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Nutraceutical Potentials of *Cissus Populnea* Linn Cultivated in the Southern Guinea Savanna Agroecology of Nigeria

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Materials and Methods: The stems of *C. pulpunea* where sectioned and sent to the laboratory where Protocols for carbohydrates, crude fibre, crude protein, Crude Fat, mineral composition and oxalate were all determined. Means of the replicates were obtained and recorded.

Results: The percentage vitamins showed that Ascorbic acid had the highest value and the least was tocopherols. The essential and non-essential amino acid range from 0.10-14.48% for essential and 0.05-5.44% for non-essential in that order. The percentage total amino acid of *C. populnea* is 45.38%. The proximate composition of *C. populnea* are protein, ash, crude fibre, fat, nitrogen, moisture content and carbohydrate. The phytochemical results revealed the presence of some secondary metabolites found in *C populnea* to include flavonoids, phenolics, alkaloid, oxalate, glucosides, phytate, saponin, trypsin inhibitors and HCN. The mineral composition showed the presence of minerals as calcium, magnesium, potassium, phosphorus, sodium, manganese, iron, zinc and copper.

Conclusion: The study therefore supports the cultivation of *C. populnea* with the aim of improving the bioactive, components, directly providing medicinal and nutritional requirements of humans.

Keywords: cissus populnea, phytochemical screening, proximate, mineral, amino, acid vitamin analysis, medicinal plants.

I. Introduction

Man depend on plants for medicine, preservative sources of food, shelter and clothing because of the presence of secondary metabolites. Phytochemicals are biologically active plants compounds having disease hampering capabilities and preservative action. They are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health[1]. Phytochemical have anti-oxidant or hormone-like effect which help in fighting against many disease including dermatological problems, simple body ailments, cancer heart disease, diabetes, high blood pressure[2]. The medicinal value of plants lies in their secondary metabolites which produce definite physiological action on the human body. Medicinal plants have been used for centuries as remedies for human disease because they contain components of therapeutic value[3]. Many of the plant materials used in herbal medicine are readily available in rural areas and this has made it relatively cheaper than orthodox medicine[4]. Medical plant has been found to be helpful in curing many common ailments such as ringworm, eczema, malaria, ashema, ulcer, fever etc and have always promoted the search for different extracts from plant[5]. Phytochemicals in *Ciccus populnea* are alkaloids: Alkaloids are basic nitrogenous compound with definite physiological and pharmacological activity. Alkaloids are natural plant compounds having basic characteristics and containing at least one nitrogen atom in a heterocyclic chain[5]; flavonoids are potent water soluble super antioxidants and free radical scavengers which prevent oxidative cell damage. They have strong anti-cancer activity and protects against all stage of carcinogens. Flavonoids in the body are known to reduce the risk of heart disease[6], Tannis are a heterogenous group of complex compounds widely distributed in plants, phenols, saponins and oxalate[6]. Many physiological activities, host mediated tumor activity and wide range of anti-infective action have been trace to tannins. They are class of natural organic compound which are essential for growth and reproduction of plant and are produced as a response for defending injured plant against pathogen; Saponins are plant glycoides characterized by foaming in water when mixed and stirred. Oxalate is a common constituent of plants and several species including some crop plants, accumulate high level of this C_2 dicarboxylic acid anion. Depending on species, oxalate accumulates primarily as soluble oxalate, insoluble calcium oxalate, or a combination of these two

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Author's Contributions: This work was carried out in collaboration between both authors. Author OEB contributed to design of study, wrote protocol, performed preliminary data analysis, interpreted the data and produced initial draft and supervised the work. Author AP contributed to design of study, gathered the initial data, managed literature searches, made contributions and corrections to initial draft. Both authors read and approved the final manuscript.

forms[6]. Some minerals in *C. populnea* like calcium functions as a constituent of bones and teeth, regulation of nerve and muscle function; Potassium is net principal cation in intercellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na+/K+ - ATPase[6].

The plant *Cissus populnea* belongs to the family Amplidacea (Vitaceae) the plant is 2 to 3m high semi-circular which grows in the savannah and is widely distributed in Senegal, sudan, Uganda, Abyssinia and Nigeria[7]. It is commonly known as 'okoho' by the Idomas, Igbo and Igala tribes of Nigeria, 'Dafara' (Kano, Zaria); 'Latutuwa' (Katsina) by the Hausa language of the indicated towns of Northern Nigeria; 'Ajara' or 'Orogboro' by the Yoruba tribes of Northern Nigeria and Southern Nigeria[8]. The plant *Cissus populnea* is also called food gum. The gum is used for soup and as soup thickener. It is also widely used as medicine for the treatment of general disease and indigestion and as a drug binder. *Cissus populnea* has been used locally to treat many ailments such as veneral, stomach and skin infections, sore breast, interstinal parasites, oedema and eye problems resulting from attack from black cobra and also used as laxative or purgative[8], economically it has been used as binder in food and in lining dye pits. This study was aimed at evaluating the phytochemicals, screening, proximate composition, mineral and functional properties of *Cissus populnea* as a medicinal plant

II. Materials and Methods

a) Study Area and Sample Collections

The stem of *Cissus populnea* was collected from Anwule village of Oglewu (Longitude 7.1493 and Longitude 8.0276) district in Ohimini Local Government Area of Benue State. and was sent for analysis to International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The study was carried out in June 2018 – September 2018.

b) Preparation of Sample

The stem of *Cissus populnea* was sliced into strands of smaller size and thoroughly washed to remove the adhering and foreign material. The fresh stem was cut into file strips with a kitchen knife and was air dried. It was then reduced to a fine powder using mortar and sieve. The powdered sample was put inside an air tight polyethylene container, neatly labeled and kept in a refrigerator pending analysis.

c) Proximate Analysis

The recommended method of association of analytical chemist's Proximate analysis of *Cissus populnea* stem was to determine the moisture content, crude fat, Ash contents, crude fibre, crude protein, sugar and starch using various methods[9].

d) Moisture content determination

This is a measure of the percentage moisture[10, 11] loot due to drying at room temperature.

2.0g sample was weighed into aluminum dishes and dried in the oven at 103-105°C for 4-6hours. Dish was transfer into desiccator and allow to cool then weighed on cooling at room temperature[9].

$$\% \text{ moisture} = \frac{W1-W2}{weig\,ht\ of\ sample} x \frac{100}{1}$$

Where

W1 = Initial weight of crucible + sample

W2 = Final weight of crucible + sample

e) Determination of carbohydrates

Determination of carbohydrates (Clegg anthrone) plant samples weighing 1g were put into a 250ml volumetric flask. Distilled wale (10ml) and 13ml of 62% perchloric acid was added and the mixture was shaken in order for it to homogenize completely. The flask was made up to 250 ml with distilled water, the solution formed was filtered through a glass filter paper. Filtrate (10ml) was collected and transferred into a 100ml test tube; this was also diluted to volume with distilled water. The hydroxyl used solution was pipette into a clean fat tube and 5ml of anthrone reagent was added, they were then mixed together. The whole mixture was read at 630nm wave length using the 1m distilled water and the 5ml anthrone prepared as blank. Glucose solution of 0.1ml was prepared and this was treated with the Anthrone reagent. Absorbance of the standard glucose with calculated suing the formula below[12];

$$\% \text{ CHO} = \frac{25\ x\ Absorbance\ \ of\ sample}{(Absorbance\ \ of\ glucose\ )x\ 1g\ of\ smple\ \ used}$$

f) Ash content determination

2.0g of dried ground sample was weighed into a dish which has been previously ignited and weighed. The sample was ignited on a hot plate in a fume cup board to char the organic matter. The dishes were placed in the muffle furnace and maintained at 600°C for 6hours until completely ashed. The dish was directly transferred to the desiccators and allowed to cool then weighed[13].

$$\text{ASH content (\%)} = \frac{W2-W1}{weig\,ht\ of\ sample}\ x\ 100$$

Where

W2 = Weight of dish + ash

W1 = Weight of dish

g) Crude fibre determination (Trichloro-acetic Acid (TCA) method)

1.0g of defatted sample was weighed into digestion beaker and 100ml of TCA digestion reagent was added. Place on the heating unit of digester open water supply to reflect condenser. Bring to boiling and reflux for exactly 40 minutes, counting from the time boiling commenced. Beaker was removed from the heater and allowed to cool a bottle bit, then filtered through Whatman No 4 (15.0 cm diameter), residue was washed with hot water and once with industrial spirit. The filter paper was opened out, the residue was removed with a spatula and was transferred to a previously ignited and pre-weighed dish then dried overnight in an oven at 150°C. desiccators were transferred and allowed to cool before weighing. Ash in a muffle furnace at 600°C for 6hours, then allowed to cool before reweighing[10].

$$\text{Crude fibre \%} = \frac{weig\,ht\ of\ crude\ \ fibre}{weig\,ht\ of\ sample}\ x\ 100$$

h) Crude Protein Determination

The crude protein was determined using micro-Kjeldah method. 1.0g of sample and catalyst mixture were weighed, 15ml of concentrated sulphuric acid was added from dispenser and mixed carefully by swirling the tube with a tube mixer. The digestion tube stand was placed with the prepared sample in front of the digester and the exhaust caps was fit.

The vacuum source was turned on to maximize air flow and ensure the fumes are contained. The tube was placed in the preheated digester inside the hood. As soon as the sample solution was cooled sufficiently, 75ml distilled water was diluted and mixed. 25ml boric acid was measured into receiver flask, then placed in the upper position on the form of the distilling unit. Prepared digestion tube was fixed to the corresponding tube bolder, 50ml of NaOH (55-40%) solution was dispensed automatically, steam supply was switch on to start distillation (usually 5 minutes). When the distillation was completed, digestion tube was removed with residue and placed back in the stand, receiver flask was removed and rest was distilled accordingly. Stirrer bar was added to the receiver flask solution and placed on the magnetic stirrer place of the filtrator[14].

This was filtrated 5 N standard HCl solution to a neutral grey colour. The result was expressed as

$$\%\ N = \frac{1.401\ x\ (ml\ HCL\ sample\ titre - ml\ HCL\ titre\ of\ blank) x\ NHCL}{sample\ weight}$$

i) Crude Fat (Soxhlet Extration method)

4.0g of sample was weighed accurately into a moisture dish and dried in an oven for 5-6hours at 100°C. Exactly 2-3g of the dried sample was weighed into a filter paper and wrapped properly then placed in thimble and transferred to extraction barrel-petroleum there was added through barrel into the extractor flask until it siphons over once into the extraction flask directly below it, more other was added until volume was enough to run through the duration of extraction. Water inlet tap was opened to make sure all joints are tight and flask was healed on the regulated heating mantle for 6hours at a condensation rate of at least 3-6 drop per seconds[9]. After extraction was completed, thimble was removed from the extractor barrel and dried in the oven, barrel was replaced and continued distilling until the extraction flask was almost dried then flask containing oil was then detached and dried in the oven overnight at 70°C to constant weight.

$$\text{Crude fat \%} = \frac{W2-W1}{W1}\ x\ \frac{100}{1}$$

Where *W1* = Weight of evaporating dish or extraction flask

W2 = Weight of evaporating dish and content after drying

S = Sample weight in g.

j) Determination of Amino acids using waters 616/626 LC (HPLC) Instrument

The sample preparation and determination were carried out in the following four Stages[9]:

1. *Hydrolysis*

The samples (0.5g) were weighed into a sterile furnaces hydrolysis tube 5 nmols Norleucine was added to the samples and then dried under a vacuum. The tube was again placed in a vial containing 10.05 N HCl with a small quantity of phenol, thereby hydrolyzing the protein by the HCl vapours under vacuum. This stage of hydrolysis of the sample lasted for between 20 – 23 hours at 108°C. After the hydrolysis, the samples were dissolved in ultra-pure water grade, containing ethylene diamine tetraacetic acid (EDTA). The EDTA chelates the metal was present in the samples. The hydrolysed samples now are stored in HPLC amino acid analyzer bottles for further analytical operations.

2. *Derivatisation*

The hydrolyzed samples were derivalised automatically on the water 616/626 HPLC by reacting the five amino acid, under basic situations with phenylisothiocyanate (PITC) to get phenylthiocarbamyl (PTC) amino acid derivatives. The duration for this is 45 minutes per sample, as calibrated on the instrument. A set of standard solutions of the amino acids were prepared from Pierce Reference standards H (1000

μmol) into autosampler crops and they were also derivatised. These standards (0.0, 0.5, 1.0, 1.5, 2.0 μmol) were used to generate a calibration file that was used to determine the amino acids contents of the samples. After the derivatisation, a methanol solution (1.5 N) containing the PTC-amino acids was transferred to a narrow bore waters 616/626 HPLC system for separation.

k) HPLC separation and Quantization

The separation and quantization of the PTC-amino acids was done on a reverse phase (18 silica column) and the PTC chromophone was automatically and digitally detected at the wavelength of 254 nm. The elution of the whole amino acids in the samples took 30minutes. The buffer system used for separation was 140 mm sodium acetate pH 5.50 as buffer A and 80% acetonitrile as buffer B. The program was ran using a gradient of buffer A and buffer B concentration and ending with a 55% buffer B concentration at the end of the gradient[9].

l) Data processing/interpretation and calculation

The intensity of the chromatographic peaks areas was automatically and digitally identified and quantified using a Dionex chromeleon data analysis system which is attached to the waters 616/626 HPLC System. The calibration curve or file prepared from the average values of the retention times (in minutes) and areas (in Au) at the amino acids in 5 standards runs was used[9]. Since a known amount of each amino acid in the standard loaded into the HPLC, a response factor (Au/pmol) was calculated by the software that was interphased with the HPLC. This response factor was used to calculate the amount of each of the amino acid (in pmols) in the sample and displayed on the system digitally. The amount of each amino acid in the sample is finally calculated by the software by dividing the intensity of the peak area of each (corrected for the differing molar absorptivity's of the various amino acids) by the internal standard (Pierce) in the chromatogram and multiplying this by the total amount of internal standard added to the original sample. After the picomole by the intensity of the height of each amino acid has been ascertained by the software, the data, the digital chromatographic software extrapolate back to 5 nmoles of the internal standard (Norleucine), and displays for the total amount that was pipetted into the hydrolysis tube at the beginning of the analysis as below:

$$\frac{mg}{mL}(in\ extract) dilution\ factor\ x\ peak\ height\ intensity$$

$$\frac{mg}{mL}(in\ sample) = \frac{\mu \frac{g}{mL} in\ extract\ x\ sample\ volume}{weight\ of\ sample}$$

m) Phytochemical Screening

The extraction and analysis of *Cissus populnea* stem was screened for the presence of phytochemical constituents such as alkaloids, flavonoids, tannins, saponin, oxalate, phytate, glucosides, trypsin, phenol, using waters 616/626 HPLC.

n) Extraction and Analysis of alkaloid using waters 616/626 HPLC

10g of the sample was weighed into a 250ml beaker, 200ml of 20% acetic acid in ethanol (2:1) was added, covered and allowed to stand for 6hours to extract. This was shake for 25minutes and centrifuged at 3500 rpm (revolution per a minute). The supernatant, after filtration was concentrated on a water bath to half of the original volume, concentrated ammonium hydroxide was 4times drop wisely added for alkaloids extraction, until the precipitation was completed. The whole solution was allowed to set and the precipitate was collected and washed with dilute ammonium hydroxide, filtration was carried out and after that, the precipitate was dried[9].

o) Extraction and Analysis of Flavonoids using waters 616/626 HPLC

2.0mg of the sample was weighed in each of the extraction tubes. 2.0ml of methanol was mixed with the sample and shake for 25 minutes, it was allowed to stand for 1hour. 0.2ml of 40% aluminum trichloride in methanol, followed by a drop of acetic acid (CH_3COOH), and finally, diluted with methanol to 250ml. Shaked for 15minutes and allowed to stand for 45minutes and then transferred supernatants to a set of HPLC auto analyzer cups and stored for determination on waters 616/626 HPLC.

p) Extraction and Analysis of Glycosides using waters 616/626

0.5g of the sample was weighed into a set of plastic vails and 1.0ml of elimeth sulfoxide (DMSO) was added and vortexed for 2minutes. The vials containing the sample was transferred to the dark environment for 5minutes. 5% methanol containing hydrochlorie acid and 1.0mg was added. TBHQ was added. The vials were mixed using a vortex mixer for 5minutes. The solution was transferred to a set of centrifuge tubes and was allowed to stand for 2hours, then centrifuged for 10minutes at 3500 rpm. The supernatant was transferred to HPLC auto analyzer cups for analysis[9].

q) Extraction and Analysis of phenol using water 616/626

5.0g of the sample was weighed into a 250ml digestion tube. 40ml of 80% ethanol + 60ml of ultra-pure water was added and 10ml of the above solutions was transferred to a set of test tube; 0.8ml of 2N of the folinciocalter reagent and 2.5ml of 40% Na_2Co_3 solution was added and the final volume was made to 250ml with ultra-pure water and shacked vigorous for

10minutes and allowed to stand for 4hours. The extract was sampled to a set of HPLC auto analyzer cups and then run on water 616/626 at the water length (1nm) of 765nm as follows[9].

r) Determinations of Saponins

A spectrophotometric method described by Brunner (1984) was used for saponin analysis. 1g of the finely ground sample was weighed into a 250ml beaker and 100ml of isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5hour to ensure uniform mixturing. Subsequently, the mixture was filtered through a Whattman No. 1 filter paper into a 100ml beaker and 20ml of 40% saturated solution of magnesium carbonate was added[9].

The obtained mixture with saturated $mgCo_3$ was again filtered to obtain a clear colourless solution. 1ml of the colourless solution was pipetted into 50ml volumetric flask and 2ml of 5% $fecl_3$ solution was added.

s) Determination of Tannins

Tannins content was determined using the standard method[9]. 500mg of the sample was weighed into a 50ml plastic bottle and 50ml of distilled water was added and then shaken thoroughly for 1 hour in a mechanical shaker. The solution was filtered into a 50ml volumetric flask and made up to the mark. 5ml of the filtrate was pipetted out into a test tube and mixed with 2ml of 0.1m $fecl_3$ in 0.1N HCl and 0.008m potassium ferocyanide. The absorbance was measured at 120nm within 10mins.

t) Determination of Oxalate

5.0g of sample was weighed out. 100ml distilled water was added which was heated for 1 hour and allowed to cool, make up to 100ml with distilled water, then measured 25ml of sample into a flask, added 20ml of 2m H_2SO_4. Heat the sample on the heating mantle, put the thermometer as you are heating till 70_oC temperature, then bring down the flask. Titrate the sample with KMNO4. For oxalate standard, measure 25ml of oxalic acid into another flask and treat standard as sample[9].

u) Mineral composition

Mineral content of *C. populnea* were determine using atomic absorption and flame spectrometry according to standard method[9].

Wet digestion of sample was done by taking the powered sample in a digesting glass to which 12ml of HNO_3 was added and kept the mixture overnight at room temperature. 4ml of perchloric acid was then added to the mixture and digested in a fume block. The temperature was increased gradually, starting from 50°C to 300°C, where the appearance of white fumes indicated the completion of digestion. The mixture was left to cool down, the contents of the tube was transferred to volumetric flask and the volume of the content were increased to 100ml by adding distilled water. The wet digestion was transferred to labeled plastics. The digest stored and used for mineral determination. Determination of Iron (Fe), Zinc (Zn), Calcium (Ca), manganese (Mn) and magnesium (Mg) was analysed using atomic absorption spectrophotometer. Different electrode lamps were used for each mineral. The equipment was standard solution for each mineral before and during determination to check if it was working properly. The dilution factor for all mineral except p and mg was 100.

For determination of Mg, the original solution was further diluted to 0.5ml original solution and adding distilled water to obtain a volume of up to 100ml.

For the determination of calcium, 1ml of lithium oxide solution was added to the original solution to unmask Ca from Mg. The concentration of the minerals recorded are converted to Mg by multiplying the absorbency (in ppm) with a dilution factors and dividing by 100.

$$\text{MW} = \frac{Absorbency\ \ (ppm)}{Weight\ of\ sample} \text{x100}$$

Determination of sodium (Na) and potassium (k) was done by flame photometry. Standard solution of 20, 40, 60, 80, and 100mg/ml used both for Na and k. The calculation for total mineral intake involve the same procedure given involve the same procedure given in atomic absorption spectrophotometer above.

Phosphorus (P) in the sample was determined by spectrophotometry by mixing ammonium molybdate with 250ml of distilled water in a beaker (solution A). antimony potassium titrates 0.2g was taken and dissolved in 500ml H_2SO_4 solution in a volumetric flask, enough distilled water was added to make the solution up to 1000ml (solution B). The two solutions were mixed in a 200ml volumetric flask to get the mix reagent. The volume of the mixed reagent was increased to 200ml by a distilled water.

Ascorbic acid (0.7g) was mixed with 140ml of the mixed reagent in a beaker left until dissolved to make the colour reagent. Digested sample (1.0g) was taken into a plane bottle labeled property and 4ml of distilled water was added to make a diluted volume of 5ml colour reagent (5ml) was added to this volume and the total volume of this mixture (final solution) was increase to 25ml. The dilution factor of this solution was 2500. After sometime the colour of this final solution turned blue. Sample from the final blue solution was taken cuvette and read using a spectrophotometer. Reading of phosphorus was recorded in ppm. The calculation for the total mineral intake involve the same procedure as given in atomic absorption spectrotometry.

III. Results

a) *Phytochemical screening of the stem of Cissus populnea*

The result of phytochemical screening from table 1 below, showed the presence of some secondary metabolites which are alkaloid, flavonoid, saponin, oxalate, Phytate, phenol, Glucosides, Trypsin, Tannins.

Table 1: Phytochemical Composition of *Cissus populnea*

Parameters	Stem of *C. populnea* (g/100g)
Pmm saponin	1.636
Pmm phytate	2.140
Pmm tannins	4.015
Ppm trypsin-inhibitor	1.216
Ppm total hemagykutinin	0.977
Ppm hydrogen cyanide (HCN)	0.024
g/100 total Phenolics	86.750
g/100 total Flavonoids	92.248
g/100 total Glucosides	5.893
g/100 total Alkaloids	11.131

Ppm (Part per million)

b) *Proximate Analysis Result of Plant Sample*

The result of proximate analysis showed the presence of Nitrogen, protein, moisture content, ash, crude fibre, fat and carbohydrate. Among all carbohydrate gave the highest value with 48.574% while Nitrogen gave the lowest value of 2.190% as shown in table 2.

Table 2: Proximate Composition of *Cissus populnea*

Parameter	Stem (%)
Nitrogen	2.190
Protein	13.690
Moisture Content	13.033
Ash	9.66
Crude fibre	11.331
Fat	3.705
Carbohydrate	48.574

c) *Mineral Analysis Results of Plant Sample*

Mineral analysis result showed the presence of calcium, potassium, magnesium, phosphorus, sodium, manganese, Iron, zinc, copper contents with Fe having the highest content in *Cissus populnea* with Ni with the least content (Table 3).

Table 3: Mineral Content of *Cissus populnea*

Elements/parameter	Stem (%)
Ca %	3.877
Mg %	0.598
K %	0.082
P %	0.48
Ppm Na	0.086
Ppm mn	85.781
Ppm Fe	94.798
Ppm Zn	41.957
Ppm Cu	5.655
Ppm Sn	0.024
Ppm Pb	0.024
Ppm Cd	0.017
Ppm Se	0.003
Ppm Cr	0.069
Ppm Co	0.016
Ppm Ni	0.002
Ppm As	0.020
Ppm Hg	0.019
Ppm Ag	0.004

Cissus populnea showed the presence of water soluble and fat-soluble vitamins. Vitamins were also present in *C. populnea,* Vitamin B2 (riboflavin) as the highest and vitamin E (tocopherols) having the smallest content (Table 4).

Table 4: Vitamins content of *Cissus populnea*

Types of vitamins	Vitamins(%)	
Water soluble	Vitamin B1 (thiamine)	2.347
	Vitamin B2 (riboflavin)	7.567
	Vitamin B3(niacine)	0.621
	Vitamin B6(pyridoxin)	1.829
	Vitamin B12 cobalamin)	3.128
	Vitamin C(ascorbic acid)	9.102
Fat soluble	VitaminA beta carotene)	5.641
	Vitamin E(tocopherols)	0.062

Twenty amino acid were present in *C. populnea* with asparagines having the least content and phenylamine the highest content of amino acid Table 5).

Table 5: Amino Acids content of *Cissus populnea*

Elements/parameter	Stem
Threonine %	3.26
Leucine %	3.19
Isoleucine %	0.90
Lysisne %	0.11
Methonine %	0.10
Phenylamine %	14.48
Tyrosine %	0.34
Valine %	3.76
Argine %	0.68
Histindine %	0.43
Alanine %	0.87
Aspartic acid %	0.11
Asparagines %	0.05
Glutamic acid %	0.76
Glutamine %	5.44
Glycine %	3.11
Proline %	2.48
Serine %	2.10
Tryptophane %	2.76
Crotine %	0.47
% total Amino acids	45.38

Glycoside analysis showed glucoside having the highest content and Metoprolol acid with the least (Table 6).

Table 6: Glycosides contents of *Cissus populnea*

Elements/parameter	Stem
g/100 glycyrhizic acid	2.624
g/100 glycyrrhetinic acid	0.003
g/100 18-beta-glycyrrhetinic acid	0.013
g/100 E-strphanthin acid	0.067
g/100 Digoxin acid	0.005
g/100 Digitoxin acid	0.049
g/100 Oleandrin acid	0.004
g/100 varapamil acid	0.095
g/100 Infedipine acid	0.0255
g/100 Amedipine acid	1.326
g/100 lisinopril acid	0.748
g/100 Enalapril acid	0.030
g/100 captopril acid	0.023
g/100 Hydrochlorathiazide acid	0.008
g/100 furosemide acid	0.034
g/100 propranolol acid	0.0569
g/100 Atenonol acid	0.023
g/100 Metoprolol acid	0.017
g/100 total glucosides	5.893

Alkaloids of various kinds were also present in the stem of *C. populnea* with Ricinine as the highest quantity and Quinoline with the least (Table 7)

Table 7: Alkaloids constituent of *Cissus populnea*

Elements/parameter	Stem
g/100 caffeine	0.496
g/100 colchicine	0.069
g/100 cinchonine	0.017
g/100 Rauwolfia	0.062
g/100 morphine	0.045
g/100 Aptmorphine	0.045
g/100 Atropine	0.035
g/100 Apoatrpine	0.087
g/100 Quinine	0.235
g/100 Narcotine	1.222
g/100 codaine	0.689
g/100 papaverine	0.028
g/100 Nicotine	0.021
g/100 conine	0.007
g/100 Piperine	0.031
g/100 Ricinine	1.918
g/100 strychine	0.003
g/100 vincristine	0.015
g/100 eserine	0.080
g/100 Pilocarpine	0.006
g/100 Ehedrine	0.058
g/100 lobelline	0.004
g/100 Tubocurarine	0.112
g/100 Reserpine	0.301
g/100 Vinblastine	1.567
g/100 Piperidine	0.884
g/100 Heroin	0.035
g/100 Emetine	0.027
g/100 Guinidine	0.009
g/100 Peletrevine	0.040
g/100 Pyridine	0.736
g/100 Quinoline	0.001
g/100 Aeridine	0.006
g/100 Cocaine	0.031
g/100 Eryotamine	0.002
g/100 Norpseudoephedine	0.022
g/100 Nornicotine	0.002
g/100 Cinchoridine	0.043
g/100 Hyoscine	0.116
g/100 Berberine	0.601
g/100 Psychotrine	0.339
g/100 Theobromine	0.014
g/100 Theophylline	0.010
g/100 cephaline	0.003
g/100 B-carboline	0.015
g/100 Phenylethylamine	1.039
g/100 Total Alkaloids	11.131

All phenolic components were acidic with valnilic acid having the highest content and castarinol C1 acid with the least value (Table 8).

Table 8: Phenolics constituents of *Cissus populnea*

Elements/parameter	Stem
g/100 Cinnamic acid	4.054
g/100 Piperonic acid	2.317
g/100 veratoe acid	2.317
g/100 valnilic acid	18.243
g/100 genticitic acid	0.097
g/100 galic acid	0.494
g/100 salicylic acid	0.159
g/100 carreic acid	0.234
g/100 sinamic acid	16.806
g/100 ferulic acid	0.420
g/100 homogentisic acid	3.262
g/100 pyrogallic acid	3.424
g/100 syringic acid	0.290
g/100 Benzoic acid	0.424
g/100 Izoferulic acid	0.204
g/100 mendelic acid	2.466
g/100 salicitic acid	0.389
g/100 P-OH-Phenyloacetic acid	0.299
g/100 m-OH-benzoic acid	0.056
g/100 homovanillic acid	0.797
g/100 protocatelic acid	7.098
g/100 P-cumaric acid	0.508
g/100 Galic acid	0.793
g/100 cafein acid	3.680
g/100 sinagic acid	2.366
g/100 singlic acid	8.578
g/100 P-OH-benzoic acid	0.957
g/100 caffeic acid	0.090
g/100 cattaric acid	0.275
g/100 coumaric acid	0.005
g/100 contaric acid	1.100
g/100 cyanidin 30-glucoside	0.009
g/100 castarinol C1 acid	0.0025
g/100 castarinol C2 acid	0.058
g/100 castarinol C3 acid	0.027
g/100 castarinol C4 acid	0.055
g/100 cutissin acid	0.023
g/100 Astringin acid	2.432
g/100 catechin acid	0.878
g/100 Aesculetin acid	0.305
g/100 ethyl/caffeati acid	0.501
g/100 ethyl/gallon acid	0.005
g/100 ferteric acid	0.036
g/100 Total phenolics	86.750

Flavonoid constituent was not left out in the analysis. Poncirin had the highest constituent and Thearubigin with the least constituent (Table 9)

Table 9: Flavonoids constituents of *Cissus populnea*

Parameters	Stem
g/100g Hesperidin	3.418
g/100g Nanitutin	1.953
g/100g Neoriocitin	1.953
g/100g Poncirin	15.380
g/100g didymin	0.082
g/100g Enocetrin	0.417
g/100g Rhoifolin	0.134
g/100g Diosmin	0.197
g/100g Nobiletin	14.169
g/100g Acacetin	0.354
g/100g raxifolin	2.750
g/100g sinerisetrin	0.134
g/100g tangeretin	2.887
g/100g neodiosmin	0.244
g/100g Naringin	0.358
g/100g Naringinenin	0.172
g/100g Quercetin	2.079
g/100g Erodictyol	0.328
g/100g myricetrin	0.252
g/100g Kaempterol	0.048
g/100g Apigenin	5.121
g/100g Isorharmnetic	5.984
g/100g Luteolin	8.302
g/100g Daidzein	5.844
g/100g Genistein	3.102
g/100g Glycitein	1.995
g/100g anthocyanin	7.232
g/100g Catechin	5.924
g/100g Epicatechin	0.076
g/100g Theoflarins	0.232
g/100g Thearubigins	0.003
g/100g Epigallocatechin	0.927
g/100g Epicatechin gallate	0.007
g/100g Epigallo catechin gallate	0.031
g/100g Proanthocyanidins	0.021
g/100g Hesperetin	0.049
g/100g Acacetin	0.022
g/100g Taxifolin	0.046
g/100g Tangeretin	0.020
g/100g Total Flavonoid	92.248

Plants, including most feeds and foods, produce a broad range of bioactive compounds via secondary metabolism. Venation in both nutrient and non-nutrient composition of plant materials have been reported to be influenced by age, culture practices, environment, seasons and the varieties. Thus the variation in the phytochemical properties of the species may present baseline information for their utilization in synthesis of pharmacological products for medications. Plants with potent bioactive compounds are often characterized as both poisonous and medicinal and may depend on the amount taken and the content.

IV. Discussion

The result of this study may be used in formulating antimicrobial products against pathogen which shows that of *C. populnea* is rich in plant compounds/secondary metabolites which are alkaloid, flavonoid, sapinin, oxalate, phytate, phenol, Glucosides, Trypsin and Tannins. The conducted phytochemical screening has shown that the plant part (stem) contain large amount of flavonoid, phenol, alkaloids (table 1). The flavonoid content in the stem of *C. populnea* seems to be very abundant as compared to other phytochemcials. The abundance of flavonoid in the stem is also indicative of its potent antioxidant effect, which suggest that the plant may be very useful as an antibacterial, anti-inflammatory, anti-allergic, anticlinal, antithrombotic, antimultagic and vasodicatory compound[15]. Plants containing alkaloid do not feature strongly in herbal medicine, yet the alkaloid have always been an important phytocompound used in allopathic systems[16].

Saponins, although non-toxic, can generate adverse physiological response in animals that consume them. Saponins have tumour-inhibiting effects in animals[17]. Their abundance in the stem and root of *C .populnea* support the use of the plant parts in many parts of Nigeria, particularly in the north, for traditional treatments of ailments[18]. Similarly, flavonoid and tannin compounds have also some anti-bacterial, antiviral[19] and antiparasitic effect. Their destruction or modification, in turn, plays an important role in the ripening of fruit[20]. The amount of tannins reported in this work suggested that *Cissus populnea* can also be useful in the production of drug for treatment of bacterial and viral infections. Its tannin content may also be useful in treating haemochromatosis a hereditary disease characterized by excessive absorption of dietary iron, resulting in pathological increase of total iron content stored in the body. The presence of other phytochemical also supported the fact that the plant is useful in many ways, as earlier reported by several authors.

The trend of the abundance of the mineral content in the stem of *C. populnea* in table 3 is as follows Fe > Mn > Zn > Cu > Ca > Mg > Na > K > P. The macro elements play a vital role in the body metabolism. From my results potassium content was (0.082), calcium (3.877), magnesium (0.598), phosphorus (0.48), sodium (0.86), manganese (85.781), iron (94.798), Zinc (41.957), copper (5.655). The stem *C, populnea* was high in iron, manganese, zinc, copper and calcium.

Calcium and magnesium play a significant role in photosynthesis, carbohydrate metabolism, nucleic acid and binding agent of eth cell wall. Calcium aids in teeth development.

Magnesium is an essential mineral for enzyme activity. Magnesium also play a role in regulating the

acid-alkaline balance in the body. The value of sodium in the sample which is required in the body only in a small quantity[21] stated that sodium and potassium found in the Intracellular and extracellular fluid in human help to maintain electrolyte balance and membrane fluidity. The function of the cell of the immune system.

The proximate composition of the stem of *C. populnea* is presented in Table 2. Carbohydrate is the highest. However, moisture content obtained in this study for *C. populnea* stem was 13.033. The low moisture content helps to prevent the stem spoilage by microorganism. The fat content value of the stem of C. *populnea* was lower compare to other values. The ash-content is an indication of the level of inorganic mineral and organic matter present in the stem.

Crude fibre quantity in these stem is desirable because adequate consumption of dietary fibre may aid digestion. Fibre softens stool and therefore prevent constipation. Dietary fibre is also important in layering serum cholesterol level and reduce risk of disease such as coronary heart disease, hypertension, diabetes and breast cancer.

The amino acid profiles of *C. populnea* using HPLC are listed in table 5. This method identified and quantified twenty amino acids in *C. populnea.* The essential and non-essential amino acid range from 0.10 – 14.48% for essential and 0.05-5.44%, for non-essential. It is interesting to note that phenylalamine, Glutamine, tyrosine, valine, Glycine threonine, leucine, trypotophan, proline, serine, acids were the most concentrated amino acids in *C. populnea*. This observation has shown that the concentration and presence of amino acid vary from one plant to another. The medium of propagation of plant is also a key factor to the concentration of amino acid. The percentage total amino acids in *C. populnea* was 45.38%. This is an indication that *C. populnea* produced a higher concentration of amino acid.[22] reported that amino acid concentration increased in salt-stressed environment. Secondary metabolites such as alkaloid provide chemical defense for plant and amino acid are important in protein synthesis and precursor in the formation of secondary metabolism molecules[23]. Valine maintain the balance of branched chain amino acids, whereas alanine is involved on hepatic, autophagy, gluconeogenesis and transamination. Leucine regulats the protein turnover (mTOR signaling) and gene expression. Glycine, lysine, threnonine and glutamate help to maintain intestinal integrity and health.

The vitamin content of *C. populnea* as presented in Table 4. It shows that ascorbic acid content was found to be the highest followed by riboflavin then beta-carotene after which cobalamin followed and finally thiamine. The result revealed a higher percentage of vitamin in *C. populnea*. Vitamins are crucial in the functioning Vitamin C is a major antioxidant in the human body and possess the capacity to participate in enzymatic and hydroxylation reaction. It also prompts the absorption of microelements such as iron and copper, involves in trace element metabolism and process cells from damage caused by free radicals and environmental pollution.

Vitamin A is a micronutrient essential to most mammalian species. It is necessary in vision, growth and development, gene transcription, immunity dermatology[24,25] any bioactive components such as vitamin C and E, carotenoids, phenolic and thiol compound have been reported to have natural protective effect against many illnesses.

V. Conclusion

The phytochemical, proximate and mineral results present in the stem of *C. populnea* showed that certain chemical compounds in plants such as alkaloid, flavonoids, saponins, oxalates and tannins contains some bioactive component, which may have biological significance to living organisms and can be useful in pharmaceutical industry, ethnobotany and ethno-medicine. Traditionally, *C. populnea* is used for the treatment of different ailments.

VI. Significance Statement

This study discovers that *Cissus populnea* can be beneficial for not only as a food crop but also as a medicinal crop. This study will help the researcher to uncover the critical areas of medicinal properties of *C. populnea* that many researchers were not able to explore. Thus a new fact on the phytochemical screening, proximate composition, mineral content, amino acid and vitamin analyses of *Cissus populnea* linn as a medicinal plant may be arrived at.

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