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Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill. & Perr. (Vitaceae) – an important medicinal plant in central Nigeria

[*1]Soladoye M, O and [2]Chukwuma E. C

[1]Department of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria
[2]Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, Ibadan, Nigeria

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ABSTRACT

Phytochemical analysis was conducted on the leaves of Cissus populnea Guill. & Perr., a plant belonging to the family- Vitaceae/Ampelidaceae. The leaves were collected from New Bussa, Niger State, Northern Nigeria, air-dried and ground into fine powder. The powdered leaves were screened and results were properly recorded as observed. Results showed that the saponin content was the highest with about 47.3%. This is followed by anthraquinones – 33.2% and flavonoid – 6.48%. Although, results also showed that the cyanogenic glycoside content (3.65%) is indicative of its poisonous property as the human body may not withstand this large amount at a time, the small quantity of alkaloid – 2.49% (0.15±0.07mg/g) also suggests that it may be harmless in some ways. This study, however, supports the fact that leaves of C. populnea contain important compounds which may be useful in medicine, it also suggests that further research should be conducted into the plant as a whole, since some of the phytochemicals may be very dangerous to the human body whether consumed as vegetable or used as medicine.

Keywords: *Cissus*, Vitaceae, Phytochemicals, Screening, Medicine.

INTRODUCTION

Phytochemicals are natural bioactive compounds which are present in plants. These natural compounds work with nutrients and dietary fibres to protect animals and man against diseases. Since time immemorial, these plant products which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine, thus indicating that any part of a plant may contain important active compounds. [1], pointed out clearly that medicinal plants constitute the main source of raw pharmaceuticals and healthcare products while [2] also reported that extraction and characterization of several active phytocompounds from green plants have given birth to some high activity profile drugs. Such phytochemical screening of various plants had been reported by many workers [3-7].

Cissus is a genus of approximately 350 species of woody vines belonging to the grape family - Vitaceae. They have a cosmopolitan distribution, though a large number is found in the tropics. The generic name is derived from the Greek word *kissos*, meaning "ivy" [8]. [9], described members of the family as climbing shrubs or small trees or herbs from a perennial rootstock, jointed stems, often with watery juice. The leaves of *Cissus* species are simple, sometimes lobed, but not digitately compound, stems are succulent, sharply quadrangular, with sides 6-15mm. wide, constricted at the nodes

Cissus populnea Guill. & Perr is a strong woody liane or climbing shrub, 8-10m long and $7^1/_2$cm in diameter, growing in the savanna, and dispersed generally throughout West Africa from the coast to the Sudanian and Sahelian woodland, Senegal to North and South Nigeria, and across Africa to Sudan, Ethiopia and Uganda [9 & 10]. Stems when cut, exudes copious clear watery sap, flowers are cream, fruits blackish-purple when ripe. The plant has succulent roots which when dried, are useful in building [11].

C. populnea have been reported to be primarily used as vegetable, as forage including feed for fish and insects like silkworms. However, the plant has also been described as a poisonous plant used as pesticide and in fish poisoning [12]. The fibres are used as tying material and for making paper and baskets. More recently, [13] reported that the plant is also useful in treating male infertility factor in South-Western Nigeria as well as urinary tract infections. The use of this plant is very popular in central part of Nigeria; Niger, Kogi, Plateau, Adamawa, Kwara and Benue states for making vegetable soup for postnatal stoppage of blood flow; although this is yet to be documented in literature. According to [14], it is used as a diuretic in Benin Republic and as a post-harvest ethnobotanical protectant in Ghana. Previous studies on the plant have also shown that the root extracts are used for the management of skin diseases, boils, infected wounds [15] which also suggests its antibacterial activity. The gum is used for soup and as soup thickener; it is widely used as medicine for managing indigestion and in the treatment of venereal diseases and even as drug binder [16]. In the present work, quantitative phytochemical analysis was conducted on *C. populnea* with the aim of identifying and determining the actual phytochemicals and quantity of each constituent present in the leaves of the plant.

MATERIALS AND METHODS

Preparation and extraction of plant material

The leaves of *Cissus populnea* were collected from New Bussa, Niger State in July, 2011. The collected plant was taken to the Forest Herbarium, Ibadan for proper identification before further work was carried out on it. Voucher specimen, FHI 109459 was prepared and deposited in the herbarium. The leaves were then air-dried for about 10 days under shade, ground into fine powder and then subjected to phytochemical analysis which was conducted at the National Horticultural Research Institute (NIHORT), Ibadan. The quantitative phytochemical analysis of *C. polpunea* leaves was carried out in order to ascertain the presence of some active constituents employing standard conventional protocols outlined by Sofowora, Trease and Evans [17 & 18] and detailed method of extraction and purification techniques for active constituents from plants described by Harborne [19]. These active compounds include Alkaloids, flavonoids, tannins, saponins, anthraquinones, cardiac glycosides, and cyanogenic glycosides

Determination of Alkaloids

This was done by the alkaline precipitation gravimetric method described by Harborne. A measured weight 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 4h at 28^{o}C. It was later filtered via

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 conc. 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 a percentage of the weight of sample analyzed.

Determination of Flavonoids

This was also determined according to the method outlined by Harbone [19]. 5gram of the sample was boiled in 50ml of 2M HCl solution for 30min under reflux. It was allowed to cool and then filtered through whatman No 42 filter 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 flavonoid in the sample.

Determination of Tannins

Swain's method [**20**] was used for the determination of tannin contents of the powdered leaves of *C. polpunea*. 0.2 g of finely ground sample was measured into a 50 ml beaker. 20 ml of 50% methanol was added and covered with parafin and placed in a water bath at 77-80^{o}C for 1 h and stirred with a glass rod to prevent lumping. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper into a 100 ml volumetric flask using 50% methanol to rinse. This was made up to mark with distilled water and thoroughly mixed. 1 ml of sample extract was pipetted into 50 ml volumetric flask, 20 ml distilled water, 2.5 ml Folin-Denis reagent and 10 ml of 17% Na2CO3 were added and mixed properly. The mixture was made up to mark with distilled water, mixed well and allowed to stand for 20 min when a bluish-green colouration developed. Standard Tannic Acid solutions of range 0-10 ppm were treated similarly as 1 ml of sample above. The absorbances of the Tannic Acid Standard solutions as well as samples were read after colour development on a Spectronic 21D Spectrophotometer at a wavelength of 760 nm. Percentage tannin was calculated.

Determination of Saponin

The Spectrophotometric method described by Brunner was used for saponin analysis [21]. 1 g of finely ground sample was weighed into a 250 ml beaker and 100 ml Isobetyl alcohol was added. The mixture was shaken on a UDY shaker for 5 h to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. 1 filter paper into a 100 ml beaker and 20 ml of 40% saturated solution of Magnesium carbonate added. The mixture obtained with saturated $MgCO_3$ 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 50 ml volumetric flask and 2 ml of 5% $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 min for blood red colour to develop. 0-10 ppm standard saponin solutions were prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% FeCl solution as done for 1 ml sample 3 above. The absorbances of the sample as well as standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer at a wavelength of 380 nm. The percentage saponin was also calculated.

Determination of Anthraquinone contents

50 mg of the fine powder sample was soaked in 50 ml of distilled water for 16 hours. This suspension was heated in water bath at 70^{o}C for one hour. After the suspension was cooled, 50ml of 50% methanol was added to it and then filtered. The clear solution was measured by spectrophotometer at a wavelength of 450nm and compared with a standard solution containing 1mg/100ml alizarin and 1mg/100ml purpurin with the absorption-maximum 450nm

Determination of Cardiac gylcosides

Cardiac glycoside content in the sample was evaluated using Buljet's reagent as described by El-Olemy *et al* [22]. 1g of the fine powder of *C. populnea* was soaked in 10ml of 70% alcohol for 2hrs. and then filtered. The extract obtained was then purified using lead acetate and Na_2HPO_4 solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colours of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

Determination of Cyanogenic glycosides

For this purpose, the method described by Knowles and Watkins [23] was adopted. 5g of the fine powder sample of *C. populnea* was weighed into 250ml conical flask. The sample was incubated for another 16hrs at 38^{o}C and later extracted with 95% methanol. Sample was then filtered using double layer of hardened filter paper and distillation was done with Marham distillation apparatus. The extracted sample was transferred into a tow-necked 500ml flask connected with a steam generator. This was steam distilled with saturated sodium bicarbonate solution contained in a 50ml conical flask for 1hr. 1ml of starch indicator was added to 20ml each of the distillate and was titrated with 0.2N of iodine solution. The percentage hydrocyanide content was calculated.

RESULTS AND DISCUSSION

The quantitative phytochemical estimation present in *C. populnea* studied showed that the leaves are very rich in saponins, anthraquinones and flavonoids (Table 1). The presence and large amount of tannins also confirms its astrigent property. This compound can also be effective in protecting the kidneys [24]. Tannis have also shown potential antibactaerial and antiviral effects [25 & 26].

The saponin content makes the leaves an important source of detergents, surface active agents used in industrial applications and also possesses beneficial health effects [27]. Most saponins, which readily dissolve in water, are poisonous to fish. This result therefore supports earlier report by Bosch *et al* [12] and affirms its role in fish poisoning. Anthraquinone (an aromatic organic compound) content of *C. populnea* is also on the very high side as revealed from this study and shown in Figure 1. Apart from the health benefits derived from anthraquinones, they are also used in bleaching pulp for paper production because it is a building block of many dyes [28]

Table 1. Mean values (mg/g) of phytochemical screening of *C. populnea* leaves

Phytochemicals	Mean Vol (mg/g) and Standard deviation	Relative % of mean
Tannin	0.30±0.01	4.98%
Saponin	2.85±0.35	47.3%
Alkaloid	0.15±0.07	2.49%
Anthraquinone	2.00±0.14	33.2%
Total Flavonoid	0.39±0.03	6.48%
Cardiac Glycoside	0.10±0.01	1.83%
Cyanogenic Glycoside	0.22±0.01	3.65%
		100.00%

Results also revealed that cyanogenic glycoside (0.22±0.01mg/g) is relatively high in the leaves of *C. populnea*. This phytochemical has been known to be one of the most potent, rapidly acting poisons known [29] and because the human body detoxifies it so rapidly, an adult can only

withstand 0.05-0.06mg/g an hour without serious consequences. This suggests that the plant is not too safe for consumption even though it has some important bioactive compounds that may be beneficial to the body. Table 1 also shows that the flavonoid content of *C. populnea* is relatively on the high side (6.48%). This observation indicates that the plant has a high antioxidant effect. [30] earlier reported that flavonoids have antibacterial, anti-inflamatory, antiallergic, antimultagenic, antiviral, antineoplatic, anti-thrombotic and vasodilatory activities. However, the low amount of alkaloid present (0.15±0.07mg/g) is also indicative of its harmless effect based on its content. Onyeka and Nwambekwe earlier reported that alkaloid content of some edible vegetables ranged between 12.8-29.6mg/g [31]. Trease and Evans [18] have also pointed out that plants containing alkaloids do not feature strongly in herbal medicine because they are extremely toxic, yet, they have always been important in allopathic systems where the dose is strictly controlled and in homoepathy where the dose-rate is so low as to be harmless.

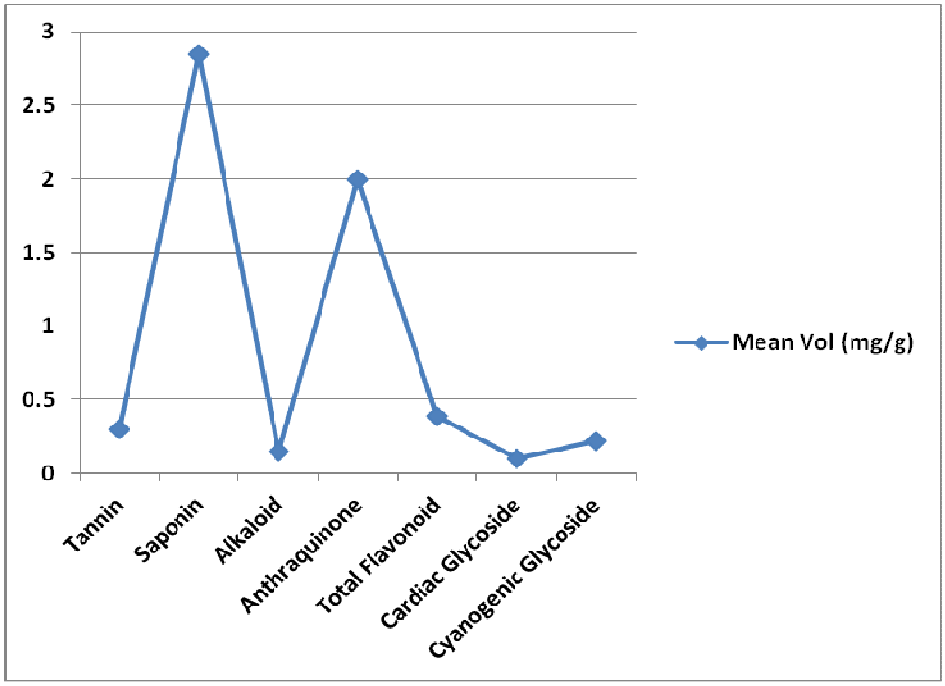

Figure 1. Mean volume of some phytochemicals in *C. populnea* compared.

Phytochemical screenings are not only used to search for bioactive agents. Plants have provided agents which serves as starting products for the partial synthesis of some useful drugs. An example is the steroidal sapogenins produced by *Dioscorea* species (or Mexican yams) and also by the *Balanites* and *Trigonellai* species. The 'Solanum alkaloids' from *Solanum* species have been used in the partial synthesis of drugs [17]. Plant steroidal sapogenins are used as starting products in the synthesis of steroidal drugs such as corticosteroids, the sex hormones, and oral contraceptives. Results from this work therefore, supports the fact that *C. populnea* possess important bioactive compounds which could be screened for several medicinal purposes.

CONCLUSION

There is no doubt that plants have continued to offer a large range of natural compounds belonging to different molecular families which have various properties to humans as earlier reported by Zabri *et al* [32]. *C. populnea* leaves appears to be rich in bioactive compounds which are widely used for various activities including traditional medicine. Findings from this work however suggest that the leaves of *C. populnea* are not consumable as the content of some

phytochemicals are extremely higher than the body can tolerate. Even though Bosch *et al* earlier reported that the plant is used as vegetable [12], it is our opinion that further analysis apart from phytochemical screenings should be conducted into *C. populnea* as a whole.

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