Nigerian Research Journal of Chemical Sciences **(ISSN: 2682-6054)**
Volume 11, Issue 1, 2023

Phytochemical Screening and Antioxidants Activities of *Cissus populnea* Leaf Extracts

Danladi, A. H., *Ushie, O.A. and Egwaikhide, P.A.

Department of Chemical Science, Federal University, Wukari, Nigeria

* Correspondence Author: ushie@fuwukari.edu.ng or afiushie@yahoo.com

ABSTRACT

Phytochemicals are important constituents of natural plants as they are capable of protecting humans against diseases. The phytochemical screening and antioxidants activities of four different solvent extracts of *Cissus populnea* leaf extracts were investigated in this study using standard procedures. The *Cissus populnea* leaf were harvested, washed, air dried, and ground into powder. Hexane, ethyl acetate, acetone, and methanol were used to extract the ground material. The findings of the study revealed that of all the four solvents, methanol had the highest yield of extracts (5.02%). Ethyl acetate had the lowest yield of extracts (1.58%) which indicated that the optimal solvent for extraction is dependent on the plant material. *Cissus populnea* leaf revealed the presence of saponins, flavonoids, steroids, anthraquinones, tannins, terpenoids, phenols, and phlobatannins with the exception of cardiac glycoside which was not detected in all extracts. The crude extracts were richer in tannins (2.560 mg/g). Result of the antioxidant activity of the crude extracts of *Cissus populnea* leaf showed higher antioxidant activity on the methanol extract (25.18 mg/mL) after the standard (Vitamin C) which showed highest activity. From the findings of this study, it was concluded that *Cissus populnea* leaf possess antioxidant activity and the extracts can be used to manage stress and some degenerative diseases. Therefore, more studies should be carried out on other plants of medicinal and industrial value.

Keywords: Medicinal plant, *Cissus populnea,* cold maceration extraction, antioxidant activities, and phytochemicals screening.

INTRODUCTION

Plants are made up of natural bioactive compounds which are called phytochemicals [1]. These compounds tend to protect animals and man against diseases by acting on nutrients and dietary fibres in the body. These plant products have been derived from plant parts such as stem bark, leaf, fruits and seeds and find extensive use in medicine [2, 3]. This however means that most plant parts contain important active compounds. The main source of pharmaceuticals and healthcare products are mostly medicinal plants [4] and the active components of this plant led to

the development of high activity profile drugs [5]. *Cissus populnea* is under-utilized in the middle belt of Nigeria and mostly prepared and eaten by the Tiv, Jukun, Idoma and Igala ethnic groups [6]. The Tiv people call it *Ager*. However, in Idoma and Igala, it is known as *Okoho*. It is called *Dafara* in Hausa and *Ogbolo* in Yoruba [7].

The current study, "Phytochemical Screening and Antioxidants Activities of *Cissus populnea* Leaf Extracts", aims to investigate the phytochemical composition and antioxidant activity of *Cissus populnea* leaf extracts. This study is significant because it provides a better understanding of the potential health benefits of *Cissus populnea* and its potential use as a natural source of antioxidants.

Several previous studies have investigated the phytochemical composition and antioxidant activity of various plant extracts, including those from *Cissus populnea*. Ajayi *et al* reported the presence of flavonoids, phenolic acids, and tannins in *Cissus populnea* leaf extracts, and demonstrated their antioxidant activity using various assays [8]. Another study by Olajide *et al* also reported the presence of various phytochemicals in *Cissus populnea* extracts, and demonstrated their anti-inflammatory and analgesic properties [9].

Overall, the current study contributes to the existing knowledge on the potential health benefits of *Cissus populnea*, and provides a basis for further investigation into its use as a natural source of antioxidants.

METHODOLOGY

Collection of Sample and Preparation

The leaves of *Cissus populnea* were collected from its natural habitat in Takum Local Government Area of Taraba State, Nigeria. The sample was collected and taken to Forestry and Wild Life Department of the Federal University Wukari, for identification. The leaves were washed thoroughly in running tap water and then with distilled water. The leaves were cut into small bits and dried for two weeks under a shade. After drying, the plant material was pounded using mortar and pestle into fine powder. Then, the powder was stored in airtight container with proper labeling and kept in the laboratory for use.

Preparation of plant extracts

The extracts of the leaves were prepared by soaking 100 g of the samples in 250 ml hexane for 72 hours with frequent agitation. The resulting mixture was filtered by gravity filtration and the

filtrate were concentrated by evaporation using rotary evaporator, then kept in a vacuum oven over night at room temperature to remove all the solvents and weighed. The procedure was repeated on the residue using ethyl acetate, acetone and methanol sequentially in order of polarity. The extracts were stored in a desiccator until required for testing.

Phytochemical Screening

Phytochemical examinations were carried out for all the extracts using standard procedures to identify constituents. Qualitative analysis of the crude extracts was carried out as described [10-12] to identify the presence of the classes of secondary metabolites (alkaloids, anthraquinones, flavonoids, tannins, saponins, glycosides, cardiac glycoside, terpenes, steroids, phenol etc.).

Detection of Alkaloids

Mayer's Test

The Mayer's test was done by treating filtrates with Mayer's reagent (potassium iodide 5.0 g). Formation of a yellow colour precipitate indicates the presence of alkaloids.

Wagner's Tests

Extracts was dissolved in 1% hydrochloric acid solution and filtered. In the Wagner's test filtrates were treated with Wagner's reagent (Potassium iodide 3.0 g). Formation of brown/reddish precipitate indicates the presence of alkaloids [13].

Dragendroff's Test

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids will be confirmed by the formation of yellow coloured precipitate.

Detection of Glycosides

Extracts were hydrolysed with dilute 1% hydrochloric acid solution, and then subjected to test for glycosides using the Modified Borntrager's test. Extracts were treated with ferric chloride solution and immersed in boiling water for 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia

solution. Formation of rose pink colour in the ammonical layer indicates the presence of anthranol glycosides [13].

Detection of Saponins

This was done by the Froth Test and Foam test. In the Froth test extracts were diluted with distilled water to a 20 ml volume. This was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins. In the Foam test, exactly 0.5 g of the extract was shaken with 2 ml of water, since the foam that was produced persists ten minutes, which indicates the presence of saponins.

Detection of Flavonoids

This was done by using the alkaline reagent and Lead acetate tests [13]. In the alkaline reagent test, extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of 1% hydrochloric acid solution, indicates the presence of flavonoids. In the Lead acetate test, extracts was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Tannins

A small quantity of the extract was mixed with distilled water and heated on a water bath. The mixture will be filtered and ferric chloride will be added to the filtrate. A blue black or brownish green indicate the presence of tannins.

Detection of Anthraquinone

Small portion (0.5 g) of the extract was boiled with 2 ml HCl for five minutes in a water bath. The resultant solution was filtered and allowed to cool at room temperature. Equal volume of chloroform was added to the filtrate. Few drops of 10% NH_3 solution were added to the mixture and heated. Formation of rose pink colour indicates the presence of anthraquinone.

Detection of Terpenoids

Exactly 0.2 g of the extract was mixed with 2 ml chloroform, and 3 ml concentrated H_2SO_4. A reddish-brown interface was formed which indicates the presence of terpenoids.

Detection of Phenol

To 1 ml of the leaf and stem extract 2 ml of distilled water was added followed by two drops of 10% ferric chloride. Formation of blue or black colour indicates the presence of phenols.

Test for steroids

Concentrated H_2SO_4 (5 drops) was added to 1ml of each extract in a test tube. The solutions were observed for a red colouration which indicates the presence of steroids in the extracts.

Test for phlobatannins

Deposition of a red precipitate when an aqueous extract of the plant part was boiled with 1 % aqueous hydrochloric acid were taken as an evidence for the presence of phlobatannins.

Test for Cardiac glocoside

Salkowski Test

Small portion of the extract (0.5g) were dissolved in 2ml of chloroform. Sulphuric acid were carefully added to form a lower layer. A reddish-brown colour at the interface which indicates the presence of a steroidal ring (i.e.aglycone portion of the cardiac glycoside)

Keller Killani test

Each of the extracts (0.5 g) was dissolved in glacial acetic acid (2.0 cm^3) containing one drop of ferric chloride solution. A test tube was tilted and 1.0 cm^3 of conc. H_2SO_4 were added. A brown ring at the interface indicates the presence of a characteristic of cardenolides.

Antioxidant Assay using DPPH (1, 1-diphenyl-1-picrylhydrazyl)

The free radical scavenging activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH [14]. About 0.1mM of DPPH in ethanol was prepared and 1ml of this solution was added to 3.0ml of extract solution in ethanol at different concentrations (0.50000, 0.25000, 0.12500, 0.06250, 0.03125mg/ml). After thirty minutes (30), the absorbance was measured at 517nm. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The same experiment was carried out on ascorbic acid which is known antioxidant. All test and analysis were run in duplicate and the results obtained were averaged. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{(A - B)}{A} \times 100$$

Where A = the Absorbance of control and B = the Absorbance of sample.

All measurements of free radical scavenging activity were performed in duplicates. The concentrations of samples resulting in 50% inhibition on DPPH (IC_{50} value) were calculated from linear regression equations.

RESULTS AND DISCUSSION

Nature and yield of crude extracts of *Cissus populnea* Leaf

The extraction of bioactive components from the leaf of *Cissus populnea* using the hexane, ethyl acetate, acetone and methanol in the order of increasing polarity gave the following yields.

The nature and the yield of the crude extracts of *Cissus populnea* leaf are presented on Table 1.

Table 1: Nature and yield of crude extracts of *Cissus populnea* leaf

Solvent	Samples	Nature/colour of the extract	Yield of extracts (%)
Hexane	CPLHE	Hard powder/Black	3.40
Ethyl acetate	CPLEAE	Solid/Dark Brown	1.58
Acetone	CPLAE	Sticky/brown	3.51
Methanol	CPLME	Wax/Black	5.02

Keywords: CPLHE = *Cissus populnea leaf* hexane extract, CPLEAE = *Cissus populnea* leafEthyl acetate extract, CPLAE = *Cissus populnea* leafAcetoneextract and CPLME = *Cissus populnea* leafmethanolnextract.

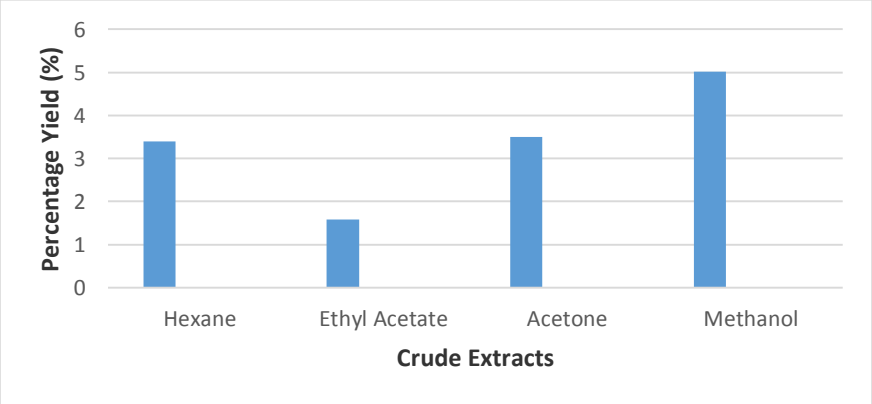

Figure 1: Extraction yield of the leaf extracts of *Cissus populnea*

Phytochemical Screening of *Cissus populnea* Leaf Extracts

Table 2 shows the results obtained from the phytochemical screening of the leaf extracts of *Cissus populnea* using hexane, ethyl acetate, acetone and methanol in order of increasing polarity.

Table2: Phytochemical Analyses of the Leaf Extract of *Cissus populne*

Phytochemicals	Reagents	Extracts			
		HECPL	EAECPL	AECPL	MECPL
Alkaloids	a) Mayer's Reagent	-	-	+	+
	b) Wagner's Reagent	+	+	+	+
	c) Dragedroff's reagent	+	-	+	+
	d) Hager Test	+	+	+	+
Saponins	a) Froth Test	-	-	+	+
	b) Foam Test	-	+	+	+
	c) Emulsion Test	+	-	-	+
Flavonoids		-	+	-	+
Tannins		++	+	+	+
Terponoids		+	+	+	++
Cardiac glycoside	a) Keller Killani Test	-	+	+	-
	b) Salkowski Test	-	+	-	-
Steroids		+	+	-	++
Anthraquinones		-	-	+	+
Phenols		+	+	+	+
Phlobatannins		+	+	+	+

Key: + indicates presence of the phytochemicals, - indicates absence of the phytochemicals, HECPL = Hexane extract of *Cissus populnea*, EAECPL = Ethyl acetate extract of *Cissus*

populnea leaf. AECPL = Acetone extract of *Cissus populnea* leaf, MECPL = Methanol extract of *Cissus populnea leaf*.

Antioxidant Activities of *Cissus populnea* leaf Extracts

The crude extracts of the *Cissus populnea* leaf were assayed for antioxidant activity using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) radical scavenging method. *Cissus populnea* leaf extracts of different solvents as presented in Table 3.

Table 3: Absorbance of *Cissus populnea* leaf extracts and Standard at 517 nm UV-Vis Spectrophotometer

Concentration	Absorbance				
(mg/mL × 10^{-2})	CPLME	CPLAE	CPLEE	CPLHE	Vitamin C
3.13	0.132	0.134	0.157	0.162	0.133
6.25	0.117	0.128	0.143	0.155	0.124
12.50	0.109	0.108	0.136	0.147	0.108
25.00	0.088	0.088	0.107	0.139	0.085
50.00	0.059	0.064	0.100	0.117	0.044

Blank = 0.185

Table 4: % Inhibition for Standard and *Cissus populnea* leaf extracts

Concentration	% Inhibition				
(mg/mL × 10^{-2})	CPLME	CPLAE	CPLEE	CPLHE	Vitamin C
3.13	28.65	27.57	15.14	12.43	28.11
6.25	36.76	31.08	22.70	16.22	32.97
12.50	41.08	41.62	26.49	20.54	41.62
25.00	52.43	52.70	32.97	24.86	54.05
50.00	68.11	65.68	45.95	36.76	76.22
IC_{50}	**25.18**	**27.19**	**55.17**	**76.39**	**22.76**

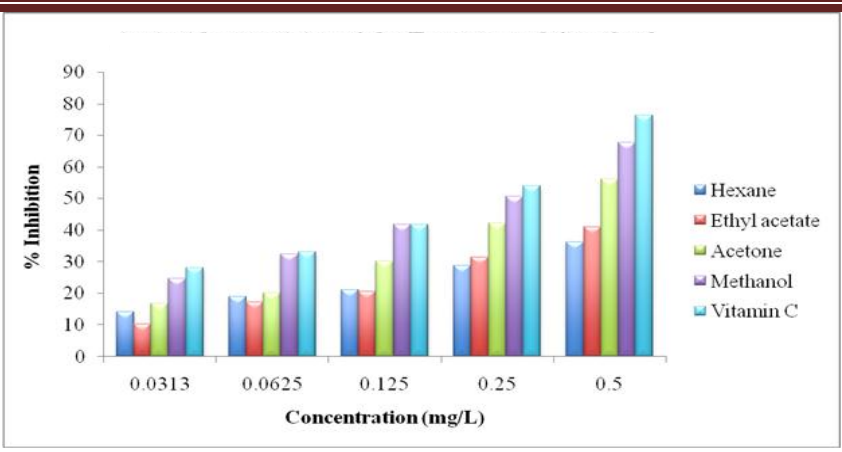

Figure 2: Percentage Inhibition of Standard and *Cissus populnea* leaf Crude Extracts.

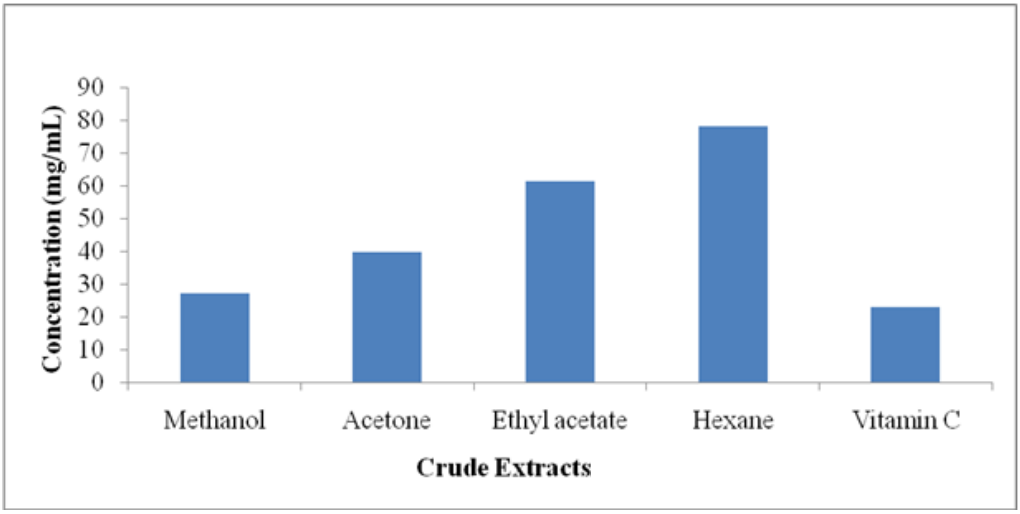

Figure 3: Maximal Inhibitory Concentration of Standard and *Cissus populnea* leaf Crude Extracts

Extraction Yield (%)

The result of extracts (Table 1) showed that all the four solvents used were able to extract some components of the plant leaf though at varying quantity. *Cissus populnea* leaf extracts; methanol had the highest yield of 5.02%, followed by acetone 3.51%, hexane 3.40% and the least was ethyl acetate with 1.58%. The difference in the percentage yield could be as a result of the difference in polarity of the solvents, the nature of the constituents involved, variety of bioactive compounds and their differing solubility properties in different solvents [15]. Hexane being the lower polar solvent was expected to have lowest yield, rather, ethyl acetate had the lowestyield. This could be that the optimal solvent for extraction depends on the particular plant materials and the compounds to be isolated [16].

Qualitative phytochemical screening

The results of preliminary phytochemical screening of the crude extracts of *Cissus populnea* leaf in Table 2 revealed the presence of alkaloids in all the extracts except ethyl acetate extracts. Saponins were present in ethyl acetate, acetone and methanol extracts except hexane extracts. Flavonoids were present in ethyl acetate and methanol extracts but absence in acetone and hexane extracts. Tannins, terpenoids, phenols, and phlobatannins were present in all the extracts but tannins and terpenoids are richlypresent in hexane extract and methanol extract respectively. Cardiac glycoside was found present only in ethyl acetate extract. Steroids were found to be present in all extracts except acetone extract and richly found in methanol extract. Anthraquinones were present only in acetone and methanol extracts but were found absence in hexane and ethyl acetate extracts. The variation in the phytochemical composition of the extracts is as a result of the fact that the bioactive components have different degrees of polarity [17] and is soluble in solvents depending on their polarity.

Cissus populnea leaf can be used for immediate treatment of sore throats, diarrhea, sexual transmitted disease, indigestion, veneral diseases, skin diseases, boils and infected wounds and for treating urinary tract infections because it has saponins, steroids, terpenoids, alkaloids, and tannins. Steroids were detected richly in methanol extracts. It is worth noting that steroidal compounds are of importance and interest in pharmacy due to their relationship in sex hormones. [18]. The presence of flavonoids, tannins, cardiac glycosides and saponins in the present study agrees with earlier reports [19].

The quantitative phytochemical screening of the crude extracts of *C. populnea* showed that the leaves were rich in tannins 2.560 mg/g, followed by phenols 0.826 mg/g, saponins 0.356 mg/g, flavonoids 0.260 mg/g and alkaloids 0.256 mg/g. Tannins are a group of polyphenolic compounds found in many plant species, including *Cissus populnea*. They are known to possess a variety of biological activities, including anti-inflammatory properties. Tannins have also been shown to scavenge free radicals and reduce oxidative stress, which can contribute to inflammation. Additionally, tannins can inhibit the release of histamine, a chemical mediator of inflammation, from mast cells [20]. The highest concentration of tannin in *Cissus populnea* leaf is an indication that the leaf possesses anti-inflammatory properties.

Antioxidant Activity of Crude Extracts

The antioxidant activity of the crude extracts of *Cissus populnea* leaf and standard (Vitamin C) are as shown on Tables 3 and 4, and Figures 2 and 3. Comparing the antioxidants activities of the crude extracts to that of the standard at the various concentrations of 0.0313, 0.0625, 0.125, 0.250, 0.500 mg/mL, the samples showed lower activity than that of the standard. The absorbance readings of the leaf extracts of *Cissus populnea* (Table 3, Figure 2) were observed that the absorbances were decreasing as the concentration increased from 0.313 to 0.500 mg/mL; while the percentage inhibition increased as the concentration increases (Table 4; Figure 3).

The maximal inhibitory concentration (IC_{50}) was observed that he standard had the least IC_{50} value of 22.76 mg/mL indicating highest antioxidant activity. Comparing to that of the extracts, methanol extract (25.18 mg/mL) was closely comparable to that of the standard, closely followed by that of acetone extract (27.19 mg/mL), then, ethyl acetate (55.17 mg/mL), and lastly hexane (76.39 mg/mL).

CONCLUSION

The results of extraction showed that the four solvents (Hexane, ethyl acetate, acetone and methanol) used can extract certain bioactive components of the leaf of *Cissus populnea* but with different percentages. The secondary metabolites present in all extracts of the plant part included saponins, flavonoids, steroids, anthraquinones, tannins, terpenoids, phenols, and phlobatannins. However, cardiac glycoside was not detected in any of the extracts. Therefore, these phytochemicals or bioactive components are the constituents of the *Cissus populnea*leaf.

DPPH was used to assess the antioxidant activity of the hexane, ethyl acetate, acetone, and methanol crude extracts of *Cissus populnea* leaf and standard (Vitamin C). Comparing the antioxidants activities of the crude extracts to that of the standard at the various concentrations of 0.0313, 0.0625, 0.1250, 0.2500, 0.5000 mg/mL, the samples showed lower activity than that of the standard. The plant part possesses antioxidant phytochemicals as hexane, ethyl acetate, acetone and methanol extracts showed antioxidant activity with scavenging ability increasing with increase in solvent polarity. Therefore, the extracts can be used to manage stress and some degenerative diseases arising from it. The leaf of *Cissus populnea* should be explored for its rich phytochemicals content as the assessment of its bioactive components showed the presence of alkaloids, steroids, tannins, flavonoids, phenols etc. Therefore, further work should isolate more bioactive components and probably other compounds of medicinal and industrial values from the plants.

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