



A COMPARATIVE ANALYSIS OF THE PHYTOCHEMICAL, PROXIMATE, AND NUTRITIONAL PROFILES OF EXTRACTS FROM VARIOUS COMPONENTS OF *CISSUS POPULNEA* STEM BARK.

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Abstract

Cissus populnea represents a species of climbing shrubs within the family *Vitaceae*, and is referred to as 'oro okoho' by the Igala tribe of Central Nigeria. This plant serves as a multifaceted medicinal resource, found extensively throughout tropical Africa. The extract derived from its stem bark has attracted considerable attention owing to its possible therapeutic benefits. This research offers an in-depth comparative examination of the phytochemical, proximate, and nutritive characteristics of *Cissus populnea* stem bark extracts with differing peel content, illuminating its prospective uses in the fields of Medicine and Nutrition. The extracts from the stem bark were meticulously prepared utilizing samples categorized as stem with peel, stem without peel, and peel alone. Systematic quantitative phytochemical screening and analysis were performed to discern and measure the bioactive compounds inherent in each extract. Furthermore, an analysis of proximate composition was conducted to evaluate the nutritional content present in each sample. The results demonstrated notable variations ($p \leq 0.05$) in the phytochemical composition, proximate composition, and vitamin contents of the extract.

Graphical Abstract



Keywords: Phytochemicals, Proximate, Nutrition, Antioxidant, Therapy

Introduction and literature review

Nigeria is richly endowed with numerous varieties of useful plants whose fruits, seeds, stems, roots, and leaves play vital roles in medicine and nutrition. Across the world, many cultures continue to rely heavily on traditional medicines, with about 38% of American adults reportedly using alternative remedies (Barnes *et al.*, 2008). The preference for alternative or complementary medicines often arises from the side effects associated with orthodox drugs or the ineffectiveness of allopathic treatments for certain ailments. Consequently, there is increasing global

interest in identifying and scientifically validating plants used in traditional medicine. Scientific authentication not only promotes the safe and effective use of these plants but also eliminates potentially harmful remedies. Encouragingly, many contemporary researchers are now dedicated to providing scientific explanations for the biological activities of traditionally used medicinal plants (Fernandes and Banu, 2012).

One such group of plants, found across continents and renowned for its medicinal applications, belongs to the genus *Cissus*. This genus, a member of the family Vitaceae, also includes the common grape (*Vitis vinifera*). The medicinal benefits of resveratrol—a bioactive compound present in grapes—are well-documented. Resveratrol exhibits anti-diabetic activity (Szkudelski and Szkudelska, 2011), neuroprotective effects against degenerative diseases (Sun *et al.*, 2010), anti-cancer properties (Athar *et al.*, 2009), cardiovascular benefits (Berthelli and Das, 2009), and contributes to longevity (Das *et al.*, 2010).

Botanical Description and Geographical Distribution

The genus *Cissus* comprises numerous species recognized for their diverse medicinal and nutritional properties. Among these, *Cissus populnea* (Guill. & Perr.), commonly known as “Asthma plant” or “Veld grape,” is a tropical species native to West Africa and widely distributed across regions with tropical and subtropical climates. It is traditionally employed in African folk medicine for treating a wide range of ailments due to its reported anti-inflammatory, antioxidant, and antimicrobial activities (Oktay *et al.*, 2003; Wangenstein *et al.*, 2004).

Cissus populnea is a woody climber with vine-like growth and distinctive leaves. With over 350 known varieties, the species thrives in tropical and subtropical zones, particularly across West Africa (Burkill, 2012; Geidam *et al.*, 2014). Its persistence and adaptability have enabled it to naturalize beyond its native range, tolerating diverse environmental conditions (WHO, 2014). All parts of *C. populnea* exude a mucilaginous substance traditionally utilized as a food raw material. The leaves also serve as both a thickener and a vegetable in soups.

In Nigeria, *C. populnea* is commonly found in both the northern and southern regions. The vernacular names include ‘Oro okoho’ (Igala – North Central Nigeria), ‘Ogbolo ajara’ (Yoruba – South West Nigeria), and ‘Dafaaraa’ (Hausa – Northern Nigeria) (Burkill, 2012). Its extensive use underscores its importance in local diets and traditional medicine. Ethnomedicinally, various parts of the plant—stems, leaves, and roots—are employed to manage respiratory disorders, digestive disturbances, wounds, and rheumatic conditions (Thomson and Kelly, 1990). These traditional practices highlight the plant’s ethnopharmacological relevance and justify scientific exploration of its chemical constituents and pharmacological potential.

The global distribution of medicinal plants is remarkable. Studies estimate that approximately 70,000 plant species, ranging from lichens to higher-order trees, possess therapeutic potential (Kuruppu *et al.*, 2019). According to the World Health Organization (WHO), about 21,000 medicinal plants are currently utilized in

various forms of traditional and modern medicine. Approximately 2,500 species are used by traditional herbal practitioners in rural areas to treat common ailments—a practice still regarded as one of the most effective approaches in Indian medicine (Panmei *et al.*, 2019). India, one of the twelve global megadiversity hotspots, ranks second in medicinal plant exports, boasting 16 agro-climatic zones and over 45,000 plant species, of which about 7,000 are medicinally valuable (Chakraborty, 2018; Prasathkumar *et al.*, 2021).

Nutritional and Phytochemical Significance

Beyond its ethnomedicinal uses, *Cissus populnea* exhibits considerable nutritional value (Onoja *et al.*, 2013; Inovova, 2000). Proximate analysis of its stem components—particularly the peel and inner stem—reveals appreciable levels of proteins, dietary fiber, minerals, and vitamins. Such findings emphasize its potential as a functional food source. Furthermore, the bioactive properties of *C. populnea* are of significant pharmacological interest. The antioxidant potential of its extracts is attributed to phenolic compounds and flavonoids (Szkudelski and Szkudelska, 2011), while its antimicrobial activities against common pathogens suggest potential applications in treating infectious diseases.

Building on the rich ethnobotanical heritage and promising pharmacological profile of *Cissus populnea*, this study aims to conduct a comprehensive evaluation of its phytochemical composition, nutritional properties, and bioactive potential across different stem components. By analyzing the whole stem extract, stem without peel, and peel alone, the research seeks to elucidate the chemical diversity and functional properties of *C. populnea*. This will contribute to scientific understanding and support its potential applications in nutrition, medicine, and functional food development.

Experimental Methods

Materials and Chemicals

Cissus populnea stem was bought from Afor market in Ankpa, Kogi State, Nigeria and was identified at the Herbarium of the Plant Science and Biotechnology Department of Kogi State Nigeria where a voucher specimen was prepared and deposited. The chemicals and reagents used in the study included gallic acid and Folin-Ciocalteu's reagent, which were obtained from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Acetic acid and ferric chloride (FeCl₃) were sourced from BDH Chemicals Ltd. (Poole, England). Additionally, sulfuric acid (H₂SO₄), sodium carbonate, aluminum chloride (AlCl₃), picric acid, ammonia, and sodium nitrite were also utilized.

Extraction of plant material

The whole stem prior to drying were divided into portions namely, samples with peel (A), without peel (B) and peel alone (C). *Cissus populnea* used as sample was characterized as with peel (WP: 67.58g), sample without peel (WOP: 29.50g) and peel alone (PO: 38.08g). About 600 ml of distilled water was added to each of the sample,

stirred and left for 24 h with occasional stirring for proper extraction. The stem was dried for about 15 days until devoid of moisture. The dried plant parts were cut into pieces, pounded and ground into fine powder which was transferred into airtight containers with proper labelling before they were screened. This screening was done to determine the biologically active compounds present in the plant parts. Procedures were adapted from earlier works as described below and reported by Sofowora, (1993) and Trease and Evans, (2005). The plant material was extracted using a thorough extraction procedure and purifying methods for active plant elements as outlined by Harborne (1998). The extract was concentrated using a rotary evaporator (IKA-Werke GmbH & Co, Germany) and then transferred into a vial of known mass and placed in a desiccator. After evaporation, the vials were weighed and the changes in mass were used to determine the mass of the extract. The crude extract was reconstituted with ethanol (0.1 g in 1litre) for experimentation

Qualitative Phytochemical screening

Every phytochemical screening was carried out using the procedures outlined by (Sofowora, 1993).

Quantitative phytochemical determination

Determination of total phenolic content

The phenolic content was determined using the Folin-Ciocalteu assay. To 20 µl of the reconstituted extract was added 80 µl of Folin-Ciocalteu reagent mix was added and the mixture was allowed to incubate for 5 minutes, then 80 µl 20% sodium carbonate solution was added. For the control, a similar mixture was constituted without the extract. After incubating all samples at room temperature for 1 hour, their absorbance was measured at 760 nm using a spectrophotometer (Agilent Technologies). Gallic acid was used as the standard established to calculate phenolic content (Bayli *et al.*, 2011).

Determination of total tannin content

The method of Rammika (2016) was employed with slight modification. To 0.1 ml of extract was added 0.5 ml Folin-Ciocalteu reagent followed by the addition of 2.5 ml 7.5% sodium carbonate solution and 0.9ml distilled water. The reaction mixture was vortexed and allowed to stand for 40 minutes and absorbance was recorded at 760 nm using a UV/visible spectrophotometer (Agilent Technologies). Blank was prepared with reagent instead of the sample. Total tannin content was determined from the calibration curve made with standard tannic acid.

Total flavonoid content estimation

To 1 ml of reconstituted extract was added 4 ml of water and allowed left to stand for 5 minutes. Then 0.3 ml of 5% Sodium nitrite and 0.3 ml of 10% Aluminum Chloride were added to the mixture and later incubated for 6 minutes at room temperature. After heating, 2 ml of Sodium Hydroxide was added to the incubated mixture and the volume was made to increase to 10 ml with distilled water. The absorbance was measured at 510 nm. Quercetin was used as a standard against a blank (0.01g of Quercetin in 100 ml of H₂O) (Vijay and Rajendra, 2014).

Estimation of alkaloids

To 20 ml of 10% acetic acid in ethanol was added 1 ml of the reconstituted extract. The mixture was covered and allowed to stand for 4 hours and then filtered. The filtrate was concentrated in a water bath to reduce a quarter of its original volume. Concentrated Ammonium hydroxide was added to the filtrate and the precipitate was collected through weighed filter paper. This was followed by rinsing the precipitate with 1% Ammonium hydroxide and then filtered. The alkaloid precipitate was dried in an oven at 60°C for 30 minutes and the filter paper was reweighed (Harborne, 1998).

Estimation of Saponins

The method of (Obadoni and Ochuko, 2001) was adopted. One ml of the extract was treated with 25 ml of 20% ethanol and this mixture was heated at 55 °C for 2 hours in a water bath with continuous stirring. The extracted residue was added to 50 ml of 20 % ethanol and reduced to 40 ml over a water bath at about 90 °C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. 60 ml of n-butanol was added. The combined n- butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight.

In-vitro antioxidant activity

Reducing power method

The method described by Oyiazu (1986) was used with some modifications. An aliquot of 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of Potassium ferricyanide (1% w/v) was added to 1.0 ml of extract (at four different concentrations 25, 50, 75 and 100 µg/ml) in methanol. The resulting mixture is incubated at 50 °C for 20 min, followed by the addition of 2.5 mL of Trichloroacetic acid (10% w/v). The mixture is centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 ml), mixed with distilled water (2.5 ml) and 0.5 ml of Ferric chloride (0.1%, w/v). 1 ml of methanol was used as a blank. The absorbance is then measured at 700 nm against blank sample. An increase in absorbance of the reaction mixture indicates the reducing power of the samples.

DPPH radical scavenging assay

The method of Omale *et al.*, (2016) was adapted for the determination of the scavenging activity of the extract on 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. To 2 ml of the extract at four different concentrations (25, 50, 75 and 100 µg/ml) by diluting 10 fold in 80% methanol was added 2 ml of 0.1 mM solution of DPPH in methanol. The mixtures were allowed to stand at room temperature in the dark for 30 minutes. Thereafter, absorbance was taken at 517 nm. Ascorbic acid was taken as the standard while methanol was used as the blank (control). The percentage of DPPH scavenging capacity was calculated at each concentration according to the formula:

$$\% \text{ DPPH scavenging activity} = \frac{A_C - A_S}{A_C} \times \frac{100}{1} \quad (1)$$

where A_c and A_s are the absorbances of the control and of the sample respectively.

Lipid profile of wistar rats fed methanol extract of *C. populnea*.

The serum level of total cholesterol TC, high density lipoprotein HDL, low density lipoprotein LDL and triglycerides TG were determined using analytical kits supplied by Randox Laboratory Ltd, UK based on enzymatic hydrolysis or oxidation (Islam *et al.*, 2018). Once the protocol in the kit were established, the following formulae were employed for calculation of the various parameters.

$$\text{Total Cholesterol (TC)} = \frac{OD_S \text{ of sample}}{OD_S \text{ of standard}} \times 200 \text{ (mg/dL)} \quad (2)$$

$$\text{HDL Cholesterol} = \frac{OD_S \text{ of sample}}{OD_S \text{ of standard}} \times \text{conc of standard} \text{ (mg/dL)} \quad (3)$$

$$\text{LDL cholesterol} = \text{Total cholesterol} - \left(\text{HDL cholesterol} + \frac{\text{Triglyceride}}{5} \right) \text{ (mg/dL)} \quad (4)$$

$$\text{Triglyceride (TG)} = \frac{OD_S \text{ of sample}}{OD_S \text{ of standard}} \times \text{conc of standard} \text{ (mg/dL)} \quad (5)$$

(OD_S is the optical density)

Statistical analysis

Data analysis was performed using the Excel 2010 spreadsheet. Results are presented as Mean \pm SEM of triplicate determinations. Statistical analysis among treatments were carried out at the significant level of $p \leq 0.05$.

Results and Discussions

The results of the phytochemical screening of *C. populnea* revealed the presence of tannins, phenols, alkaloids, saponins, flavonoids, and cardiac glycoside in the extract in varying degrees as shown in Table 1.

Table 1: Qualitative phytochemical constituents of methanolic extract of *Cissus populnea*

Phytochemicals	Bioavailability
Alkaloids	+++
Flavonoids	++
Saponins	+++
Tannins	++
Cardiac glycosides	+
Phenolics	++

The relative abundance is reflected in the number of + sign

Fig 1 depicts the results of the quantitative phytochemical composition of the methanol extract. From the Table, the whole stem (WP) revealed the highest alkaloid content (6.00 ± 0.01 mg/g) followed by the stem without peel, WOP (4.01 ± 0.11 mg/g). Peel (PO) at (3.00 ± 1.41 mg/g) had the lowest level of alkaloids. All the three fractions displayed statistically ($p \leq 0.05$) the same level of flavonoids (7.60 ± 0.10 , 7.95 ± 0.08 , 7.56 ± 0.00 mg/g) and the levels here when pooled together will give credence to the ubiquitous utilization of the plant. In the case of tannin, saponin, phenol and glycosides, the same pattern as in alkaloid was noticed. Flavonoid is the second most abundant phytochemicals determined, even higher than that found by Achikanu and Ani (2020). Overall, the presence of total phenol and cardiac glycosides supports the usefulness of *C. populnea*, especially as antioxidant.

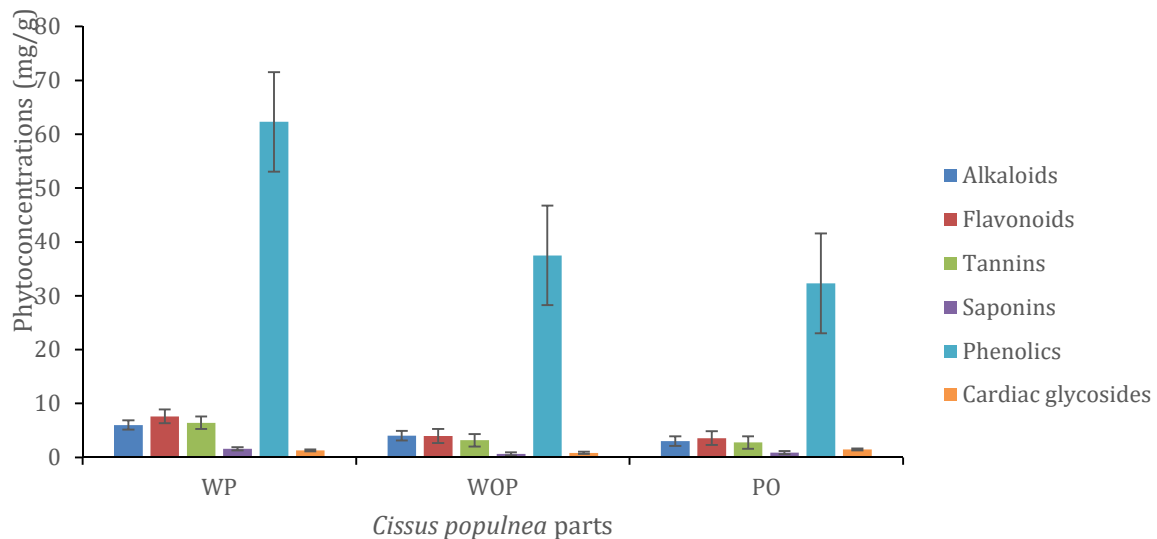


Fig 1: Quantitative phytochemical composition of methanol extract of *Cissus populnea* parts.

The results of the proximate composition of the fractions are contained in Fig 2. It is noticed that the moisture content is very high especially in the peel (PO) ($19.9 \pm 0.14\text{mg/g}$) alone where it is almost hitting the critical equilibrium moisture content value. Moisture content is a measure of the food materials to microbial contamination. The ash content for all the fractions were within the same value showing that the carbon content might be evenly distributed within the plant. However, the ash content ranged from 8.75 to 9.75 % which is lower than the findings of Williams *et al.*, (2019) which was 13.4 %. On the whole, crude fibre and carbohydrate level are high in all the fractions (24.07 ± 0.01 , 18.5 ± 0.71 , 20.3 ± 0.15 % and 31.4 ± 0.55 , 39.8 ± 0.69 , 30.4 ± 0.96 %) respectively. The fat content ranged from 7.07 – 8.20 %. High fat content in *C. populnea* is not desirable for risk of cardiovascular disorder and obesity. Protein content ranged from 5.42 to 8.36 %. This is less than the RDA for protein which is 50 g for adults weighing 70 kg and 46 g for those weighing 50 kg and for children, 18 – 20 g/day (Mann and Otori, 2014). The low protein indicate that *C. populnea* stem bark is a poor source of protein (Achikanu and Ani, 2020).

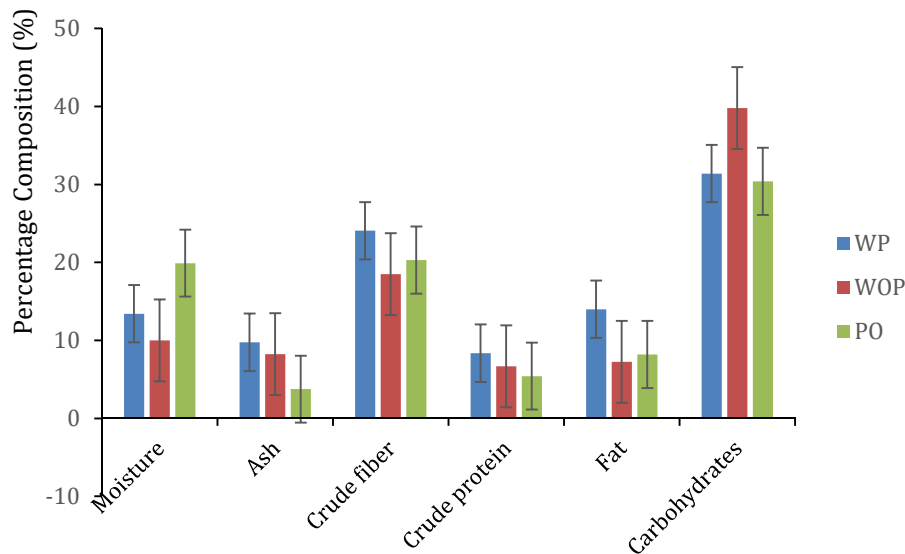


Fig 2: Proximate composition of the methanol extract of *Cissus populnea* stem bark.

The reducing power of the various fractions of *C. populnea* studied at four different concentrations 25, 50, 75 and 100 µg/ml are presented in Table 2. The highest reducing power was observed at an optical density of 0.182 corresponding to 82.00%. All the fractions however had much lower reducing power than the standard ascorbic acid which had values between 0.117 ± 0.01 absorbance unit at 25 µg/ml and 0.150 ± 0.04 absorbance unit at 100 µg/ml.

Table 2: Reducing power antioxidant of methanol extract of *Cissus populnea* and standard.

Con (µg/mL)	Methanol extract		Standard (Ascorbic acid)	
	OD (700nm)	Reducing power (%)	OD (700nm)	Reducing power (%)
25	0.057 ± 0.11	12.31	0.117 ± 0.01	17.00
50	0.068 ± 0.01	40.00	0.127 ± 0.10	21.00
75	0.112 ± 0.10	43.00	0.138 ± 0.00	38.00
100	0.182 ± 0.00	82.00	0.150 ± 0.04	72.00

Table 3 shows percentage DPPH inhibition of the extracts of *C. populnea* at various concentrations. It is observed that as concentration increases, the scavenging activity of both extract and standard ascorbic acid increases and the highest activity were recorded at 100 µg/mL. However, the percentage DPPH inhibition was higher in the extract as was observed than with the standard ascorbic acid (81.48 ± 0.50 and 71.70 ± 0.17 %) at 100 µg/ml respectively. Expectedly, the extract has a higher IC₅₀ (1.2723) than the standard ascorbic acid (1.0162).

Table 3: DPPH radical scavenging activity of methanol extract of *Cissus populnea* and standard.

Con (µg/mL)	Methanol extract			Standard (Ascorbic acid)		
	OD (577nm)	% activity of extract	IC ₅₀	OD (577nm)	% activity of AA	IC ₅₀
25	0.070	53.70	1.2723	0.009	55.16	1.0162
50	0.060	72.22		0.020	61.39	
75	0.055	77.77		0.035	68.50	
100	0.050	81.48		0.042	71.79	

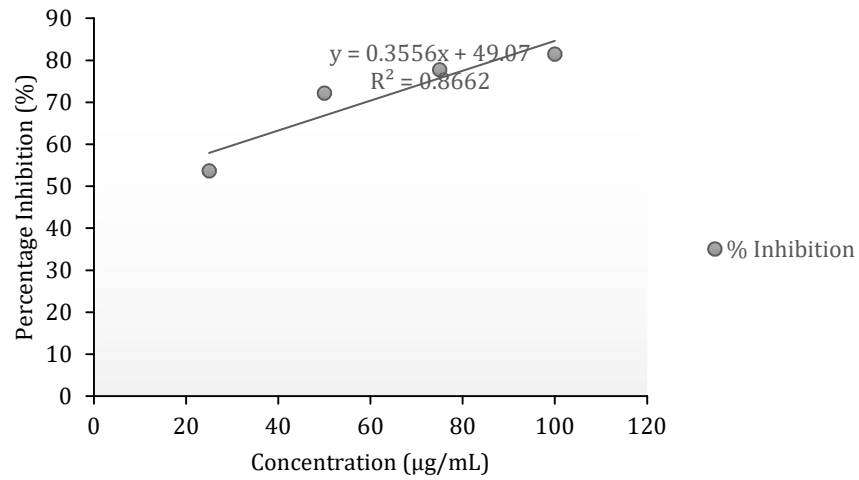


Fig 3: DPPH Inhibitory Activity of Methanol Extract of *Cissus populnea*.

The regression coefficient R^2 , of the extract (Fig 3) was found to be 0.8662 whereas that of the standard ascorbic acid (Fig 4) was found to be 0.9803 showing that both have higher radical scavenging activities even though AA exhibited a superior regression.

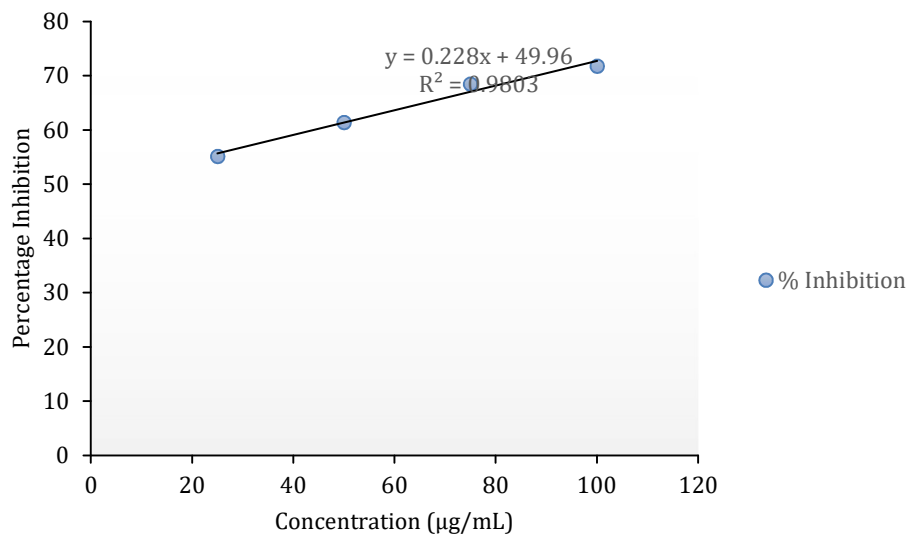


Fig 4: Ascorbic Acid Inhibitory Activity of Methanol Extract of *Cissus populnea*.

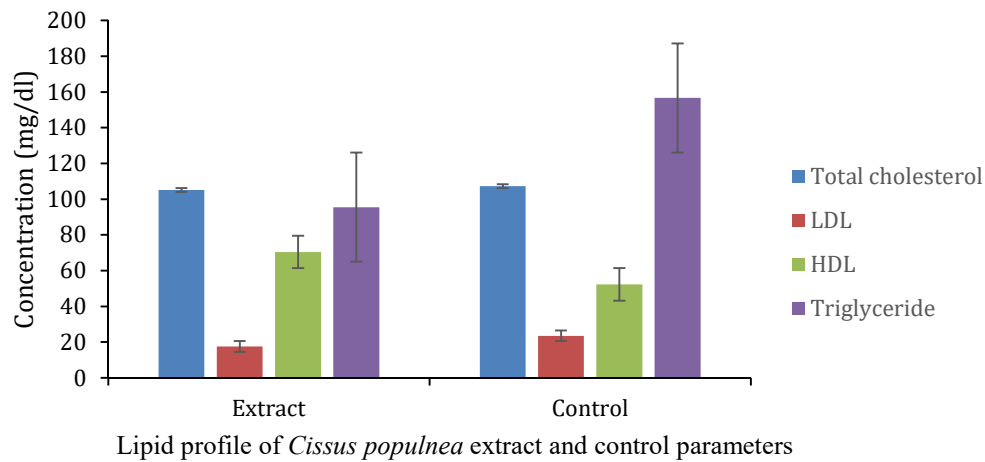


Fig 5: Lipid profile of methanol extract *Cissus populnea* and control

After administering the extracts, as represented in Figure 5, it is apparent that *Cissus populnea* extract maintained a stable total cholesterol (TC) level while showing a noteworthy 25.75% increase in high-density lipoprotein (HDL) cholesterol as compared to the control. This positive shift in HDL cholesterol can be attributed to a 39.03% reduction in triglycerides (TG) and a remarkable 25.63% decrease in low-density lipoprotein (LDL) cholesterol. These results are indicative of the health-improving effects of *C. populnea* extract when administered for a 14-day duration. In nutritional terms, this signifies an enhancement in lipid profiles, which are associated with reduced cardiovascular risk factors. The observed reduction in TG concentrations of the extract is an indication of increased rate of lipolysis favoring the production of HDL cholesterol. This has led to a lowering of serum TG and LDL cholesterol concentrations, suggesting a favorable lipid profile. These finding is consistent with the study conducted by Islam *et al.*, (2018) and underscore the potential cardiovascular benefits associated with the consumption of *C. populnea* extracts.

The stem with peel combines the effects of both the outer bark and the internal tissue, giving it a broader range of bioactive compounds. The study show that this part contains high levels of saponins, tannins, alkaloids, flavonoids, and terpenoids. These compounds are particularly effective for antimicrobial activity due to the synergistic effect of the peel and stem, this part has shown strong inhibitory action against pathogens, making it useful for treating skin infections and digestive issues caused by bacteria and fungi. It also has effective anti-inflammatory Properties due to the combination of tannins and flavonoids which makes the stem with peel suitable for managing inflammatory conditions, which could be beneficial for wound healing and gastrointestinal inflammation (Alex-Asaolu *et al.*, 2024).

Removing the outer peel exposes the inner stem, which has a different composition. This part is particularly high in polyphenols and lignans, making it beneficial for antioxidant effects, metabolic health and anti-diabetic properties. The inner stem's rich polyphenol content provides potent antioxidant properties, which are essential for cardiovascular health and may help in reducing oxidative stress. Also, due to the presence of phytosterols, which are known to lower cholesterol, the inner stem may be particularly useful in managing metabolic disorders,

including cardiovascular disease. Anti-diabetic Properties' studies have been explored revealing the stem's hypoglycemic effect, making it a candidate for further research in managing blood sugar levels (Adewole *et al.*, 2022).

The peel alone, which is essentially the outer bark, is often concentrated with certain compounds that protect the plant from environmental stressors. It contains high levels of terpenoids, tannins, and phenolic compounds, and is particularly effective for antimicrobial and astringent Properties. The peel is highly effective against pathogens and can act as a natural antimicrobial agent. This makes it suitable for topical applications for skin infections and as a natural wound dressing. Its high tannin content gives it strong anti-inflammatory as well as astringent properties, beneficial for wound healing and skin tightening, useful in managing minor cuts and skin irritations. Terpenoids and tannins in the peel are known for their pain-relieving qualities and are sometimes used in traditional medicine for treating localized pain (Edoho *et al.*, 2023). Some of these plants have been ignored because people are unaware of their crucial health promoting benefits (Anwar *et al.*, 2022). Unfortunately, no well-defined studies are currently being conducted to support the consumption of historically known wild edible plants as sources of nutrition and therapeutics (Bello *et al.*, 20019, al-Rbai, 2024).

Conclusions

The results of the present study showed that the stem bark of *Cissus populnea* contained phytochemicals with biological functions as well as proximate contents with nutritional properties which could be utilized for therapeutic purposes and food supplementation for man. This study therefore, serves as scientific proof and validation for the plants' numerous usage in traditional pharmacopeia.

Conflict of Interest

The authors declare no conflict of interest whatsoever

Author's contributions

Authors OJA and AB: Conceptualization and experimentation, OA, ESA, SMS and NPM: Experimentation, resources and validation, EKO, SFA, AF and OSC: writing and proof reading. All authors read and approved the manuscript for publication.

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Supplementary Material

Not applicable

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By checking the following box, the senior author acknowledges that they and the other authors have read, understood, and complied with the Instructions to Authors (ItA).

