pigments while the second solvent extracted saponin.

Two grammes of each of the sample were weighed into a thimble and put in a soxhlet extractor with a condenser fitted on top. Extraction was done with acetone in a 250 ml round bottom flask for three hours, after which another 250 ml round bottom flask containing methanol was fitted to the same extractor and extraction continued for another three hours. At the end of the second extraction, the methanol was recovered by distillation and the flask was oven-dried to remove the remaining solvent in the flask. The flask was allowed to cool in a desiccator and then weighed.

Test for Cardiac Glycosides

Legal Test

A small quantity of each extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside together with a few drops of 20% NaOH solution added. A deep red colour that faded to a brownish colouration indicated the presence of cardenolides in the extracts.

Test for Steroids: Salkowski Reaction

One milli-gram of the sample was dissolved in 1ml chloroform ($CHCl_3$) and 1ml conc. H_2SO_4 added, thus forming two layers, with red or yellow coloured interphase. This indicated the presence of sterols and methylated steroids.

Test for Flavonoids: Shinoda Test

A small quantity of magnesium powder and a few drop of conc. HC lwas added to an alcoholic solution of each plant extract. The appearance of orange, pink, red to purple colours indicated the presence of flavones, flavanols, the corresponding 2, 3-dihydro derivatives and/or Xanthones.

When zinc was used for the test, a deep red to magenta colour was observed for flavones while the flavanoids and flavonols gave a faint pink to magenta colour or no colour change.

Determination of the quantity of Flavonoid

Ten grammes of each sample was extracted thrice with 100 ml each of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed to a constant weight.

Test for Tannins

A small quantity of each extract was dissolved in 10 ml distilled water, boiled and filtered. To the filtrate

was added few drops of 1% Iron (II) chloride solution, a blue – black, green or blue-green precipitate was taken as evidence for the presence of tannins.

Determination of quantity of Tannins

Principle:

The Tannin content was estimated spectrophotometrically by Folin-Denis method . The method is based on oxidation of the molecules containing a phenolic hydroxyl group. The Tannin and Tannin-like compounds reduce phosphotungungustomolybdic acid in alkaline solution to produce a highly coloured blue solution; the intensity of which is proportional to the amount of Tannin and can be estimated against standard tannic acid solution at wavelength of 725 nm (Hill, 1983).

Sample preparation and extraction of Tannins

The sample was dried at 55 ± 1^0C and ground to pass through a sieve of 1 mm diameter. Tannin extraction was done using 400 mg ground sample in conical flask with 40 ml diethyl ether containing 1% acetic acid (v/v) and mixed to remove the pigment material.The supernatant was carefully discarded after five minutes and 20 ml of 70% aqueous acetone was added. The flask was then sealed with cotton plug covered with aluminum foil and placed in electrical shaker for two hours for extraction. It was then filtered through Whatman filter paper No. 1 and the sample was kept in a refrigerator at 4^0C until analysis.

Determination of Alkaloid

The gravimetric method of Harbone (1980) was adopted as specified.

Five grammes of each sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added to each sample and was allowed to stand for four hours. The extracts were filtered and concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle, and the precipitate was filtered in a weighed filter paper and washed with 1% NH_4OH solution.

The precipitate in the filter paper was dried in the oven at 60^0C for 30 minutes and reweighed.

RESULTS AND DISCUSSION

Table 1 shows the results of phytochemical screening of *A. leiocarpus*. Leaf extract contained annins, saponnins, cardiac glycoside, flavonoids, steroids, terpenoids, alkaloid and cyanogenic glycoside. Stem bark extract contained three metabolites. Tannins, phlobotannins and saponnins. Similarly, root extract contained tannins, saponnins, cardiac glycoside, flavonoids, steroids terpenoids, alkaloid and phlobotannins.

Results in Table 2 indicated the quantitative analysis of the various chemical compounds present in *A. leiocarpus* plant. Tannins were detected in large quantity in leaves and stems but in small quantity in the roots; Saponin was found in large quantity in leaves but in little quantites in stem and root extracts. Phlobotannin was found in large quantity in the stems and less quantity in the root extracts of the plant but was not detected in the leaves; steroids, terpinoids and cynogenic glycoside were only detected in large quantities in the leaves but not found in the stem and root bark extracts; Cardiac glycoside was found in large quantity in roots but less in the leaves; Alkaloid was detected in very large quantities in the leaves, small quantities in the roots and not detected in the stem.

The major bioactive chemical component of the test plant was favonoids, glycosides, saponins, Alkaloids and Steroids. The most important of these bioactive constituents of plants are Tannins, Alkaloids and Flavoniods compounds (Hill, 1983). Vegetables, so common in our environment, potato, tomato and eggplant belonging to the Solanaceae family, produce chaconine, solanine, tomatine, atropine and scopolamine which have a strong insecticidal effect in most insects. Some species have learned to tolerate the toxins (Menjivar, 2001).

The activity of Saponins against living caterpillers of *Spodoptera littoralis* and aphids (*Acrythosi phonpisum*) via treatment on artificial diets containing different concentrations of saponin revealed that saponin have insecticidal activity causing mortality and/or growth inhibition in the tested insects (Geyter *et al.*, 2007). Exploiting biological molecules like tannin and alkaloid will promote agricultural production and food security. Phytochemicals are the molecules of the future.

CONCLUSION

Results from the present investigation showed that tannins, saponins, Flavonoids, steroids, terpiniods, cynogenic glycoside, Alkaloid , Cardiac glycosides, and Phlobotannins are rich in phytochemicals, even though the phytochemical screening of the three plant parts revealed some differences in their constituents. The presence of terpinoids revealed that the plant can act as mainly as anti-feedant and growth

Table 1: Preliminary Phytochemical Analysis of the Active Compounds of Kane plant (*Anogeissusleiocarpus*)

Compound	Leaf extract	Stem extract	Root extract
Tannins	+ve	+ve	+ve
Phlobatannins	-ve	+ve	+ve
Saponins	+ve	+ve	+ve
Flavonoids	+ve	-ve	+ve
Steroids	+ve	-ve	-ve
Terpinoids	+ve	-ve	-ve
Cardiac glycosides	+ve	-ve	+ve
Alkaloid	+ve	-ve	+ve
Cyanogenic glycoside	+ve	-ve	-ve

+ve = Chemical ingredient detected **(**Present)
-ve = Chemical ingredient not detected (Absent)

Table 2: Quantitative analysis of the active biopesticide components of Kane plant (*Anogeissusleiocarpus*) in mg/100g

Compound	Leaf extract	Stem extract	Root extract
Tannins	15.38	14.82	1.61
Phlobatannins	-	10.2	2.1
Saponins	12.4	1.2	1.4
Flavonoids	13.3	-	2.3
Steroids	10.7	-	-
Terpinoids	11.6	-	-
Cardiac glycosides	1.1	-	11.2
Alkaloid	45.2	-	3.6
Cyanogenic glycoside	8.9	-	-

- = Chemical ingredient not detected

disruptor and possessed considerable toxicity towards insects (Khalid *et al*., 1989).

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