COMPARATIVE PHYTOCHEMICAL STUDIES ON THE PRESENCE AND QUANTIFICATION OF VARIOUS BIOACTIVE COMPOUNDS IN THE THREE MAJOR ORGANS OF OKOHO PLANT (*CISSUS POPULNEA* GUILL & PERR) IN BENUE STATE NORTH CENTRAL NIGERIA, WESTERN AFRICA

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ABSTRACT

Phytochemical studies were carried out on the leaf, stem and root of Cissus populnea used by the Idoma people of North Central Nigeria as soup seasoner to determine the presence and abundance of various bioactive compounds. The various plant parts were collected from five(5) different locations in Ai-kwu Otukpa in Ogbadibo Local Government Area of Benue State,Nigeria. The plant parts, the leaf, stem and root were dried at room temperature, ground into fine powder to increase their surface area and subjected to series of phytochemical analysis. SPSS software package (20.0 version) was used where descriptive statistics, correlation matrix, non parametric statistics and comparison of means using the T-test were carried out. Percentages of all the mean values of the various phytochemicals were calculated. Line of mean plot, simple line plot, box plot, area plot and radar plot were also made. Results showed high saponins content in the leaf (44.46%), followed by anthraquinone (39.63%). The stem and the root also contain large amount of alkaloids, saponins, flavonoids and tannins. However, the alkaloids content in the stem was highest, with 51.84%. It was followed by flavonoids(17.44%), saponins(15.42%), and tannins(13.29%). Similarly there was high amount of flavonoids(43.48%), alkaloids(28.95%), tannins(12.29%) and saponins (11.32%) in the root. However, Chi-square,Wallis Test and comparison of two means (T-test) revealed no significant difference in the mean values of the phytochemicals across the plant parts at 95% confidence limit.

Keywords: Cissus populnea, phytochemical, root, stem, leaf.

INTRODUCTION

Cissus populnea Guill and Perr belong to the family of Vitaceae and the genus *Cissus* which comprises of about 350 species, it is native to west tropical Africa. In Nigeria it is commonly found in the Northern and Southern parts. The vernacular names include ‘Okoho’(Idoma and Igala),'Ogbolo ajara'(Yoruba) and 'Dafaaraa'(Hausa) (Burkill 2000).

The plant is a strong woody climbing shrub, 8-10cm long and with 7½ cm in diameter, with a perennial root stock with jointed stems often with watery juice. The stock is often an annual rod drying during the dry season, covering the tree in which it is hung. The bark is cream and smooth when young, then gray and scaly condition, flaking by fibrous shell on the old foot. The branch is finely pubescent becoming glabrous, green with a branched tendril opposite of a leaf.

The leaf is alternate, oval, 5-18cm wide with acuminate or slightly pointed apex, the leaves of *Cissus* species are also simple, sometimes lobed, but not digitately compound. However, the leaf base is cordate in morphology. Petiole glabrous,3-13cm long. Inflorescence is of the panicle type which branches repeatedly. It bears flower in the rainy season which are usually green, with four petals of 3-4mm in diameter. The fruit is usually ovoid in shape, smooth, dark purple at maturity. The stems are succulent, sharply quadrangular, with sides 6-15mm wide, constricted at the nodes (Eggli, 2002).

Cissus populnea Guill and Perr have a cosmopolitan distribution, though a large number is found in the tropics. From the coast to the sudanian and Sahelian woodland, with an average annual rainfall of 250 and 100 per annum, with an average annual rainfall of 250 and 1000 per annum, but in such cases it depends solely on ground water levels. The mean annual temperature range is 25^{o} C- 30^{o} C (but with usually low or temperature). The plant lives in grove and forest galleries of Sahel savannah and sudano- Guinean on all soil types (Burkill, 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. Among the numerous varieties of plants is *Cissus populnea* (*Inavova et al.,* 2005). Bioactive ingredient made from the plant has proven medicinal properties. Experts in the evaluations of *Cissus populnea*, reported that they have antimicrobial activities which cure many sexually transmitted diseases infections that could be responsible for male infertility (Ojekale *et al.,* 2006). Other studies have also shown that the essential oil from the stem powder of the plant inhibit the growth of several germs of bacteria origin and as such may correct male infertility arising from the bacterial infections.

According to Belmain *et al.,(* 2000) the plant is used as diuretic and as a post- harvest ethnobotanical protectant in Ghana. Previous studies on the plant have also shown that the root extracts of the plant have been used for the treatment of skin diseases, boils, infected wounds and for treating urinary tract infection, thus suggesting anti bacteria potency of the plant (Kone *et al.,* 2004). The root is also used in the southern part of Nigeria as an arrow – poison antidote (Gill, 1992). In Niger, Kogi, Plateau, Kwara and Benue state of Nigeria, the plant is used for making vegetable soup for post natal stoppage of blood flow (Soladoye and Chukwuma, 2012).The gum obtained from the plant has been evaluated for its potential uses as a dispersant in pharmaceutical liquid system (Iwe *et al.,* 1993). However, further studies conducted on the plant shows that it is a poisonous plant used as pesticides and fish poisoning, therefore suggesting it ichtyotoxic properties (Bosch, 2002). The roots are used by the Yoruba to cure sore breasts of women at childbirth and male coital adjunct disease (Burkill, 2000). Studies from herbaria collections indicates that the plant is confined to the savannah zone of the country and thus is more abundant in the northern region where it is used by the Fulani to feed cattle, ostensibly to increase milk production(Brotherton,1969). The fibre are also used for binding material and for making papers and baskets.

Chengaiah *et al.,*(2010) carried out phytochemical analysis on the leaf of *Lawsonia inermis* Linn using extraction and spectroscopic methods. The alkaloids obtained from the plant has potent medicinal benefits . Idowu *et al.,(* 2010) also carried out phytochemical analysis on the root of henna which is also of medicinal use. A Phytochemical studies conducted on *Seamum radiatum* also showed that the plant is effective in the treatment of catarrh, eye pains, bruises and erupted skins (Bankole *et al,*. 2007). In view of the foregoing, it is pertinent to conduct phytochemical analysis on *Cissus populnea* to quantify and determine its

basic bioactive component of medicinal values since reports on the plants are limited. Therefore the overall aim of this study was to test and quantify the presence of alkaloid, tannins, saponins, anthraquinone and flavonoids in the root, leaves and stem of *Cissus populnea* in different locations in Benue State Nigeria where the plant is in high demand as a delicacy.

MATERIALS AND METHODS

Sample Collection and Preparation

For the purpose of phytochemical analysis five (5) *Cissus populnea* plants were randomly selected from five (5) different locations within Benue State. The plant materials were dried at room temperature for 16 days. The dried plant parts were pounded with a local mortar and pestle to increase their surface area.

Phytochemical Analysis

Phytochemical analysis was carried out on the leaves, stem and root and the presence of alkaloid, flavonoids, tannins, saponins and anthraquinone were tested and investigated. The investigation was followed by quantitative analysis of each bioactive ingredient in each part of the plant to generate 75 data sample size. The procedures followed earlier works on plant analysis as used by Sofowora (1993) and Trease and Evans (2005). A detailed method of extraction, as well as purification techniques for active plant ingredient described by Harborne (1973) was also employed for extraction of plant materials.

(A) Determination of Alkaloids

Five (5) grams of each of the powdered sample was weighed into a 250ml beaker of 200ml of 10% acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extraction was concentrated on water bath to one quarter of the original volume. Drop-wise addition of concentrated ammonium hydroxide to the extract followed until the precipitation was complete. The entire solution was allowed to settle and collection of the precipitated was done by filtration (Harborne 1973; Obadoni and Ochuko, 2001) and then weighed. The residue collected was the alkaloids.

(B) DETERMINATION OF FLAVONOIDS

To determine the flavonoids content in the leaves, stem and root of *Cissus populnea*, the aluminum chloride colorimotric method was employed. One ml of each plant parts was mixed 3ml of methanol, 0.2ml of 10% aluminum chloride , 0.2ml of 1m potassium acetate, and 5.6ml of distilled water . The entire mixture was allowed to stand at room temperature for 30mintues, while the absorbance was measured at 420nm. The total flavonoid content in each plant part was expressed.

(C) DETERMINATION OF TANNINS

Tannin method was determined using the method outlined by Van-Burden and Robinson (1981). Five hundred mg of each of the sample was weigh into a 50ml plastic bottle and 50ml of distilled water was added and then shaken thoroughly for 1 hour in mechanical shaker. The solution was filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipette out into a tests tube and mixed with 2ml of 0.1m $FeCl_3$ in O.1N HCl and 0.008m

potassium ferocyanide. The absorbance was measured at 120nm within 10minutes (Edeoga *et al.*, 2005).

(D) DETERMINATION OF SAPONINS

One gram of the finely grand sample was weigh into a 250ml beaker and 100ml of isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5 hours to ensure uniform mixing. Subsequently, the mixture was filtered through a whatman No.1 filter papper into a 100ml beaker and 20ml of 40% saturated solution of magnesium carbonate was added. The obtained mixture with saturated $MgCO_3$ was again filtered to obtained a clear coulourless solution. One ml of the colourless solution was pipetted in 50ml volumetric flask and 2ml of 5% $FeCl_3$ solution was added and made up to the mark distilled water and then allowed to stand for 30minutes for the development of red colour. Standard saponin solution of 0-10ppm was prepared from saponin stock solution and each standard solution was treated similarly with 2ml of 5% FeCl solution. The absorbance of the sample as well as the standared saponin solution was read after colour development on a spectronic 21D spectrophotometer, at a wave length of 38nm and percentage of saponin was calculated.

(E) DETERMINATION OF ANTHRAQUINONE

50mg of each powdered sample was soaked in 50ml of distilled water for 16 hours and then heated in a water bath at 70^{o} C for 1 hour. The suspension was allowed to cool, after which 50ml of 50% methanol was added to it and then filtered. The clear solution was measured with a spectrophotometer at a wavelength of 450nm and then compared with standard solution containing 10mg/100ml alizarin and 1mg/ 100ml purpnin with the absorption maximum of 450nm.

DATA ANALYSIS

The result was statistically analysed using the SPSS (Statistical Package for Social Scientists) software of 20.0 version (latest version). Graphically analysis was done using the Box plot, Line of mean plot, Simple line plot, Area plot and Radar plot.

RESULTS

The results of the various phytochemicals investigated are as presented in Table 1 which also shows the mean values and percentage (%) of mean of the various phytochemicals present in the leaf, stem and root of *Cissus populnea*.

Box plot charts of the phytochemicals are presented in figures 5. Figure 4 shows the line of mean plot, while figure 3 gives the radar plot values of the phytochemicals. Figure 1 simple line of the phytochemicals in the different organs whereas figure 2 shows area plot of the phytochemicals in the different organs. Table 2 displays overall mean value of the phytochemicals in the different organs.

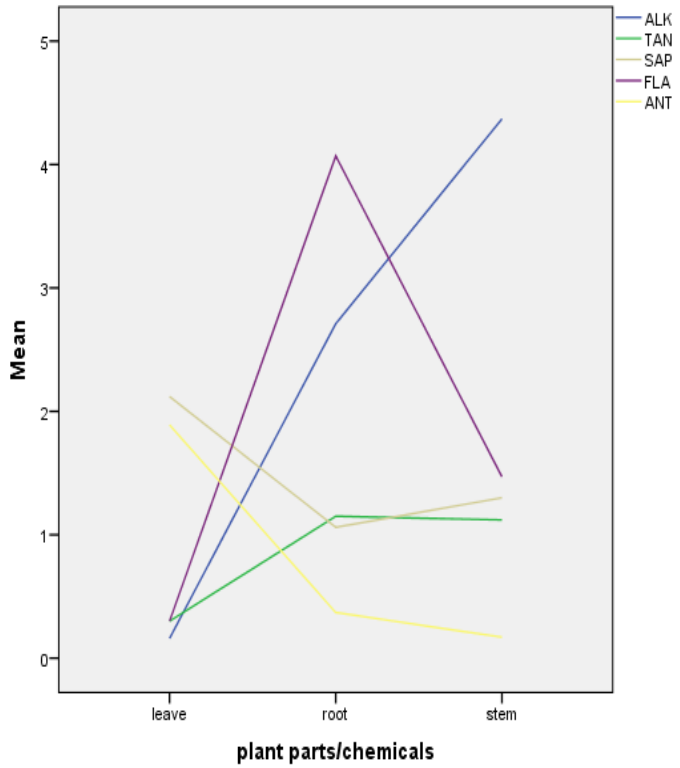


Fig 1 : Simple line plot of the phytochemicals

Table 1: Percentage mean of Phytochemicals

Phytochemicals (mg)	MEAN	% MEAN	
alkaloids	0.158	3.31	
tanin	0.3	6.29	Leaf
Saponins	2.12	44.46	
Flavonoids	0.3	6.29	
Anthraquinone	1.89	39.63	
Alkaloids	4.37	51.84	
Tanins	1.12	13.29	
Saponins	1.3	15.42	Stem
Flavonoids	1.47	17.44	
Anthraquinone	0.17	2.02	
Alkaloids	2.71	28.95	
Tanin	1.15	12.29	
Saponins	1.06	11.32	Root
Flavonoids	4.07	43.48	
Anthraquinone	0.37	3.95	

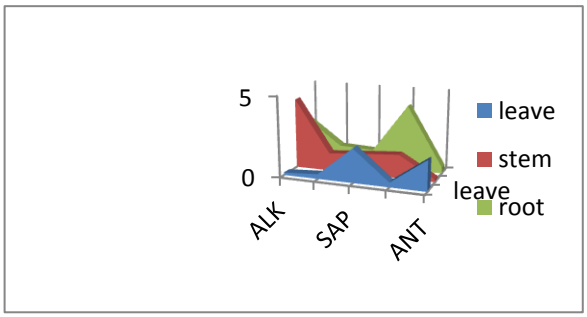


Fig. 2: Area plot of the phytochemicals

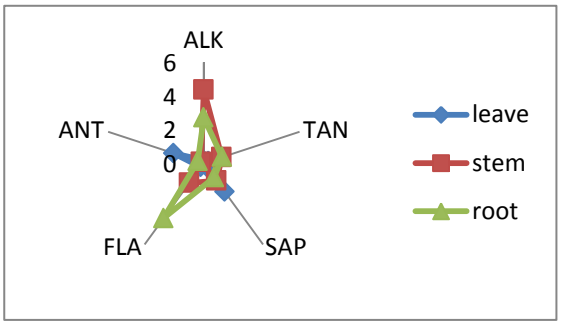


Fig. 3: Radar plot of the phytochemicals

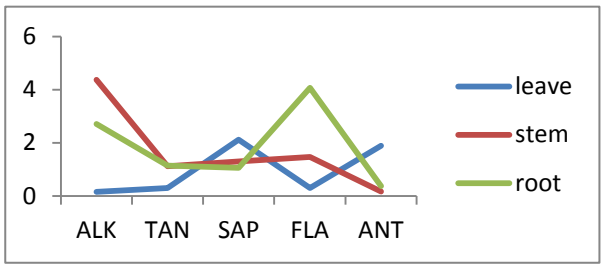
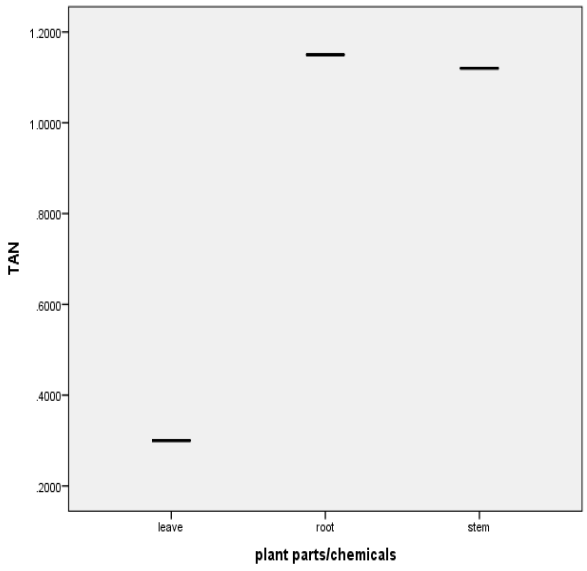
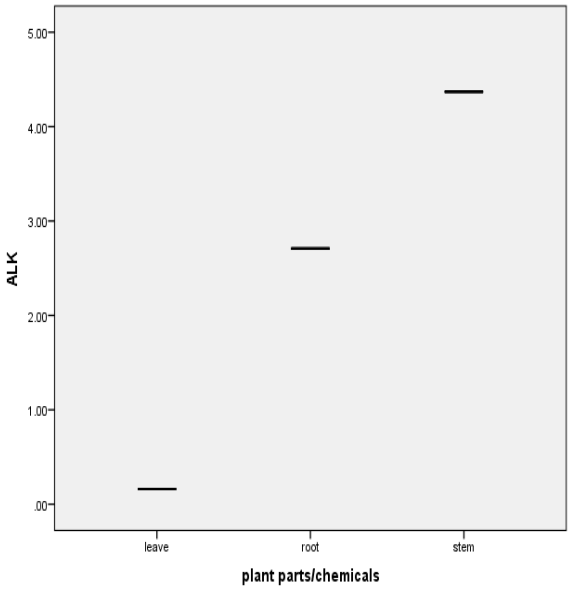


Fig. 4: Mean line plot of the phytochemicals





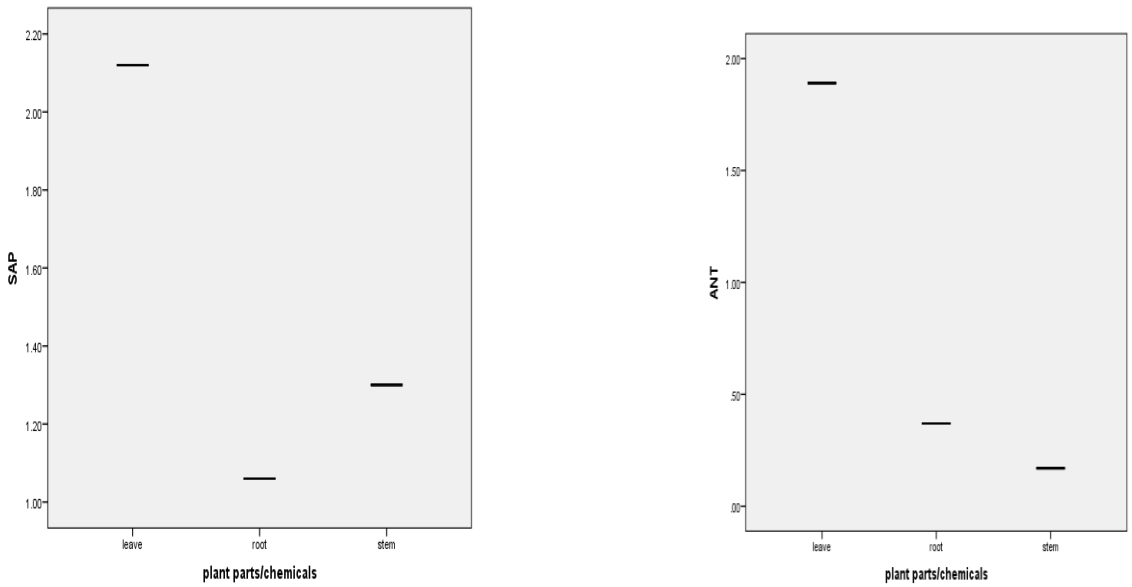


Figure 5 : Box plot chart of the phytochemicals

Table 2: Overall mean values of the phytochemicals in different plant parts

Phytochemicals(mg)	ALK	TAN	SAP	FLA	ANT
Leave	0.16	0.3	2.12	0.3	1.89
Stem	4.37	1.12	1.3	1.47	0.17
Root	2.71	1.15	1.06	4.07	0.37

KEY:
ALK-Alkaloids
TAN-Tannins
SAP-Saponins
FLA-Flavonoids
ANT-Anthraquinone

DISCUSSION

The results obtained from this study has clearly revealed that the leaf, stem and root of *Cissus populnea* contain some proportions of alkaloids, tannins, saponins, flavonoids and anthraquinone. The percentage mean value of tannin present in the stem is13.29% been the highest and lowest in the leaf with 6.29% value (figure 1, 2 and 3).

The saponin contents in the plant varies with the highest occurrence in the leaf (44.46%) and lowest occurrence in the root (11.32%).

The alkaloids content in the plant also varies among the leaf, stem and root, with the stem having the highest value (51.84%) followed by the root (28.95%) and the leaf been the lowest (3.31%), which shows that the stem and root of the plant contain abundant alkaloids. . All the figures presented in this work have also shown that the flavonoid content in the plant varies. The percentage mean value of the root ,stem and leaf are 43.48%,17.44% and l6.29% respectively.

The anthraquinone content varies among the different plant parts with the leaf having highest percentage mean value of 39.63%, root (3.95%) and lowest occurrence in the stem (2.02%). The high content of saponin (44.46%) and anthraquinone (39.65%) in the leaf of *Cissus populnea* has suggested the industrial values of these bioactive ingredients present in the leaf (Kakuda *et al*., 2004). It is also in agreement with earlier report on the leaf been high in saponin but ichtyotoxic in nature (Bosch *et al*.,2004). The use of this chemical in the treatment of tumour has been significant in most reports (Akindahunsi and Salawu,2005) which suggests the exploitation of the leaf in this regard and might be beneficial.

This finding is in conformity with the work of Soladoye and Chukwuma (2012) where saponins was found in abundance in the stem and root of *Cissus spp*. In their report, wider range of different phytochemicals were analysed in which saponins content was higher than most phytochemicals but saponin content on the leaf of this plant was not reported and here reported for the first time.

Meanwhile, the saponin content in the leaf (44.46%), root (15.4%) and the stem (11.3%) observed in this study is higher than anthraquinone content among the five phytochemicals analysed.

The stem contains high level of alkaloids (51.84%) and that agrees with earlier report on the medicinal value of the plant by using the stem in the treatment of various ailment and as anaesthetics. Osibite *et al*. (2010) carried out the assessment of anti-malaria activity of essential oil from the stem powder of *Cissus populnea* and the role of the alkaloids extracted as medication for male infertility. The outcome of their findings is supported in this work since the stem contain high alkaloids content. Trease and Evans (2005) had earlier pointed out that plant parts containing high alkaloids content are not recommended in herbal medicinal practices because such regions are extremely toxic when consumed in large quantity.

This study has also revealed that the root should be exploited for it flavonoids content (43.48%) when compared with other phytochemicals. Flavonoids have been shown to inhibits topoisomerase enzymes and to induce DNA mutation in the mixed-lineage leukamia (Mandel *et al*., 2007).

CONCLUSION

Cissus populnea leaves , stem and root appear to be rich in bioactive compounds which are widely used for various activities including traditional medicines. Though statistically not significant, graphically and descriptive analyses have revealed that the leaf, stem and root should be exploited for their immense values embedded in saponins, alkaloids and flavonoids respectively.

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