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Evaluation of phytochemicals and *in vitro* antioxidant activity of *Cissus populnea* (Stem)

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Abstract

The medicinal properties of plants are often linked to the presence of phytochemicals and their antioxidant potentials. In this research, aqueous extracts of *Cissus populnea* stem were qualitatively analyzed for various phytoconstituents and evaluated for *in vitro* antioxidant activity using three different assessment methods. The screening revealed the presence of all tested phytochemicals, except glycosides and tannins, which were not detected. Across all three antioxidant assays, the antioxidant activity of the extracts increased proportionally with concentration. Using the nitric oxide, hydrogen peroxide, and ferric ion inhibition assays, the extracts at concentrations ranging from 100 µg/ml to 1000 µg/ml demonstrated up to 50% of the antioxidant activity exhibited by standard ascorbic acid at equivalent concentrations. These findings suggest that the stem of *C. populnea* may serve as a valuable source of bioactive compounds.

Keywords: Antioxidant activity, bioactive compounds, *Cissus populnea*, phytochemicals

1. Introduction

The use of plant parts for medicinal purposes is as old as humanity itself. Nearly every part of a plant, from the roots buried underground to the aerial portions above ground, has found therapeutic application, depending on the species. According to the USDA (2022) [1], approximately 40% of conventional medicines in the Western world are derived from plants that have been utilized by humans for centuries. This medicinal value is largely attributed to the presence of phytochemical constituents (Naseem, 2014) [2] and the antioxidant capabilities of these plants (Krishnaiah *et al.*, 2011) [3]. The occurrence of these phytochemicals is not accidental but is thought to serve as a natural defense mechanism against predators, parasites, and harsh environmental conditions (Molyneux *et al.*, 2007) [4]. *Cissus populnea* is a climbing plant (liana) belonging to the Vitaceae family (also known as Ampelidaceae), with pale woody stems reaching up to 7.5 cm in diameter and extending as long as 8 meters (Soladoye and Chukwuma, 2012) [5]. The plant exudes a clear, watery sap when cut and typically withers during the dry season. The young bark is creamy and smooth but becomes gray, scaly, and fibrous with age. Its flowers are cream-colored, and the fruits ripen to a blackish-purple hue. *C. populnea* is commonly found across the savanna regions of Africa, with distribution from Senegal to Mozambique (Ouoba *et al.*, 2018) [6].

This plant is highly versatile. In Nigeria, the stem is traditionally used by the Idoma people of Benue State as a soup thickener (Falayi, 2021) [7]. Its applications extend further: the leaves serve as fodder for fish and insects, the root is used for treating skin infections, boils, and wounds due to its antimicrobial properties (Kone *et al.*, 2004) [16], and it has demonstrated anti-sickling activity in sickle cell patients (Moody *et al.*, 2003) [9]. In Southwestern Nigeria, the aqueous extract of the stem is employed to enhance libido and male fertility. Supporting this use, Ojekale *et al.* (2015) [10] confirmed the spermatogenic properties of the stem bark extract in Wistar rats. The medicinal potential of *C. populnea*

stems from its rich phytochemical profile, which includes alkaloids, flavonoids, tannins, saponins, and phenols (Merecz-Sadowska *et al.*, 2020) ^[11]. Several studies have attempted to profile these phytochemicals in various parts of the plant (Adebowale *et al.*, 2013; Soladoye and Chukwuma, 2012) ^[12]. Of particular interest is the antioxidant potential of *C. populnea*, as antioxidants play a crucial role in combating degenerative diseases caused by oxidative stress.

Despite its traditional uses, there remains a significant gap in scientific knowledge regarding the specific phytochemical composition and antioxidant potential of the *C. populnea* stem. Given the increasing demand for natural antioxidants and phytotherapeutics, a comprehensive analysis of the *in vitro* antioxidant capacity and phytochemical profile of the *C. populnea* stem is essential to explore its potential as a source of bioactive compounds for pharmaceutical and therapeutic applications.

While *C. populnea* has been historically used to manage oxidative stress-related conditions, its stem has not been extensively studied. Identifying the bioactive compounds within the stem could not only contribute to the development of novel antioxidant therapies but also support the use of *C. populnea* in the food and cosmetic industries as a natural preservative or additive.

2. Materials and Methods

2.1 Apparatus

Spatula, Whatman filter paper, water bath, oven, sample bottles, beakers, test tubes, sieve, funnel, measuring cylinder, hand grinder, detergent, aluminum foil, Petri dishes, hand gloves, swab sticks, and autoclave.

2.2 Reagents

H₂SO₄, Ethanol, Acetic Acid, FeCl₃, HCl, Magnesium Ribbon, Distilled water, Chloroform, KOH, Wagner's reagents, Meyer's reagents, lead acetate, acetic acid anhydride, acetic acid glacial

2.3 Sample collection

Stem part of *Cissus populnea* were collected from farmland in Ugbokpo village, Apa local government, Benue State, Nigeria, on 11th may 2024 in the morning. They were authenticated at herbarium, Department of Biological Science Sa'adu Zungur University Bauchi with Batch number: BASUG/00156, And it was taken to Abubakar Tafawa Balewa University, Bauchi, Nigeria for the experiment.

2.4 Sample preparation

The stem was cut into smaller pieces without prior washing and spread out in my room, where it was air-dried at room temperature for three weeks until completely dried. The dried pieces were first crushed into a coarse powder using a local mortar, and then ground into a fine powder using a laboratory blender.

2.5 Extraction of sample

"The pulverized sample will be collected, and 100 g of it will be weighed and added to 600 mL each of ethanol and distilled water. Each mixture will be subjected to intermittent stirring and left to stand for 48 hours before filtration. Filtration will be carried out using Whatman filter paper. The residue will be discarded, while the filtrate will

be air-dried to allow for evaporation, yielding the concentrated extract. The resulting concentrate will be stored in a clean bottle and kept in a refrigerator until use

2.6 Qualitative phytochemical screening

2.6.1 Test for alkaloids

Wagner's test (iodine-potassium iodide reagent): Approximately 1 mL of the extract will be treated with a few drops of Wagner's reagent. The formation of a reddish-brown precipitate indicates the presence of alkaloids (Vishnu *et al.*, 2019) ^[13].

2.6.2 Test for glycosides

To 2ml of extract is mixed with about 0.4 ml of glacial acetic acid containing traces of ferric chloride and 0.5 of conc. H₂SO₄ will be added the production of blue colour is positive for glycosides (Vishnu *et al.*, 2019) ^[13]

2.6.3 Test for cardiac glycosides (Keller-Killani test)

To 1.5 mL of the solvent extract, 2 mL of glacial acetic acid will be added, followed by a drop of ferric chloride solution. Subsequently, 1 mL of concentrated H₂SO₄ will be carefully added. The formation of a brown ring at the interface indicates the presence of deoxy sugars of cardenolides. Additionally, a violet ring may appear beneath the brown ring, and a green ring may gradually develop in the acetic acid layer (Vishnu *et al.*, 2019) ^[13].

2.6.4 Test for phenol

Lead acetate test: To 5 ml of extract 3ml of 10%lead acetate solution will be added and mixed gently. The production of bulky white precipitate is positive for phenols (Vishnu *et al.*, 2019) ^[13]

2.6.5 Test for tannins

To 5 ml of extract few drops of neutral 5% ferric chloride solution will be added, the production of dark green colour indicates the presence of tannins (Vishnu *et al.*, 2019) ^[13]

2.6.6 Test for flavonoids

To the aqueous solution of the extracts 10% ammonia solution is added and is heated. The production of fluorescence yellow is positive for flavonoids (Vishnu *et al.*, 2019) ^[13]

2.6.7 Test for phytosterols

The extract is dissolved in 2 ml of acetic anhydrite and to which 1 or 2 drops of concentrated H₂SO₄ is added along the sides an array of colour change indicates the presence of phytosterols (Vishnu *et al.*, 2019) ^[13]

2.6.8 Test for saponins

To 0.5 mg of extract will be vigorously shaken with few ml of distilled water. The formation of frothing is positive for saponins (Vishnu *et al.*, 2019) ^[13]

2.6.9 Test for steroids

To 2 ml of extract with 2 ml of chloroform and 2 ml of concentrated H₂SO₄ are added, the appearance of red colour and yellowish green fluorescence indicates the presence of steroids (Vishnu *et al.*, 2019) ^[13]

2.6.10 Test for terpenoids (Salkowski test)

To 3 ml of the extract was taken and 1 ml of chloroform and

1.5 ml of concentrated H₂SO₄ are added along the sides of the tube. The reddish brown colour in the interface is considered positive for the presence of terpenoids (Vishnu *et al.*, 2019) ^[13]

2.7 Determination of invitro antioxidant Activity

2.7.1 Ferric Reducing Antioxidant Power

The antioxidant capacity of the extracts was assessed based on their ability to convert iron (III) to iron (II), using the procedure described by Oyaizu (1986). A 0.5 mL portion of the plant extract at concentrations of 125, 250, 500, 1000, and 2000 µg/mL was combined with 1 mL of phosphate buffer (0.2 M, pH 6.6) and 1 mL of a 1% aqueous solution of potassium ferricyanide (K₃Fe(CN)₆). The mixture was thoroughly shaken and incubated at 50 °C for 30 minutes. Following incubation, 1 mL of a 10% trichloroacetic acid (TCA) solution was added to terminate the reaction, and the mixture was centrifuged at 3000 rpm for 10 minutes. Subsequently, 1.5 mL of the supernatant was mixed with 1.5 mL of distilled water and 0.1 mL of a 0.1% FeCl

2.7.2 Hydrogen peroxide scavenging activity

This uses the method as reported by Halliwell *et al.*, (1987) ^[14] with minor modification from (Awah *et al.*, 2010) ^[15].

Principle: The 2-deoxyribose assay was employed to assess the hydroxyl radical (OH•) scavenging activity of the extract, with each reaction mixture containing specific final concentrations of the reagents.

Procedure: The hydrogen peroxide scavenging activity was determined based on the method of Halliwell *et al.* (1987), with slight modifications as described by Awah *et al.* (2010). In summary, 0.1 mL of the extract was mixed with 1 mL of a reagent solution consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The mixture was incubated at 95 °C for 90 minutes. After cooling to room temperature, 200 µL of the reaction mixture was transferred to a transparent cuvette, and absorbance was measured at 695 nm. Ascorbic acid was used as the standard reference compound.

$$\% \text{H}_2\text{O}_2 = (\text{Ab}-\text{As}/\text{Ab}) * 100$$

2.7.3 Nitric oxide (NO) radical scavenging assay

Nitric oxide (NO) was quantified following the method described by Green *et al.* (1982).

Principle: NO generated from sodium nitroprusside (SNP) reacts with oxygen to form nitrite ions, which were subsequently measured.

Procedure: The nitric oxide scavenging activity was evaluated using the procedure of Green *et al.* (1982). NO was produced from SNP and detected as nitrite through the Griess reaction. The reaction mixture consisted of 2 mL of 5 mM SNP (prepared in 0.1 M phosphate buffer, pH 7.4) and 0.5 mL of the plant extract or ascorbic acid (used as a standard). The mixture was incubated at 37 °C for 2 hours. Afterwards, 0.1 mL of the reaction solution was transferred into a 96-well microplate, followed by the addition of 0.1 mL of Griess reagent (comprising 1% sulfanilamide and 0.1% naphthylethylenediamine dihydrochloride in 5% phosphoric acid). The mixture was kept in the dark at room temperature for 10 minutes, after which the absorbance was recorded at 530 nm. The nitric oxide scavenging capacity

was calculated as a percentage relative to the absorbance of the blank at 530 nm.

2.8 Data analysis

All data were analyzed statistically. Results were expressed as Mean±Standard Deviation, and one-way ANOVA was employed to assess differences among the groups. Statistical significance was determined at a p-value less than 0.05, corresponding to a 95% confidence level ($p < 0.05$). Tukey's HSD post-hoc test was applied for pairwise comparisons among group means.

3. Results

3.1.1 Qualitative phytochemical analysis

Table 2 shows the result of the qualitative phytochemical analysis of the stem of *Cissus populnea*. The stem contained moderate concentrations of alkaloids, phenols and phytosteroids of the phytochemicals present. Cardiac glycosides, Flavonoids, Saponins, Steroids, and Terpenoids were slightly concentrations present. Glycosides and tannins were not detected.

Table 2: Preliminary qualitative photochemical analysis of extracts of *C. populnea* stem

Extract/Phytochemicals	Presence
Alkaloids	++
Glycosides	-
Cardiac glycosides	+
Phenols	++
Tannins	-
Flavonoids	+
Phytosteroids	++
Saponins	+
Steroids	+
Terpenoids	+

+ = Slightly present

+++ = Heavily present

++ = Moderably present

ND = Not detected

3.1.2 In-vitro antioxidants activities

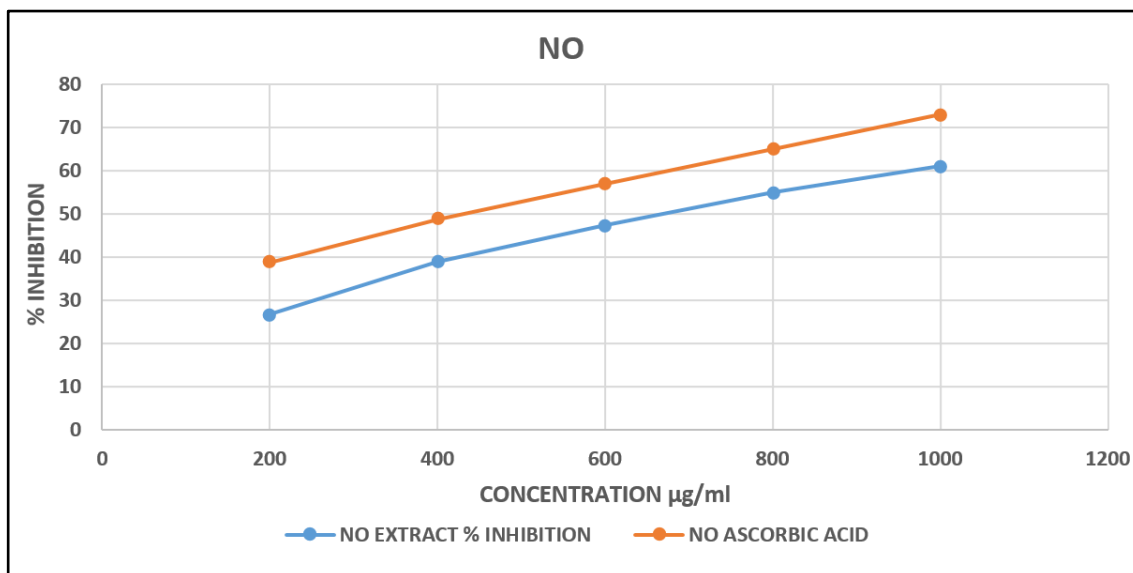


Fig 1: Shown the IC_{50} of L. ascorbic acid and *Cissus populnae* stem extract against Nitric oxide (NO) Scavenging activity.

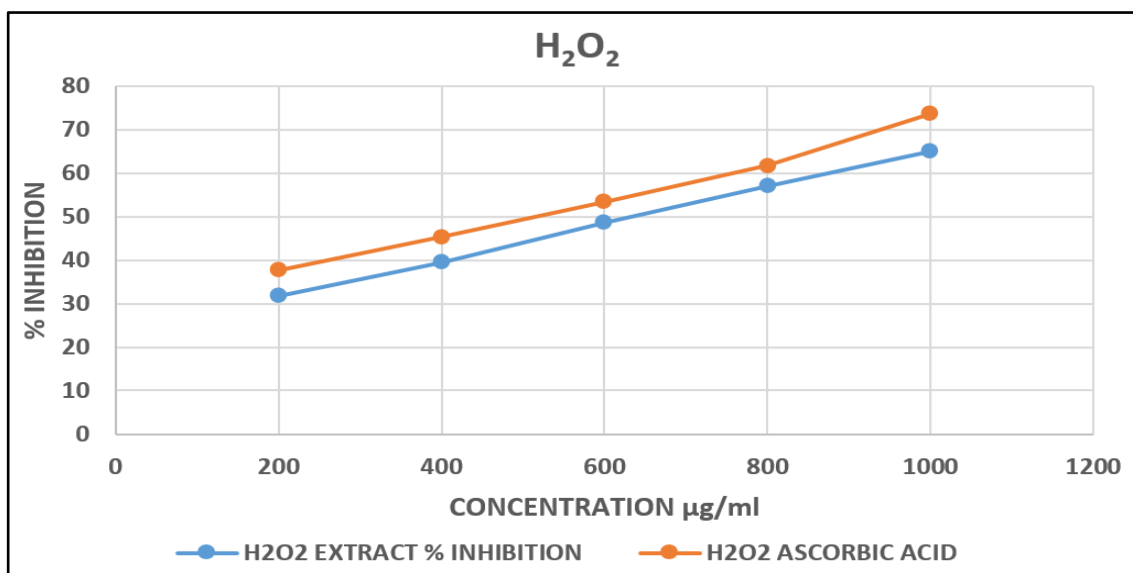


Fig 2: Shown the IC_{50} of L. ascorbic acid and *Cissus populnae* stem extract against hydrogen peroxide (H₂O₂) Scavenging activity.

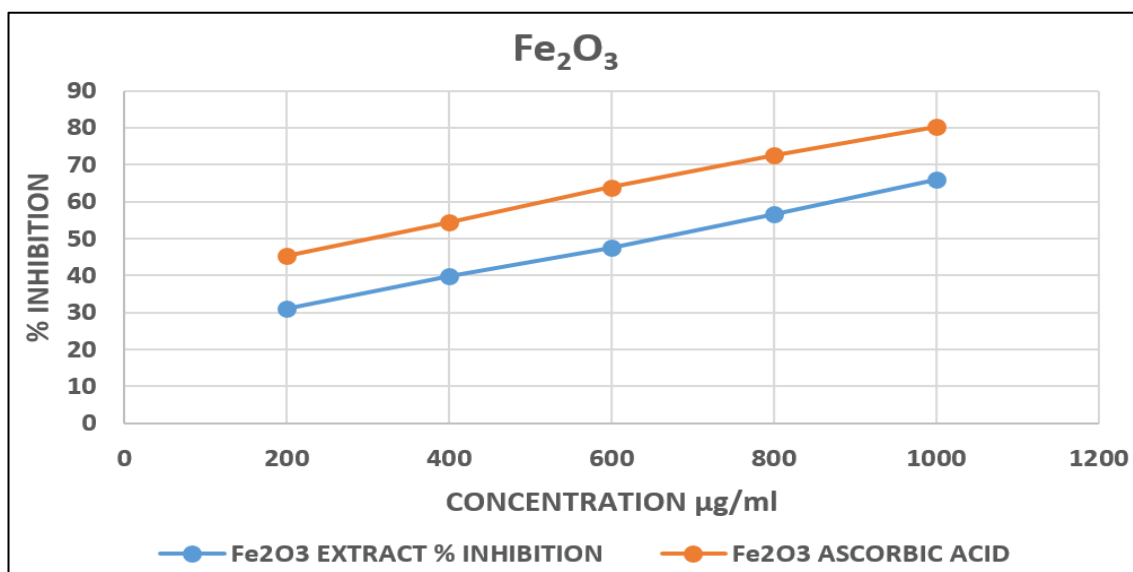


Fig 3: Shown the IC_{50} of L. ascorbic acid and *Cissus populnae* stem extract against ferric oxide Scavenging activity.

3.2 Discussion

The results of the analyses carried out preliminary phytochemical screening of *C. populnea* revealed the presence of alkaloids, phenols and phytosteroids in moderate, and Cardiac glycosides, Flavonoids, Saponins, Steroids, and Terpenoids were slightly present, while Glycosides and tannins were not detected. This work is similar to findings of (Uduak Aletan, *et al.* 2022) work on Phytochemical analysis and invitro antioxidant activity studies of crude extracts of *Cissus populnea* stem, using the same extraction were shown that the *C. populnea* contain tannins, phenols, alkaloids, saponins, flavonoids, cardiac glycoside and reducing sugars slightly while Anthraquinones, Steroids and Terpenoids where not detected. According to the findings obtained from the present study.

Cissus populnea stem revealed significant antioxidant properties such as hydrogen peroxide resistance ability and Nitric Oxide radicals scavenging activity, and ferrous ions chelating activity. Hence, the extracts studied showed appreciate antioxidant ability, but it is not potent compared to the standard ascorbic acid (Jaouadi *et al.*, 2022) [8]. The inhibitory concentration at fifty (IC₅₀) show their ability of scavenging, and the higher the IC₅₀ the lower the scavenging ability. With this the IC₅₀ of *Cissus populnea* stem extract against nitric oxide (NO) is (66) while ascorbic acid is (42), the IC₅₀ of *Cissus populnea* stem extract against hydrogen peroxide (H₂O₂) is (62) while ascorbic acid is (50), and the IC₅₀ of *Cissus populnea* stem extract against ferric oxide (Fe₂O₃) is (63) while ascorbic acid is (30), which is showing that ascorbic acid has scavenging ability than *Cissus populnea* stem extract.

4. Conclusion

Phytochemical screening carried out on aqueous solvent extracts of the *Cissus populnea* stem showed the presence appreciable quality of alkaloids, phenols and phytosteroids followed by Cardiac glycosides, Flavonoids, Saponins, Steroids, and Terpenoids, while Glycosides and tannins were not detected. The extracts studied showed moderate antioxidant ability at high concentrations when compared to the standard ascorbic acid. According to the findings obtained from the present study, *Cissus populnea* stem revealed significant antioxidant properties against hydrogen peroxide resistance ability, Nitric Oxide radicals scavenging activity, and ferrous ions chelating activity. Hence, this study indicates *Cissus populnea* stem contain essential phytochemicals and possess a remarkable antioxidant activity, which would make them valuable sources of antioxidant compounds.

5. Recommendation

My recommendation include

1. Promotion of sustainable harvesting: Encourage responsible harvesting practices to ensure the long-term availability of *Cissus populnea* for traditional medicine and modern applications.
2. Development of herbal formulations: Create standardized herbal extracts or supplements from *Cissus populnea* for use in preventing or managing oxidative stress-related diseases.
3. Supporting traditional medicine: Recognize and respect the traditional knowledge and uses of *Cissus populnea*,

and collaborate with traditional healers to develop evidence-based herbal remedies.

4. Fostering education and awareness: Educate the public about the benefits and safe use of *Cissus populnea*, and raise awareness about the importance of antioxidant-rich plants in maintaining health.
5. Encourage further research: Support further studies on *Cissus populnea* to explore its potential in various applications, such as: Cosmetics: Antioxidant-rich extracts for skincare products. Food industry: Natural preservatives or functional food ingredients. Pharmaceuticals: Development of new drugs or lead compounds.

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