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Phytochemical analysis, invitro antioxidant activity and GC-MS studies of crude extracts of *Cissus populnea* stem

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***Abstract**: The medicinal capabilities of plants has been attributed to the presents of phytochemicals and their antioxidant activities. In this study, five solvent extracts (aqueous, ethanol, butanol ethyl acetate, and n-hexane) of Cissus populnea stem were evaluated qualitatively and quantitatively for some phytoconstituents and in vitro antioxidant activities using three methods of antioxidant assessment. GC-MS analysis was also carried out on the aqueous extract for the identification of the bioactive compounds. The ethyl acetate extract revealed the presence of all the phytochemicals tested for in the qualitative phytochemical screening. The butanol extract indicated the highest quantity of alkaloids (91.90 ± 2.13 mg/100g), flavonoids (67.24 ± 1.56 mg/100g), and saponins (60.13 ± 1.41 mg/100g) while the highest level of tannins (65.25±0.14 mg/100g) and phenols (15.35±0.27 mg/100g) were recorded in the aqueous extract. The n-hexane extract recorded the least quantity of all the phytochemicals studied. In all three methods of in vitro antioxidant assessment used, antioxidant activity increased with increased concentration in all the extracts. For the percentage, DPPH and nitric acid inhibition methods all the extracts at 100 µg/ml showed antioxidant capacity up to 50% of that showed by the standard, ascorbic acid at the same concentration. The GC-MS analysis of the aqueous extract revealed the presence of 36 compounds belonging to 15 known classes of compounds while 2 of the compounds were unclassified. The major chemical constituents were 9, 12-Octadecadienoic acid (Z, Z)-(21.17%), n-Hexadecanoic acid (20.33%), Stigmastam-3, 5, 22-trien (7.78%) and 2',6'-Dihydroxyacetophenone (6.61%) . These data show that C. populnea stem is a potential source of bioactive compounds*

Keywords: *Cissus populnea,* Phytochemicals, Antioxidant, GC-MS analysis

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1.0 Introduction

The use of plant parts as medicine is as old as man. Every plant part, from the roots which are mostly hidden in the ground to the aerials parts found above the ground, has found one use or

another as medicine depending on the plant. According to USDA (2022) in the Western world, 40% of the orthodox medications come from plants that have been used by humans for centuries. The reason for this has been attributed to the phytochemical constituents (Naseem, 2014) as well as the antioxidant ability of the plants involved (Krishnaiah *et al.*, 2011). The presence of these phytochemicals is not unconnected to their use by these plants either as means of defense from natural predators or parasites and even harsh climatic conditions (Molyneux *et al.*,2007).

Cissus populnea is a liana with pale woody stems up to 7½ cm in diameter and up to 8 m long which belongs to the wild grape family known as Vitaceae (Amplidaceae)(Soladoye and Chukwuma, 2012). When cut, the stem which is usually annual, exudes copious clear watery sap and withers in the dry season, covering the trunk from which it hangs. The bark when young is creamy, smooth, then gray and scaly, flaking off into fibrous skins over the old bark. The flowers are cream-coloured and the fruits are blackish-purple when ripe. The plant is found in the savanna region with distribution from Senegal to Mozambique (Ouoba, *et al*., 2018)

C.populnea is a very versatile plant. In Nigeria, the stem is used as a soup thickener by the Idoma people of Benue State (Falayi, 2021). According to Soladoye and Chukwuma (2012), the leaves of this plant have been used as fodder for fish and insects, The root is known to exhibit antimicrobial properties thus its effect against skin diseases, boils and infected wounds (Kone *et al.*, 2004).The root has also been shown to possess anti-sickling properties on hemoglobin from sickle cell patients (Moody *et al*.,2003), thus may be promising potential in the management of sickle cell crisis. In Southwest, Nigeria the aqueous extract of the stem of this plant is used to improve libido and fertility, especially in men, in this regard Ojekale *et al*. (2015) in their study affirmed the spermatogenic properties of the stem bark extract in *wistar* rats.

It has been shown that the medicinal potentials of plants are attributed to the phytochemical constituents of the plant (Merecz-Sadowska, *et al*., 2020). It is therefore not unusual that effort has been made by some authors to determine the phytochemical constituents of some parts of *C. populnea* (Adebowale *et al.*, 2013; Soladoye and Chukwuma, 2012). Moreover, of importance is the fact that many medicinal plants are sources of antioxidants. Antioxidants have been involved in the management of degenerative diseases elicited by oxidative damage. There is still a dearth of information on the antioxidant ability as well as phytochemical constituents of this plant. Therefore this work aims to carry out the phytochemical screening, *in vitro* antioxidant ability of various solvent extracts as well as the GC-MS studies of aqueous extract stem of *C. populnea*.

2.0 Materials and Methods

2.1 Plant material collection

Stem parts of *C. populea* bearing the leaves were purchased from Mushin Market in the Mushin Local government area of Lagos State. The plant was authenticated by a Taxonomist at the Department of Botany, University of Lagos, Lagos state, Nigeria.

2.2 Pre-extraction treatment

The stem was washed and cut into smaller pieces spread on brown paper and allowed to air dry for 2 weeks at room temperature until properly dried. The dried pieces were then crushed in a local mortar to a coarse powder and then blended to powder by a laboratory mill.

2.3 Preparation of the extracts

A quantity of 250 g of the powdered sample was extracted using the solvents (n-hexane, butanol, ethyl acetate, ethanol and distilled water) in order of polarity. In order to allow for

complete extraction, each sample-solvent mixture was allowed to stand for 48 hours with intermittent shaking. The sample was dried after each solvent extraction. For the non-polar solvents, the extracts were filtered and some of the solvents were allowed to evaporate and then transfer to vials of known mass. Here the remaining part of the solvent was allowed to evaporate leaving behind the crude extract. The aqueous extract was concentrated using a rotary evaporator 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 deduce the mass of the extract. Each crude extract was reconstituted with petroleum spirit (0.1g in 1litre) for those from the nonpolar solvents) while those from polar solvents were reconstituted using distilled water (0.1g in 1litre) and stored in the refrigerator for further analysis.

2.4 Phytochemical screening

2.4.1 Test for saponins

A quantity of 5ml of the reconstituted extract was diluted with 5ml distilled water, 3 minutes of vigorous lengthwise shaking in a graduated cylinder should result in the formation of a stable, persistent froth. (Sofowora,1993).

2.4.2 Test for phlobatannins

An aliquot of 2 ml of the reconstituted extract was added to a test tube with 2 ml of 1% aqueous hydrochloric acid (HCl) then boiled for 3 minutes. This was observed for the formation of a red precipitate which shows the presence of phlobatannins in the extract (Sofowora,1993)

2.4.3 Test for tannins

A few drops of 0.1% ferric chloride was added to 3 ml of the reconstituted extract. The formation of a brownish green or a blue-black colour indicates the presence of tannins (Sofowora,1993)

2.4.4 Test for steroids

A quantity of 2 ml acetic anhydride and 2 ml of sulphuric acid (H_2SO_4) were added to 5 ml of reconstituted extract in a test tube. The mixture was a colour change from violet to blue or green indicating the presence of steroids (Sofowora,1993)

2.4.5 Test for alkaloid

To 5 ml of the reconstituted extract was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. Mayer’s reagent (Potassium mercuric iodide) was added. The formation of a yellow coloured precipitate confirmed the presence of alkaloids (Sofowora,1993)

2.4.6 Test for reducing sugar

To 2 ml of the extract was added 1 ml each of Fehling solutions A and B. The mixture was boiled for 5- 10 minutes in a water bath. The reddish brown colour due to the formation of cuprous oxide indicated the presence of reducing sugar (Sofowora, 1993)

2.4.7 Test for anthraquinones

Approximately 5 ml of each herbal extract was boiled with 10 ml of aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene. The benzene layer was then separated and 10% ammonia solution was added to half of its volume. A pink, red or violet colouration in the ammonia phase (lower layer) indicated the presence of anthraquinone derivatives in the extracts.

2.4.8 Test for cardiac glycosides

Salkoski test was used to identify cardiac glycosides. 0.5 ml of the extract was dissolved in 2 ml of chloroform and sulphuric acid was carefully added to form a lower layer. Formation of reddish-brown colour at the interface indicated the presence of steroidal ring (i.e. aglycone portion of the cardiac glycoside).

2.5 Quantitative phytochemical determination

2.5.1 Determination of total phenolic content

The phenolic content was determined using the Fiolin- Ciocalteau assay. To 20 µl of the reconstituted extract was added 80 µl of Fiolin - Ciocalteau reagent mix was added and the mixture was allowed to incubate for 5 minutes later, 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. Gallic acid was used as the standard established to calculate phenolic content (Bayili *et al.*, 2011).

2.5.2 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 vortexed and was allowed to stand for 40 minutes and absorbance was recorded at 760 nm using a UV/visible spectrophotometer Blank was prepared with reagent instead of the sample. Total tannin content was determined from the calibration curve made with standard tannic acid.

2.5.3 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 H_2O) (Vijay and Rajendra, 2014).

2.5.4 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).

2.5.5 Estimation of Saponins

The method Obadoni and Ochuko (2001) adopted. One ml of the extract was treated with 25 ml of 20% ethanol and this mixture was heated at 55 ºC 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.

2.6 In-vitro antioxidant activity

2.6.1 Reducing power method (RP)

The method described by Oyaizu (1986) was used with slight 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 ºC 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.

2.6.2 Quantitative DPPH radical scavenging assay

The method of Gyamfi *et al*., (1999) 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 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:

$$\%\ \text{DPPH scavenging activity} = \frac{A_{control} - A_{Sample}}{Absorbance\ of\ control} \times 100 \quad (1)$$

where $A_{Control}$ and A_{sample} are the absorbances of the control and of the sample respectively.

2.6.3 Nitric oxide inhibition

Two (2) ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract at various concentrations (25,50,75,100 µg/ml). The mixture was then incubated at 25 ºC for 150 min. Three milliliters (3 ml) of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)] is added to the mixture. The same mixture without the extract is used as the control. The mixture is then incubated at room temperature for 30 min and its absorbance pouring into a cuvette is measured at 546 nm (Alam *et al*., 2012). The amount of nitric oxide radical inhibition is calculated following this equation 2

$$\%\ \text{inhibition of nitric oxide} = \frac{A_{control} - A_{Sample}}{Absorbance\ of\ control} \times 100 \quad (2)$$

where $A_{Control}$ and A_{sample} are the absorbances of the control and of the sample respectively.

2.7 GC-MS Determination of phytochemical characterization aqueous extract of C. populnea

GC-MS analysis of the determination of the levels of phytochemicals in the aqueous extract was carried out using Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with an electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5% phenyl methylsiloxane (30m length x 0.32mm diameter x 0.25µm film thickness) (Agilent Technologies). Helium (99.99%) was used as the carrier gas at a constant flow of 1.49 ml/min. The extracts (1 µl) were injected in splitless mode at an injection temperature of 300 °C. Oven was initially programmed at 40 °C for (1 min) then ramped at 12 °C/min to 300 °C (10 min).The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230 °C, quadrupole temperature of 150 °C and transfer line temperature of 280 °C. Acquisition of ion was via Scan mode (scanning from *m/z* 45 to 550 amu at 2.0 s/scan rate). The percentage composition of the crude extract's constituents were expressed as percentage by peak area. The identification and characterization of chemical compounds was based on the GC retention time. The separated components were identified through mass spectral comparison with available mass spectra libraries.

3.0 Results and Discussion

The results of the different analyses carried out on the various extracts of *C.populea* are

presented in this section. The preliminary phytochemical screening of *C. populea* revealed the presence of tannins, phenols, alkaloids, saponins, flavonoids, cardiac glycoside and reducing sugars in all the extracts studied. Anthraquinones were however absent in the aqueous and butanol extracts while terpenoids were not detected in the aqueous and ethanol extracts. Steroids were only absent in the aqueous extract while the hexane extract showed no presence of saponins. Among all the extracts the ethyl acetate extract showed the presence of all the phytochemicals screened.The results are presented on Table 1.

Table 1: Preliminary qualitative photochemical analysis of various extracts of *C. populea* stem

Extract/ Phytochemicals	**Aqueous**	**Ethanol**	**Butanol**	**Ethyl acetate**	***n*-Hexane**
Tannins	+	+	+	+	+
Anthraquinones	-	+	-	+	+
Phenols	+	+	+	+	+
Phlobatannins	+	+	+	+	+
Alkaloids	+	+	+	+	+
Saponins	+	+	+	+	-
Flavonoids	+	+	+	+	+
Steroids	-	+	+	+	+
Terpenoids	-	-	+	+	+
Cardiac glycosides	+	+	+	+	+
Reducing sugar	+	+	+	+	+

+ indicates presence; - indicates absence

Table 2 shows the results of the quantitative analysis of the various extracts of *C. populea* . The aqueous extract revealed the highest levels of tannin (65.25 ± 0.14 mg/100g) and phenol (15.35 ±0.27 mg/100g) while the least quantity of tannin (1.85 ± 0.69 mg/100g) and phenol (1.19 ±0.23 mg/100g) was observed in the hexane extract. The butanol extract produced the highest quantity of alkaloid (91.90 ±2.13 mg/100g respectively), flavonoid (67.24 ±1.56 mg/100g) and saponin (60.13 ±1.41 mg/100g). Saponin was not detected in the hexane extract.

Table 2: Results of the quantitative phytochemical screening of *C. populae*

Extract/Phytochemical	**Aqueous**	**Ethanol**	**Butanol**	**Ethyl acetate**	**n-Hexane**
Tannins(mg/100g)	65.25±0.14	47.16±2.33	29.37±3.30	33.46±0.28	1.85±0.69
Phenols(mg/100g)	15.35±0.27	14.58±0.73	10.38±0.18	4.78±0.28	1.19±0.23
Reducing sugars(mg/100g)	20.74±0.18	21.36±0.25	14.24±0.44	5.05±0.33	0.91±0.26
Alkaloids (mg/100g)	63.28±1.42	65.78±0.71	91.90 ±2.13	90.89±0.71	31.64±0.71
Flavonoids(mg/100g)	46.30±1.04	48.13±0.52	67.24±1.56	66.50±0.52	23.15±0.52
Saponins(mg/100g)	48.11±1.42	47.61±0.71	60.13 ±1.41	57.63±0.71	0.00

Values represent mean ± SD

Table 3 shows the reducing power of the various extracts of *C.populea* studied at four different concentrations 25, 50, 75 and 100 µg/ml. The ethanol extract showed the highest

reducing ability with an absorbance of 0.032 ± 0.001 absorbance unit at 25 µg/ml to 0.091 ± 0.001 absorbance unit at 100 µg/ml with a maximum reducing activity at 75 µg/ml. The hexane extract showed the least reducing ability with absorbance ranging between 0.010 ± 0.001 absorbance unit at 25 µg/ml and 0.021 ± 0.001 absorbance unit at 100 µg/ml. All the extracts however had much lower reducing power than the standard ascorbic acid which had values between 0.255 ± 0.001 absorbance unit at 25 µg/ml and 0.559 ± 0.049 absorbance unit at 100 µg/ml.

Table 3: The reducing power of *C.populea* extracts

Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	0.016±0.001	0.023±0.001	0.028±0.001	0.037±0.002
Ethanol	0.032±0.001	0.054±0.004	0.087±0.002	0.091±0.001
Butanol	0.011±0.001	0.019±0.001	0.033±0.001	0.043±0.002
Ethyl acetate	0.018±0.001	0.023±0.001	0.034±0.001	0.037±0.003
Hexane	0.010±0.001	0.013±0.001	0.014±0.001	0.021±0.001
Ascorbic acid	0.255±0.001	0.438±0.012	0.465±0.008	0.559±0.049

Values represent mean ± SD

Table 4 shows percentage DPPH inhibition of the various extracts of *C.populea* studied. The Hexane extract showed the highest percentage DPPH inhibition at lower concentrations 41.11 ±0.84 % and 45.26 ±0.67 % at 25 µg/ml and 50 µg/ml respectively. However, at higher concentrations the values (49.53 ± 1.34 to 51.78 ± 0.84) were almost similar to those obtained from other extracts (50.00 ± 1.01% to 53.44±1.17) for the ethanol extract, (46.56 ±4.86 to 53.44±1.51) for the aqueous extract at 75 µg/ml and 100 µg/ml respectively. Most of the extracts showed the maximum percentage DPPH inhibition at 75 µg/ml. However, the percentage DPPH inhibition was not as high as was observed with the standard ascorbic acid (84.72 ± 0.50 and 90.64 ± 0.17 %) at 75 µg/ml and 100 µg/ml respectively.

Table 4 : Percentage DPPH inhibition of *C.populea* extracts

Extract	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
Aqueous	20.85±3.69	37.91±1.68	46.56±4.86	53.44±1.51
Ethanol	23.82±0.17	39.34±0.34	50.00±1.01	53.44±1.17
Butanol	21.68±1.17	24.41±0.67	46.45±0.34	47.39±0.34
Ethyl acetate	21.21±0.17	23.82±0.50	46.92±1.68	48.46±2.18
Hexane	41.11±0.84	45.26±0.67	49.53±1.34	51.78±0.84
Ascorbic acid	73.34±0.50	75.12±1.34	84.72±0.50	90.64±0.17

Values represent mean ± SD

Table 5 shows the percentage nitric acid inhibition of the various extracts of *C.populea*. The hexane extract showed the highest percentage inhibition (11.91 ± 0.50% to 54.72 ± 0.67%) compared to the rest of the extracts. However, at 100 µg/ml the percentage nitric oxide inhibition of all the extracts ranged between 49.76 ± 0.33% and 54.72 ± 0.67%. These values were nonetheless below the standard ascorbic acid.

The GC-MS analysis of the aqueous extract of *C. populnea* stem revealed the presence of 36 compounds belonging to 15 known classes of compounds while 2 of the compounds were unclassified. The classes include alkaloid, ester, ketone, fatty acids, alcohol, steroid, fatty

alcohols, alkane, alkene, terpene, aldehyde, carboxamides acid, ether, lactam, fatty acyl, and organosulfur. The major chemical constituents were 9, 12-Octadecadienoic acid (Z,Z) (21.17%), n-Hexadacanoic acid (20.33%), Stigmastam-3,5,22-trien (7.78%) and 2',6'-Dihydroxyacetophenone (6.61%). The chromatograph of the GC-MS analysis is presented on Fig 1, while Table 6 shows the different compounds, their retention time, percentage peak area, molecular formula, molecular weight and the class of compound they belong.

Table 5: Percentage of nitric oxide inhibition

Sample	25(µg/mL)	50(µg/mL)	75(µg/mL)	100(µg/mL)
Aqueous	5.54±0.83	20.75±0.33	39.62±1.67	49.76±0.33
Ethanol	4.95±0.67	20.99±1.33	40.21±0.17	50.47±0.33
Butanol	9.67±0.67	37.38±0.50	42.92±0.33	52.12±2.67
Ethyl acetate	8.49±0.33	37.50±0.67	42.69±0.33	53.77±0.67
Hexane	11.91±0.50	48.70±0.50	51.53±0.50	54.72±0.67
Ascorbic acid	60.97±0.50	74.53±0.33	80.42±0.33	88.68±0.33

Values represent mean ± SD

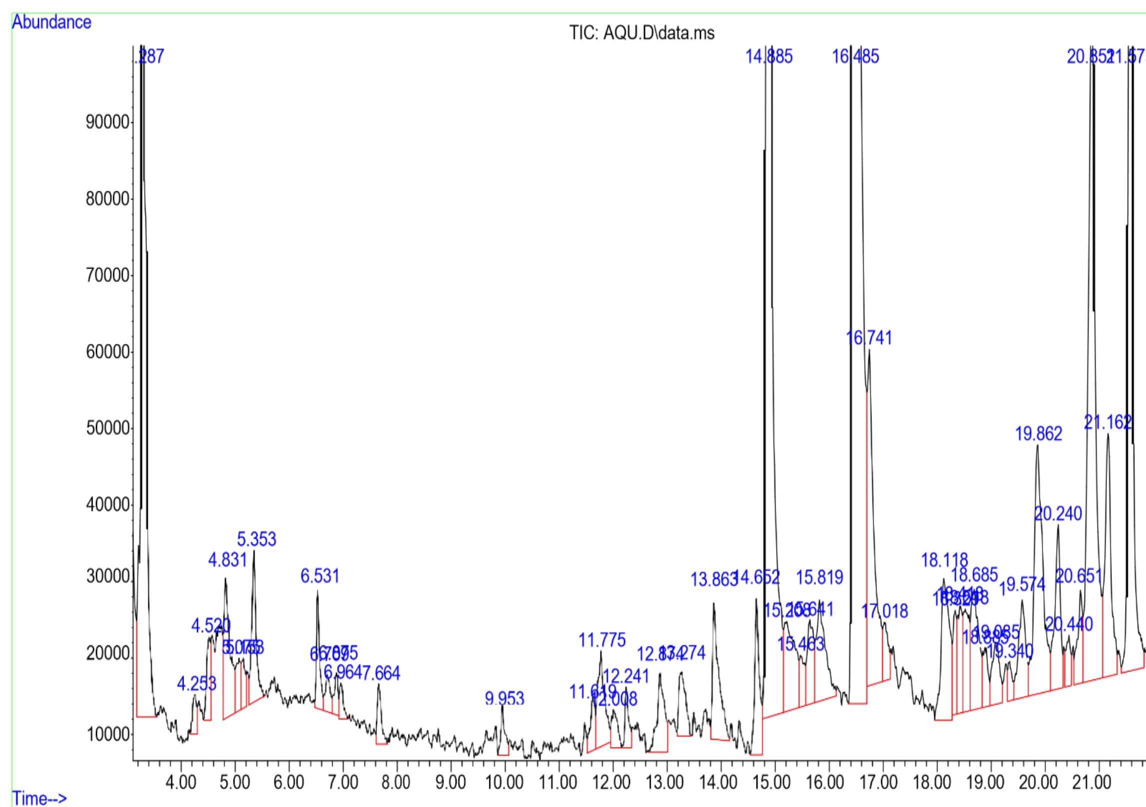

Fig 1: Chromatograph of GC-MS analysis of the aqueous extract of *C.populnea*

Table 5: Phytoconstituents of the aqueous extract of *C.populnea*

S/no	R/T	Area %	Organic Compound	CF	M/W	compounds
1	3.287	6.61	2',6'-Dihydroxyacetophenone	$C_8H_8O_3$	152.15	Ketone
2	4.253	0.024	Glutaric acid, myrtenyl 2-ethylhexyl ester	$C_{23}H_{38}O_4$	378.5	Ester
3	4.52	0.54	4-(Methylamino)butyric acid	$C_5H_{11}NO_2$	117.14	Alkaloid
4	4.52	0.54	1-Methyl-2-pyrrolidone	C_4H_9NO	87.12	Alkaloid
5	4.831	1.35	4-Trimethylsilyl-9,9-dimethyl-9-silafluorene	$C_{17}H_{22}Si_2$	284.54	Not classified
6	5.075	0.39	2-Pyrrolidinone, 1-(3,7,11-trimethyldodecyl)	$C_{19}H_{37}NO$	295.5	lactam
7	6.709	0.29	2-Propenal, 3-(dimethylamino)	$C_6H_{11}NO$	113.58	aldehyde
8	6.875	0.24	Phosphoroic acid, methyl- diethyl 1-methylenepropyl ester	$C_8H_{17}O_4P$	208.19	ester
9	6.964	0.21	Formic acid, 2-methylhex-3-yl ester	$C_8H_{16}O_2$	144.21	ester
10	9.953	0.31	Ethyl 4-t-butylbenzoate	$C_{13}H_{18}O_2$	206.28	ester
11	11.619	0.40	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	$C_{14}H_{24}O$	208.34	terpene
12	11.775	1.03	4-Dehydroxy-N-(4,5-methylenedioxy-2-nitrobenzylidene)tyramine	$C_{16}H_{14}N_2O_4$	298.29	Alkaloid
13	12.008	0.35	2-Myristynoyl-glycinamide	$C_{16}H_{28}N_2O_2$	284.44	carboximidic acid
14	12.241	0.35	E-3-Pentadecen-2-ol	$C_{15}H_{30}O$	226.39	alcohol
15	13.274	0.69	Spiro[4.5]dec-6-ene	$C_{10}H_{16}$	136.23	alkene
16	13.863	1.38	Pentadecanoic acid	$C_{15}H_{30}O_2$	242.4	fatty acid
17	14.652	1.17	2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-.(4aalpha,7beta,8abeta)-	$C_{14}H_{24}O$	208.34	Ketone
18	14.885	20.33	n-Hexadecanoic acid	$C_{16}H_{32}O$	240.42	fatty acid
19	15.641	0.85	12-Oxatricyclo[4.4.3.0(1,6)]tridecane-3,11-dione	$C_{12}H_{16}O_3$	208.25	ketone
20	15.819	1.42	Hexahydropyridine, 1-methyl-4[4,5dihydroxyphenyl]-	$C_{12}H_{17}NO_2$	308.19	alkaloid
21	16.485	21.17	9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O$	280.44	Lipids (fatty acylcs)
22	16.741	3.43	2-Methyl-Z,Z-3,13-octadecadienol	$C_{19}H_{36}O$	280.5	Alcohol (fatty alcohols)
23	17.018	0.48	Cyclopropaneoctanoic acid, 2-[[2-(ethylcyclopropyl) methyl]cyclopropyl]methyl]-,methyl ester	$C_{22}H_{38}O_2$	282.5	ester
24	18.118	1.92	Cis-8-ethyl-exo-tricyclo[5.2.1.0(2)]decane	$C_{11}H_{18}$	150.26	alkane
25	18.418	0.83	4-(3-methoxyphenoxy)-4-Allyl-5-furan-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione	C_9H_9N0S	179.04	organosulfur
26	18.685	1.48	Cyclododecanol, 1-aminomethyl-	$C_{13}H_{27}NO$	213.35	alcohol
27	19.085	0.74	[1,2,4]Triazolo[1,5-a]pyrimidine-6-carboxylic acid,	$C_{10}H_{12}N_4O_3S$	268.29	ester

			7-hydroxy-5-methyl-2-(methylthio)-, ethyl ester			
28	19.34	0.26	Indole-2-one, 2,3-dihydro-N-hydroxy-4-methoxy-3,3-dimethyl-	$C_{11}H_{13}NO_3$	207.22	alkaloid
29	19.574	1.1	(3-Chlorophenyl)(furan-2-ylmethyl)amine	$C_{11}H_{10}ClNO$	207.66	alkaloid
30	19.862	3.46	17-(1,5-Dimethylhexyl)-10,13-dimethyl-1,7,8,9,10,11,12,13,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-4-one	$C_{27}H_{44}O$	384.63	Unknown
31	20.24	1.46	1,2,5-Oxadiazol-3-amine, 4-(3-methoxyphenoxy)	$C_9H_9N_3O_3$	207.19	ether
32	20.44	0.36	1-Hexadecyne	$C_{16}H_{31}$	222.41	alkene
33	20.651	0.72	3-Methyl-N-(5-methyl-4,5-dihydro-1,3-thiazol-2-yl)-2-pyridinamine	$C_{10}H_{13}N_3S$	207.31	alkaloid
34	20.851	7.78	Stigmastan-3,5,22-trien	$C_{29}H_{46}$	394.7	terpene
35	21.162	2.29	.beta.-Sitosterol acetate	$C_{31}H_{52}O_2$	456.7	steroids
36	21.573	6.97	Cholest-5-en-3-ol, (3.alpha.)-	$C_{27}H_{46}O$	386.65	steroids

The presence of some secondary metabolites in plants has endowed them with therapeutic roles which were being tapped into by folklore medicine. Most of the therapeutic activities have been given scientific support by various studies into these plants. In this study, various solvent extracts of *Cissus populea* stem have been studied for their phytochemical constituents as well as their antioxidant activities. GC-MS analysis was also carried out on the aqueous extract.

In the initial phytochemical screening, the ethyl acetate extract of the showed the presence of all the phytochemicals determined as shown in Table 1. Ethyl acetate has the ability to dissolve both polar and non-polar substances thus the presence of all the phytochemicals tested in the extract. The other extracts studied were missing one of the constituents tested except the aqueous extract from which three of the phytoconstituents were missing. The results are in line with the report from Al-Owaisi, *et al.,* (2014) and Larayetan *et al.,* (2019) which have shown that different phytochemicals occur in different plant species depending on the solvents used for extraction.

The butanol extract produced the highest quantity of alkaloids (91.90 ±2.13 mg/100g), flavonoids (67.24 ±1.56 mg/100g) and saponins (60.13 ±1.41 mg/100g). Alkaloids have been shown to have a wide range of pharmacological capabilities like antimalarial (Uzor, 2020) antiasthmatic (Fu *et al.,* 2019) and anticancer (Mondal *et al.,*2019) properties. Flavonoids also possess pharmacological potential. They are known to have antiviral, anticancer and anti-inflammatory activities (Karak, 2019). Saponins are also known for their multiple biological activities-fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant and immunomodulatory effects (Juang and Liang, 2020). Hitherto, interest had been in the aqueous extract of the *C. populea* stem. Harnessing the other extracts could lead to more pharmacological value for this plant. Tannins were seen to be appreciable in the aqueous extract (65.25±0.14 mg/100g). They also have applications for anticancer, virucides, antioxidant, and antidiabetic wound healing abilities (Pizzi, 2021). Hexane extract had the least of all phytochemicals tested. This is attributed to the non-polar nature of the solvent. Different bioactive compounds exhibit different effective antioxidant activities that play a fundamental role in terminating the peers of free radical sequence reactions which if not dismissed will cause a lot of ailments

(Mahomoodally *et al.,* 2020). The various studies on the antioxidant abilities of the various extracts revealed that although these extracts were not strong antioxidants compared to the standard, ascorbic acid, they still exhibited the antioxidant ability of up to 50% of ascorbic acid at high concentrations. It was however observed the hexane extract had a higher percentage DPPH inhibition ability (41.11±0.84%) at low concentrations (25 µg/ml) than the other extracts which ranged between 20.85±3.69% (aqueous extract) to 23.82±0.17% for the ethanol extract at 25 µg/ml. However at high concentrations 100 µg/ml all the extracts showed almost similar percentages DPPH inhibition ability ranging between 47.39±0.34 and 53.44±1.51%. Antioxidant activities of plants have in most instances been associated with the phenol content (Larayetan *et al*., 2019). It is obvious from the level of phenols in the extracts in this study, especially the hexane extract that the antioxidant activity exhibited could not have been due to their phenolic content. In the reducing power assay, the extracts exhibited much lower reducing ability than the standard ascorbic acid as shown in their absorbance. The antioxidant activity by this method was in this order Ethanol extract > ethyl acetate extract > aqueous extract > butanol extract >hexane extract.

The GC-MS analysis of the aqueous extract of *C.populnea* confirms the presence of 36 different chemical compounds belonging to 15 class compounds. Some of these compounds have established bioactivity and therapeutic importance. *n*-Hexadecanoic acid has been shown to have antioxidant, anti-inflammatory, and hypocholesterolemic activities (Kalpana *et al.,*2012). The compound beta.-Sitosterol acetate, is an acetate of the compound, beta.-Sitosterol which has been isolated and is known to have aphrodisiac properties in sexually naive *wistar* rats (Watcho *et al*., 2012).

4.0 Conclusion

Phytochemical screening carried out on five solvent extracts of the *Cissus populnea* stem showed the presence appreciable quantity of flavonoids, alkaloids, saponins and tannins in the aqueous, ethanol, butanol and ethyl acetate extracts. All the extracts studied showed moderate antioxidant ability at high concentrations when compared to the standard ascorbic acid. GS-MS analysis of the aqueous extract revealed the presence of 36 compounds belonging to 15 known classes.

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