Phytochemical analysis of the stem and root of *Cissus populnea* (*Vitaceae*) – an important medicinal plant in Central Nigeria

Michael O. Soladoye[1] & Emmanuel C. Chukwuma[2]

[1] Department of Biological Sciences, Bowen University, Iwo, Osun State, Nigeria, e-mail: debosoladoye@yahoo.co.uk (corresponding author)

[2] Forest Herbarium (FHI), Forestry Research Institute of Nigeria, PMB 5054, Ibadan, Nigeria, e-mail: chukwumaemma@yahoo.com

Received: April 07, 2012 ▷ Accepted: July 04, 2012

Abstract. The phytochemicals in the stem and root of *Cissus populnea* were examined. Specimens were collected from New Bussa, Niger State, Nigeria and identified at the Forest Herbarium Ibadan (FHI). The stem and root were air-dried, ground into fine powder and subjected to series of phytochemical screenings. Results revealed that both plant parts contain alkaloids, flavonoids, saponins, and tannins in large quantity. However, the alkaloid content in the stem was the highest, with 49.8 %. It was followed by flavonoids (15.4 %), saponins (12.8 %) and tannins (11.6 %). Similarly, there was also significant amount of flavonoids (39.5 %), alkaloids (26.7 %), tannins (11.3 %), and saponins (10.5 %) in the root. While cardiac glycosides recorded the least amount in the stem, phytosteroids had the least amount in the root. The occurrence and quantity of other phytochemicals, as recorded in this study, also suggest that *C. populnea* may serve as a potential source of useful drugs in the near future.

Key words: *Cissus populnea*, medicine, phytochemical analysis, root, stem

Introduction

Cissus populnea Guill. & Perr. has been described by Burkill (2000) as a strong woody liane, 8–10 m long, 7.5 cm in diameter, distributed generally across West Tropical Africa, from the coast to the Sudanian and Sahelian woodland. It belongs to the family *Vitaceae/Ampelidaceae* and the genus *Cissus* which comprises of about 350 species. The plant occurs in Northern and Southern Nigeria. Studies from herbarium collections indicate that it is confined to the savannah zones of the country and thus is more abundant in the northern region where it is used by the Fulanis to feed their cattle, ostensibly to increase milk production, as reported by Brotherton (1969). All parts are mucilaginous, yielding a viscid sap, which is occasionally drunk from freshly cut stems (Bouquet & Debray 1974). The plant is also used in Niger, Kogi, Plateau, Adamawa, Kwara, and Benue states of Nigeria for making vegetable soup for post-natal stoppage of bleeding (Soladoye & Chukwuma 2012). In Nigeria, a decoction of the stem made with native natron (Hausa: *Kanwa*) and often with the addition of *Alchornea cordifolia* (Schumach.) Müll. Arg. is used for the treatment of venereal diseases. The viscid sap is used for other herbal remedies, while the root is used as antidote for arrow-poison. The roots are also used by the Yorubas to cure sore breasts of women at childbirth, and as a male coital adjunct (Burkill 2000). Phytocompounds derived from plant parts have been utilized in drug production over the years, thus indicating that any part of a plant may contain active components. Recently, there has been continual revival of interest in the

use of medicinal plants in many developing countries, including Nigeria, since herbal medicines have been reported to be safe and without adverse side effects, as compared to synthetic drugs. Edeoga & al. (2005) reported that the medicinal value of these plants lie in some chemical substances which produce a definite physiological impact on the human body. This paper examines the phytochemicals present in the stem and root of *Cissus populnea,* with a primary objective of determining the actual content of each phytocompound found responsible for traditional management of ailments.

Methodology

Collection, preparation and extraction of plant material

Cissus populnea was collected from New Bussa, Niger State, Nigeria and was identified at the Forest Herbarium, Ibadan (FHI). A voucher specimen (FHI 109459) was prepared and deposited at the same Herbarium which is internationally recognized and listed by Holmgren & al. (1990). The stem and root were dried for about 15 days until devoid of moisture. The dried plant parts were ground into fine powder and transferred into airtight containers with proper labelling. They were then subjected to phytochemical screening which was carried out at the National Horticultural Research Institute (NIHORT), Ibadan, Nigeria. This screening was done to determine the biologically active compounds present in the plant parts. Procedures were adapted from earlier works for plant analysis as described below and reported by Sofowora (1993) and Trease & Evans (2005). A detailed method of extraction, as well as purification techniques for active plant constituents described by Harborne (1998) were also employed for the extraction of plant material.

Phytochemical analysis

Determination of alkaloids

A weighed amount (5 g) of each powdered sample of the plant parts was transferred into a 250 ml beaker. Two hundred ml of 20 % acetic acid was added and then covered to stand for 4 hrs. Filtration was done, and concentration of the extracted content to one quarter of original volume was applied using a water bath. Drop-wise addition of concentrated ammonium hydroxide to the extract followed until the precipitate was complete. The entire solution was allowed to settle and collection of the precipitate was done by filtration (Harborne 1998; Obadoni & Ochuko 2001) and then weighed.

Determination of flavonoids

To determine the flavonoid content in the stem and root of *C. populnea,* the aluminium chloride colorimetric method was employed. One ml of each plant extract was mixed with 3 ml of methanol, 0.2 ml of 10 % aluminium chloride, 0.2 ml of 1M potassium acetate, and 5.6 ml of distilled water. The entire mixture was allowed to stand at room temperature for 30 min, while the absorbance was measured at 420 nm. The total flavonoid content in each plant part was expressed in terms of standardized quercetin equivalent (mg/g of each extracted compound) (Aiyegoro & Okoh 2010).

Determination of tannins

Tanin content was determined using the method outlined by Van-Burden & Robinson (1981). Five hundred mg of the sample was weighed into a 50 ml plastic bottle and 50 ml of distilled water was added and then shaken thoroughly for 1 hr in a mechanical shaker. The solution was filtered into a 50 ml volumetric flask and made up to the mark. Five ml of the filtrate was pipetted out into a test tube and mixed with 2 ml of 0.1M $FeCl_3$ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min (Edeoga & al. 2005).

Determination of saponins

A spectrophotometric method described by Brunner (1984) was used for saponin analysis. One gram of the finely ground sample was weighed into a 250 ml beaker and 100 ml of Isobetyl alcohol was added. The mixture was shaken in a mechanical shaker for 5 hr to ensure uniform mixing. Subsequently, 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 was added. The obtained mixture with saturated $MgCO_3$ was again filtered to obtain a clear colourless solution. One 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 the mark with distilled water and then allowed to stand for 30 min for the development of red colour. Standard saponin solutions of 0–10 ppm were prepared from saponin stock solution and each standard solution was treated similarly with 2 ml of 5 % FeCl solution. The absorbances of the sample as well as the standard saponin solutions were read after colour development on a Spectronic 2lD Spectrophotometer, at a wavelength of 380 nm, and percentage of saponin was calculated.

Determination of anthraquinone

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

Determination of cardiac glycosides

Buljet's reagent (El-Olemy & al. 1994) was used to evaluate the cardiac glycoside content in the examined plant parts. For this purpose, 1 g of each powdered sample was soaked in 100 ml of 70 % alcohol for 2 hrs before filtration. Using lead acetate and Na_2HPO_4 solution, the obtained extracts were purified before the addition of freshly prepared Buljet's reagent. The difference between the intensity of colours of the experimental and blank samples (distilled water and Buljet's reagent) gave the absorbance, which is proportional to the concentration of glycosides.

Determination of cyanogenic glycosides

Five ml of fine powdered sample of each plant part were weighed into a 250 ml conical flask and incubated for 16 hrs at 38 °C before being extracted with 95 % methanol. The sample was filtered by double layer of hardened filter paper and then distilled with Marham distillation apparatus. The extracted sample was transferred into a low-necked 500 ml flask connected to a steam generator. It was then steam-distilled for 1 hr with sodium bicarbonate solution in a 50 ml conical flask. One ml of starch indicator was added to 20 ml of the distillate and of iodine-titrated solution, after which percentage hydrocyanide content was calculated.

Determination of phytosteroids

For this purpose, the crude extract of each plant material was mixed with 2 ml of chloroform and concentrated sulphuric acid (H_2SO_4) was added sidewise. The presence of steroids was noticed from the red colour produced in the lower layer of chloroform. To confirm further the presence of this phytochemical, another test was performed by mixing each crude extract of the plant materials with 2 ml of chloroform. Two ml of concentrated H_2SO_4 and of acetic acid were then poured into the mixture and the development of greenish coloration indicated the presence of steroids.

Determination of terpenoids

The crude extract of each plant part was dissolved in 2 ml of chloroform and evaporated to dryness. Two ml of concentrated H_2SO_4 was then added to it and heated for 2 minutes. A greyish colour indicated the presence of terpenoids.

All tests were carried out in triplicate for each plant part, and the results are presented as Mean±SD. However, the contents were estimated and expressed in mg/g.

Results and discussion

The results from this study showed that the stem and root of *Cissus populnea* are rich in plant compounds. The conducted phytochemical screening has shown that the two plant parts contain large amounts of alkaloids, flavonoids, tannins, and saponins (Tables 1 and 2). The alkaloid content in the stem of *C. populnea* seems to be very abundant (4.70 ± 0.03 mg/g or 49.8 %) as compared to other phytochemicals. This phytochemical was also found to be abundant in the root, although the flavonoid content was the highest there, with 4.13 ± 0.06 mg/g or 39.5 %. Trease & Evans (2005) maintained that plants containing alkaloids do not feature strongly in herbal medicine, yet the alkaloids have always been an important phytocompound used in allopathic systems. The abundance of flavonoids in the stem and root is also indicative of its potent antioxidant effect, which suggests that the plant may be very useful as an antibacterial, anti-inflammatory, antiallergic, antiviral, antithrombotic, antimultagic, and vasodilatory compound as reported by Alan & Miller (1996).

Table 1. Phytochemical constituents of the stem of *Cissus populnea*.

Phytocompounds	Mean and standard deviation (mg/g)	Mean volume (%)
Tannins	1.10 ± 0.04	11.6 %
Flavonoids	1.46 ± 0.08	15.4 %
Cardiac glycosides	0.15 ± 0.06	1.54 %
Alkaloids	4.70 ± 0.03	49.8 %
Saponins	1.21 ± 0.04	12.8 %
Anthraquinones	0.16 ± 0.03	1.69 %
Terpenoids	0.27 ± 0.02	2.81 %
Phytosteroids	0.15 ± 0.01	1.59 %
Cyanogenic glycosides	0.26 ± 0.04	2.75 %

Table 2. Phytochemical constituents of the root of *Cissus populnea*.

Phytocompounds	Mean and standard deviation (mg/g)	Mean volume (%)
Tannins	1.18 ± 0.01	**11.3 %**
Flavonoids	4.13 ± 0.06	**39.5 %**
Cardiac glycosides	0.27 ± 0.01	2.54 %
Alkaloids	2.79 ± 0.16	**26.7 %**
Saponins	1.10 ± 0.05	**10.5 %**
Anthraquinones	0.24 ± 0.04	2.25 %
Terpenoids	0.36 ± 0.03	3.45 %
Phytosteroids	0.13 ± 0.01	1.24 %
Cyanogenic glycosides	0.26 ± 0.01	2.51 %

Saponins, although non-toxic, can generate adverse physiological responses in animals that consume them. Akindahunsi & Salawu (2005) pointed out clearly that saponins have tumour-inhibiting effect in animals. Asl & Hossein (2008) also reported that there is evidence of saponins in traditional medicine preparations, where oral administration might be expected to lead to hydrolysis of glycoside from terpenoids. 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 treatment of ailments (Soladoye & Chukwuma 2012).

Similarly to flavonoids, tannin compounds have also some antibacterial (Akiyama & al. 2001), antiviral (Lu & al. 2004) and antiparasitic effect (Kolodziej & Kiderlen 2005). Their destruction or modification, in turn, plays an important role in the ripening of fruit (McGee 2004). The large amount of tannins reported in this work suggests that *Cissus populnea* can also be useful in the production of drugs for treatment of bacterial and viral infections. Its tannin content may also be useful in treating haemochromatosis, a hereditary disease characterised by excessive absorption of dietary iron, resulting in pathological increase of total iron content stored in the body. The presence of other phytochemicals also supports the fact that the plant is useful in many ways, as earlier reported by several authors. Although phytosteroids seem to be in small amount in the examined plant parts, they are important in drug production, due to their relationship with such compounds as sex hormones (Okwu 2001).

Further results illustrated in Fig. 1 have revealed that there is simultaneous increase and decrease in the amount of phytochemicals in the stem and root. It is also evident that the content of cardiac glycosides, anthraquinones and phytosteroids in both plant parts was almost equal. Similarly, there is a slight difference in the amount of terpenoids and cyanogenic glycosides in the samples. However, this study has shown that the root contains more flavonoids, tannins, anthraquinones, terpenoids, and cardiac glycosides but less alkaloids, saponins and phytosteroids than the stem. While cardiac glycosides have shown the least amount in the stem, phytosteroids have the least amount in the root. In our earlier work (Soladoye & Chukwuma 2012), the anthraquinone content of the leaves of *C. populnea* was very high (2.00±0.14 mg/g or 33.2 %). Alkaloids were also low in content (0.15±0.07 mg/g or 2.49 %), but that was not the case for the stem and root as evidenced from this study. While saponins were the phytochemicals in highest amount in the leaves (Soladoye & Chukwuma 2012), alkaloids and flavonoids were most abundant in the stem and root, respectively. The present study has, therefore, shown clearly that the content and kind of phytochemicals in different plants and plant parts may vary significantly.

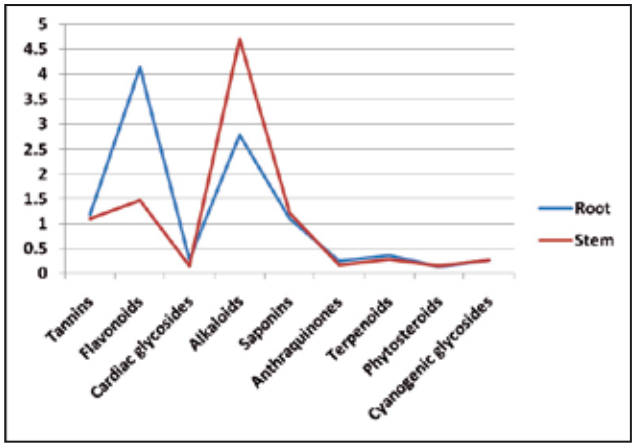

Fig. 1. Graphical presentation of actual phytochemicals in the stem and root of *Cissus populnea*.

Conclusion

The large amount of alkaloids, flavonoids, saponins, and tannis in the stem and root of *Cissus populnea* has shown that these plant parts are good sources of the above-mentioned compounds. The presence of these phytochemicals in *C. populnea* has undoubtedly contributed to its traditional medicinal value, and thus confirmed that it can serve as a potential source of useful drugs in the near future, if further pharmacological or pharmacognostic studies are conducted. Investigations are under way on the antimicrobial activities of this plant for treatment of ailments, as reported by earlier authors and traditional medical practitioners. While it is important to conduct such researches, we also suggest that conservation of medicinal plants should be taken seriously, so as to salvage the rich but threatened flora of Nigeria.

References

Aiyegoro, O.A. & Okoh, A.I. 2010. Preliminary phytochemical screening and in vitro antioxidant activities of aqueous extract of *Helichrysum longifolium* DC. –BMC Compl. and Alt. Med., **10**: 21.

Akindahunsi, A.A. & Salawu, S.O. 2005. Phytochemical screening and nutrient-antinutrient composition of selected tropical green leafy vegetables. – African J. Biotechnol., **4**: 497-501.

Akiyama, H., Fujii, K., Yamasaki, O., Oono, T. & Iwatsuki, K. 2001. Antibacterial astion of several tannins against *Staphylococcus aureus*. – J. Antimicrob. Chemother., **48**(4): 487-491.

Alan, L. & Miller, N.D. 1996. Antioxidant flavonoids: structure, function and clinical usage. – Alt. Med. Review, **1**: 103-111.

Asl, M.N. & Hossein, H. 2008. Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. – Phytotherapy Res., **22**(6): 709-724.

Bouquet, A. & Debray, M. 1974. Plantes médicinales de la Côte d'Ivoire. Travaux et Documents No **32**, ORSTOM, Paris, France.

Brotherton, J.G.H. 1969. The nomadic Fulani. – Nigerian Field, **34**:126-136.

Brunner, J.H. 1984. Direct spectrophotometric determination of saponin. – Anal. Chem., **34**: 1314-1326.

Burkill, H.M. 2000. The Useful Plants of West Tropical Africa, vol. 5. Families S-Z, pp. 296-297. Royal Botanic Gardens, Kew.

Edeoga, H.O., Okwu, D.E. & Mbaebie, B.O. 2005. Phytochemical constituent of some Nigerian medicinal plants. – African J. Biotechnol., **4**: 685-688.

El-Olemy, M.M., Al-Muhtadi, F.J. & Afifi, A.F.A. 1994. Experimental phytochemistry: A laboratory manual, pp 21-27. King Saud Univ. Press, Saudi Arabia.

Harborne, J.B. 1998. Phytochemical Methods. 3rd ed. Chapman & Hall Ltd, London.

Holmgren, P.K., Keuken, W. & Schofield, E.K. 1990. Index Harbariorum Part I. The Habaria of the World. 8th ed. Reg. Veg., New York.

Kolodziej, H. & Kiderlen, A.F. 2005. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitised RAW 264.7 cells. – Phytochemistry, **66**(17): 2056-2071.

Lu, L., Liu, S.W., Jiang, S.B. & Wu, S.G. 2004. Tannin inhibits HIV-1 entry by targetting gp41. – Acta Pharmacol. Sin., **25**(2): 213-218.

McGee, H. 2004. On Food and Cooking: the Science and Lore of the Kitchen. Scribner, New York.

Obadoni, B.O. & Ochuko, P.O. 2001. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. – Global J. Pure and Appl. Sci., **8b**: 203-208.

Okwu, D.E. 2001. Evaluation of the chemical composition of indigenous spices and flavouring agents. – Global J. Pure and Appl. Sci., **7**: 455-459.

Sofowora, A. 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd ed.. Spectrum Books Ltd., Ibadan, Nigeria.

Soladoye, M. O. & Chukwuma, E.C. 2012. Quantitative phytochemical profile of the leaves of *Cissus populnea* Guill. & Perr. (*Vitaceae*) – an important medicinal plant in Central Nigeria. – Arch. Appl. Sci. Res., **4**(1): 200-206.

Trease, G.E. & Evans, M.C. 2005. Pharmacognosy. 14th ed., pp 53, 431 and 512. Elsevier, New Delhi, India.

Van-Burden, T.P. and Robinson, W.C. 1981. Formation of complexes between protein and tannin acid. – J. Agric. Food Chem., **1**: 77.