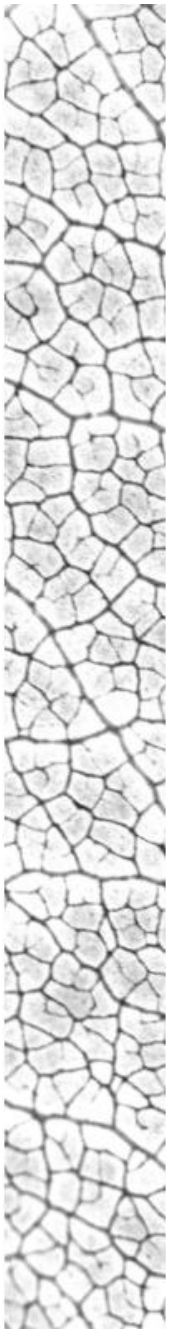

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Spectral characterization, antioxidant and anti-genotoxic properties of methanol and ethanol stem extracts of *Cissus araloides* hook

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Abstract

Purpose: This study investigated the phytocomponents, functional groups, antioxidant and anti-genotoxic properties of methanol and ethanol stem extracts of Cissus *araloides*.

Method: The phytocomponents' analyses of the extracts were determined using a gas chromatograph interfaced with a mass spectrometer (GC-MS) while functional groups were obtained using Fourier Transform Infra-red spectrometer (FT-IR). The antioxidant activity of the extracts was evaluated using ferric reducing antioxidant power (FRAP), 2, 2-diphenyl-l-picrylhydrazyl (DPPH), peroxidase activity, and 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. Anti-genotoxicity activity of the extracts was carried out using *Allium cepa* injected with 20µ Lethidium bromide solution.

Results: The results indicated the presence of saponins, flavonoids, tannins, steroids and carbohydrate in the methanol and ethanol stem extracts of *C. araloides* 25 compounds were detected from methanol extract. The functional -OH and C=C groups revealed by FTIR was validated by GC-MS analysis to indicate significant amounts of Benzoic acid-2,6-dihydroxy-, Chenodiol, Estriol, Ginkgolide A, Oxazepam, PGF2α and Resorcinol-2-acetate, that is typical of polyphenolic compounds. The methanol extract showed peroxidase activity (IC_{50} = 490.07µg/ml), ABTS (IC_{50} = 89.19 µg/ml) and FRAP (IC_{50} = 441.43µg/ml). Significant decrease ($p<0.5$ was indicated in fragmented DNA level of *Allium cepa* roots growth in ethidium bromide treated with *C. araloides* stem extracts when compared with ethidium bromide control. However, fragmented DNA was lower in *A. cepa* roots growth in methanol extract when compared with ethanol extract. Hence, extracts of *C.araloides* stem can be viewed as a new perspective in developing antioxidant agent.

Conclusion: Therefore it is recommended that *C. araloides* be utilized in natural products to strengthen our health.

Keywords: anti-genotoxicity, antioxidant, *Cissus araloides*

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INTRODUCTION

Phytochemicals are derivatives from plants. They include phenolic acids, ascorbic acid, tocopherols, and bioflavonoids. They are important for numerous body functions and have antioxidant characteristics that have been used in the treatment of several diseases (Rokayya et al. 2013; Smits et al 2019; Larki, et al. 2020; Dongarwar, et al. 2019; Usman, et al. 2019).

The antioxidant potential of compounds found in food products may be linked to the occurrence of hydroxyl group (Apak, et al.2018). Higher plants are sources of antioxidant compounds that play a dominant role in human health maintenance. (Castanas 2008, Kampa and Petrou, et al 2018). Antioxidants prevent oxidative damage to macromolecules such as nucleic acids, lipids and proteins. Antioxidants molecules also help to scavenge free radicals that are produced from biochemical reactions (Granda and De Pascual-Teresa 2018). Studies have shown that ethidium bromide induces genotoxicity by causing several types gene of mutations and DNA lesions (Szumilak et al, 2016, Wurmb-Schwark et al. 2006, Zhang et al 2018).

Thus, substances with antioxidant properties have emerged in the treatment of diseases related to DNA damage and oxidative stress. (Floyd and Hensley 2002, Schmidt et al 2016, Yang et al 2018). In pharmaceutical analysis, gas chromatograph interfaced with mass spectrometer (GC-MS) and Fourier Transform Infra-red spectrometer (FT-IR) have both played important roles in structural elucidation of compounds as well as phyto-constituents and functional group determination

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respectively (Li et al. 2018, Zariwala et al. 2018,). Spectroscopy is a major tool for biomedical application and field clinical evaluation. (Zhang et al 2019)

Cissus araloides hook is a climber that belongs to the family *Vitaceae*. The plant grows in the tropical regions of Nigeria, Togo, Benin, Niger and Ghana (Dalziel 1965) and has been used in the treatment of bacterial infections (Irvine, 1961). Various species have been investigated from the genus *Cissus*. Dhanasekaran (2020) isolated stilbenoids from *C. Quandrangularis* and Shan *et al.* (2009) reported the presence of stilbenes, triterpenoidsand steroids in stem of *C. pallida*. This study is therefore aimed at evaluating the phytocomponents, antioxidant and anti-genotoxic properties of methanol and ethanol stem extract of *C. araloides.*

MATERIALS AND METHODS

Plant material

Dry healthy onion (*Allium cepa*) was purchased from Abraka market, Delta State, Nigeria. The stem of *C. araloides* was collected in January, 2018 (during the dry season) from Abraka, Nigeria and identified by Dr Ade Akinnibosun from the department of Botany, university of Benin, Edo State, Nigeria, with a voucher number UBH-CO41.

Preparation of extract

The collected stems of *C. araloides* were washed with distilled water and dried to constant weight at room temperature. The dried sample was processed in to powdery form, using mortar and pestle. 100.00 g of *C. araloides* powder was soaked in 400mL of methanol and ethanol respectively for 48 h. Extracts were filtered using a funnel and What-man No 1 filter paper. Each filtrate was concentrated to dryness using a rotary evaporator and water bath under reduced pressure at 40°C. The dry samples were reconstituted with distilled water (1 g/9 mL) and different concentrations (100, 200, 300 and 400 µg/mL) prepared from the reconstituted sample for antioxidant analysis.

Phytochemical screening

Phytochemical screening for detecting bioactive agents was performed using standard procedures (Hemalatha and Sathiya Vinotha 2019). After the addition of specific reagents to the solution, the tests were detected by visual observation of colour change or by precipitate formation.

Determination of total phenolic content

The protocol of Liu and Yao (2007) was used in the determination of Total phenolic content. 0.5 mL of the diluted stem extract was dissolved in 100 µL of Folin-Ciocalteau reagent and 6 mL of distilled water. It was vortexed for 1 min, and 2 mL of 15% Na_2CO_3 was added. The mixture was vortexed again for 30 second made up to 10 mL with distilled water. After 1 h 30 mins, the absorbances of the samples were read at 750 nm with UV spectrophotometer. Gallic acid solution was used for the preparation of standard calibration curve. Total phenolics contents of samples were expressed as milligrams of gallic acid equivalent (mg GAE)/100 g of dry weight.

Total flavonoid assay

Assay for Total flavonoid in the extracts was by the method of Ebrahimzadeh *et al*. (2008). 5 ml of 2% aluminium trichloride in methanol was mixed with 5 ml of the extract. Absorption was read at 415 nm after 10 min against a blank sample consisting of a 5 ml extract solution with 5 ml methanol without $AlCl_3$. A standard curve with rutin (0-100 mg/l) as standard was used in the calculation of total flavonoid content.

2, 2-diphenyl-1-picrylhydrazyl) scavenging assay

The protocol of Ursini *et al*. (1994) was used in the assay of the free radical scavenging ability of the sample against DPPH (2,2-diphenyl-1-picrylhydrazyl). The extracts (50 µg) was diluted with 3 mL ethanol and mixed with 3 mL DPPH solution. The reaction mixture was shaken, and then incubated in dark for 30 min. The absorbance of the solution was measured against a blank at 517 nm.

Percentage (%) inhibition of DPPH was calculated using following equation:

$$\% \text{ Inhibition} = [(A_0 - A1)/A_0] \times 100$$

Where $A_0 =$ the absorbance of the blank sample and $A_1 =$ the absorbance of the tested sample.

Assay for Ascorbate oxidase activity

Ascorbate oxidase activity in the spice extracts was assayed for by the protocol of Vines and Oberbacher (1965). The samples were mixed [1:5 (v/v)] with phosphate buffer (0.1 M/ pH 6.5) and centrifuged at a speed of 3000 rev for 15 min at 5 ºC. The supernatant obtained were used as the enzyme source. 0.1 mL of the enzyme extracts were added to 3.0 mL of the substrate solution (8.8 mg ascorbic acid in 300 ml phosphate buffer, pH 6.5) and change in absorbance was determined at 265 nm for every 30 sec for 4 min.

Assay of peroxidase activity

The protocol of Addy and Goodman, (1972) was used in the assay for Peroxidase activity. The reaction mixture consisted of 3mL of buffered pyrogallol and 0.5mL of 1% hydrogen peroxide. To this, 0.1mL of sample extract was added and the absorbance was read at 430 nm.

ABTS radical scavenging activity

Assay for ABTS radical-scavenging activity of the extract was by the protocol of Re *et al*. (1999). The mixture of 5 mL of ABTS stock solution and 5 mL of 2.45 mM potassium persulphate ($K_2S_2O_8$) solution, was stored in the dark at room temperature for 16 h to produce ABTS radical cation (ABTS+). Before use, this solution was diluted with water to get an absorbance of

0.700 ± 0.020 at 734 nm and equilibrated at 30°C.The plant extract at various concentrations were then diluted with dimethyl sulphoxide (DMSO) to produce a sample solution. Exactly 1mL of this sample solution was homogenized with 9 mL ABTS+ solution and the mixture incubated at room temperature for 6 min and its absorbance was read at 734 nm. ABTS scavenging ability was expressed as IC_{50} (µg/ml) and the % inhibition calculated according to the formula:

ABTS scavenging activity = $(A_0 - A_1)/A_0] \times 100$

Where; A_0 is the absorbance of the control. A_1 is the absorbance of the sample.

Ferric reducing antioxidant power assay (FRAP)

FRAP was determined according to the protocol of Benzie and Strain (1996). Precisely 300 mM sodium acetate buffer (pH 3.6) was mixed with 10.0 mM TPTZ (tripyridyltriazine) solution and 20.0 mM $FeCl_3.6H_2O$ solution in a ratio of 10:1:1 in volume. Different concentrations (100, 200, 300 and 400 µg/ml) were then added to 3 ml of FRAP reagent and the reaction mixture incubated at 37°C for 30 min. The increase in absorbance at 593 nm was measured.

Antigenotoxic property of *C. araloides* using *Allium cepa* (onion)

The outer papery brown layer of 12 onion bulbs (5–10 g, 20–25 mm diameter), was peeled away and the dried basal root plate were cleaned. About 20 µL of the prepared ethidium bromide (0.03g/2mL) solution was injected into the *A. cepa* and then submerged on a 50 mLtest tube containing water (control) and reconstituted ethanol and methanol stem extracts of *C. araloides* which was allowed to stand for 5 days. Root growth was observed and then harvested for DNA fragmentation assay.

The experimental groupings were as follows:

Group A: Control (*A. cepa* without Ethidium bromide)

Group B: Ethidium bromide and *A. cepa*

Group C: Ethidium bromide and*A. cepa* plus ethanol extract

Group D: Ethidium bromide and *A. cepa* plus methanol extract

DNA fragmentation assay

DNA fragmentation assay was estimated using Khan,(2017)) method with some modifications. About 50 mg of *A.cepa* roots was homogenized in 10 mL of aTris–hydrochloric acid-EDTA (TE) pH 8.0. The TE solution consist of 5 mmol dm^{-3} Tris–hydrochloric acid, 20 mmol dm^{-3} EDTA and 0.2% triton X-100. One millilitre aliquot of sample was centrifuged at high speed (27,000 × g for 20 min) to allow proper separation of the intact chromatin (pellet, B) from fragmented DNA (supernatant, T). Diphenylamine solution that was freshly prepared was used to assay for DNA content of the pellet and the supernatant fractions. Samples readings were monitored at 620 nm with spectrophotometer. The amount of % fragmented DNA was calculated with the following formula;

$$\% \text{ Fragmented DNA} = \text{T x}100/(\text{T} + \text{B}).$$

Characterisation of extracts

Fourier Transform Infrared Spectrophotometer (FTIR)

Dried powder of methanol extract was used for FTIR analysis. In order to prepare translucent sample discs, about 10 mg of extract was encapsulated in 100 mg of potassium bromide pellet. Which was loaded into the FTIR spectroscope (IR Affinity 1, Japan) with a scan range of 400 to 4000 cm^1 and resolution of 4 cm^1.

Gas Chromatography-Mass Spectrometry

GC-MS was carried out according to the method of Dhivya and Manimegalai (2013). The extract was processed for separation with ether acetate and n-butanol using liquid – liquid extraction method in separator funnel. The extract was evaporated using rotary evaporator and dried at room temperature for one week. About 40 mg of extract was weigh and diluted with 10 ml 50 % DMSO (Dimethyl sulphate) prior to GC-MS analysis. Extract solution was filtered through sterile 0.22 mm Whatmann filter paper and kept in amber vial at 4 ^{0}C. Chemical constituents were analyzed by triple quadruple gas chromatography mass spectrometry. Analysis was performed in TIC scan mode. Oven temperature was set at 50 ^{0}C for 2 min and programmed at 50 ^{0}C to 230 ^{0}C at a rate of 4 ^{0}C/minutes and at hold 230 ^{0}C for 2 minutes resulting in the complete elution of peaks analyzed. Injector and detector temperatures were 350 ^{0}C, the carrier gas was helium. The Identification of constituents was based on comparison of constituent of analyte with National Institute of Standards and Technology (NIST) Libraries using mass Hunter Software.

Statistical Analysis

All results were represented as means ±SD and all data were analyzed using analysis of variance (ANOVA). Linear relationships between variables were evaluated using Pearson correlation. Significant difference between means were determined at 5% ($p< 0.05$) confidence level using least significant difference (LSD) Post hoc test.

RESULTS AND DISCUSSION

Qualitative phytochemical and antioxidant properties of *C. araloides* stem extracts

Methanol and ethanol stem extract of *C. araloides* revealed the presence of saponins, flavonoids, carbohydrate, tannins, and steroids and absent of alkaloid (**Table 1**). Tannin is recognized as one of the major phyto-constituent present in medicinal plants; it is mainly used for fruit juice, beer, wine clarification in food industries (Sanhueza et al. 2017, Kalam et al. 2019). Phenols and flavonoids are known for their antioxidant

Table 1. Qualitative phytochemicals of *C. araloides* stem extracts

Constituents	Ethanol	Methanol
Saponins	+	+
Flavonoids	+	+
Tannins	+	+
Steroids	+	+
Alkaloids	-	-
Carbohydrate	+	+

Key; Present (+), Absent (-)

Table 2. DPPH, peroxidase activity and ascorbate activity of *C. araloides* stem extracts

Concentration (µg/mL)	DPPH (%)		Peroxidase activity (units/g dry wt)		Ascorbate oxidase activity (units/g dry wt)	
	Methanol	Ethanol	Methanol	Ethanol	Methanol	Ethanol
100	10.17 ± 5.027 [a]	6.41± 2.77 [a]	22.62 ± 1.56 [a]	18.50 ± 1.79 [a]	40.16 ± 5.01 [a]	32.04 ± 2.011 [a]
200	15.52 ± 5.02 [b]	13.37 ± 3.49 [b]	25.40 ± 5.00 [a]	21.27 ± 10.35 [a]	43.00 ± 2.63 [a]	38.50 ± 5.11 [b]
300	18.63 ± 8.49 [b]	16.75 ± 4.05 [b]	26.35 ± 5.64 [a]	24.66 ± 4.02 [a,b]	48.34 ± 8.45 [b]	42.36 ± 2.49 [b]
400	22.36 ± 1.59 [b,c]	21.41± 4.93 [c]	32.88 ± 2.20 [b]	28.51±3.77 [b]	55.18 ± 5.17 [c]	49.08 ± 4.06 [c]

Triplicate values are represented in mean ± SD. Values with different superscript letter (a, b, c) differ significantly at $p < 0.05$

effects and study have shown that they inhibit the initiation and progression of cancer (Nnama et al. 2017) and reduction of heart disease (Boussahel et al. 2018). Saponins are useful in the treatment of yeast and fungal infections as well as protection of plants against microbial attack (Smith and Dilger, 2018).

Antioxidant properties of *C. araloides* stem extracts

DPPH scavenging effect

The DPPH method revealed that the scavenging of the free radicals was found to be 10.17, 15.52, 18.63 and 22.36 % at 100,200, 300, 400 µg/ml respectively for the methanol extract as seen in **Table 2**. The percentage inhibition of the DPPH radical by the ethanol extract was 6.41, 13.37, 16.75, 21.41 % at 100,200, 300, 400 µg/ml respectively. There was no significant difference ($p>0.05$) in DPPH of methanol extract when compared with ethanol extract at varied concentrations (**Table 2**). Ascorbate oxidase and DPPH was positively correlated ($r^2 = 0.9227$), peroxidase activity and DPPH had positive correlation of $r^2 = 0.8648$. In the DPPH assay, the IC_{50} was 545.33 µg/ml for methanol extract while that of the ethanol extract was 519.33 µg/ml (**Table 5**). Peroxidase activity had $IC_{50}$of 490.07 µg/ml and 517.68 µg/ml in methanol and ethanol stem extracts respectively while the ascorbate oxidase activity IC_{50} was 158.02 µg/ml for methanol extract and 211.48 for ethanol extract. Contrary to the IC_{50} values obtained from methanol and ethanol stem extracts using DPPH assay, the IC_{50} values obtained for peroxidase activity as well as ascorbate oxidase activity conformed with Apiamu and Asagba (2019) report, where the inverse relationship between IC_{50} value and antioxidant potential of the plant extract may suggest that methanol was a better extractant of bioactive compounds with antioxidant activity relative to ethanol. Also it was discovered that antioxidants fight against/scavenge free radicals in various diseases (Al-Jadidi and Hossain, 2018). 2,2′-diphenyl-1- picrylhydrazyl radical (DPPH) is mainly used to measure the electron donation ability of natural products (Vikas et al, 2017). The method is based on decolourizing the DPPH solution or scavenging of DPPH through the presence of a radical species/antioxidant. The colour change is proportional to the concentration the antioxidants. The study showed that DPPH scavenging of *C. araloides* stem extracts were in dose dependent manner and showed no significant difference in percentage of inhibition.

Peroxidase and ascorbate oxidase activity

The peroxidase activity in the examined methanol extract of *C. araloides* stem were 22.62, 25.40, 26.35 and 32.88 at 100 to 400 µg/mlwhile that of ethanol extract of *C. araloides* stem were 18.50, 21.27, 24.66, 28.51 at varied concentrations (**Table 2**). The ascorbate oxidase activity in methanol extract of *C. araloides* stem were 40.16, 43.00,48.34 and 55.18 at 100-400 µg/ml respectively while ethanol extract ascorbate oxidase were 32.04, 38.50, 42.36, 49.08, and 32.88 at 100-400 µg/ml. The results of this study revealed that methanol extract of *C. araloides* stem had higher peroxidase and ascorbate oxidase activity than that of ethanol extract (**Table 2**). Ascorbate oxidase and peroxidase activity of methanol extract were positively correlated with DPPH thus suggesting their antioxidant potential. Ascorbic acid is mainly involved in the regulation of growth (Nam et al 2019). Peroxidases are heme containing enzymes that oxidize a variety of proton donor compounds such as diphenols, polyphenols aminophenols, among others, in the presence of hydrogen peroxide.

FRAP and ABTS radical scavenging activity

In **Table 3**, *C. araloides* stem methanol extract was found to be effective in scavenging the FRAP radical. The percentage inhibition of this radical was concentration-dependent. At 100, 200, 300 and 400 µg/ml, the inhibition of the methanol extract was 20.42, 23.42, 28.77 and 31.28 % and that of ethanol extract were 9.85, 14.93 18.23, 28.26 % respectively. The IC_{50} of FRAP was 441.4 µg/ml in *C. araloides* stem methanol extract while that of ethanol stem extract was 450.15 µg/ml (**Table 5**). In ABTS assay, methanol extract was

Table 3. FRAP and ABTS of *C. araloides* stem methanol and ethanol extracts

Concentration (µg/mL)	FRAP (units/g dry wt)		ABTS (units/g dry wt)	
	Methanol	Ethanol	Methanol	Ethanol
100	20.42 ± 5.56 [a]	9.85 ± 2.00 [a]	46.08 ± 12.98 [a]	40.67 ± 13.88 [a]
200	23.42 ± 2.68 [a]	14.93 ± 5.55 [b]	50.44 ± 12.38 [a]	45.35 ± 4.29 [b]
300	28.77 ± 9.94 [b]	18.23 ± 3.92 [b]	56.89 ± 9.29 [b]	50.95 ± 13.79 [c]
400	31.28 ± 2.80 [b]	28.26 ± 1.91 [c]	62.80 ± 14.74 [c]	60.09 ± 11.38[d]

Triplicate values are represented in mean ± SD. Values with different superscript letter (a, b, c) differ significantly at $p < 0.05$

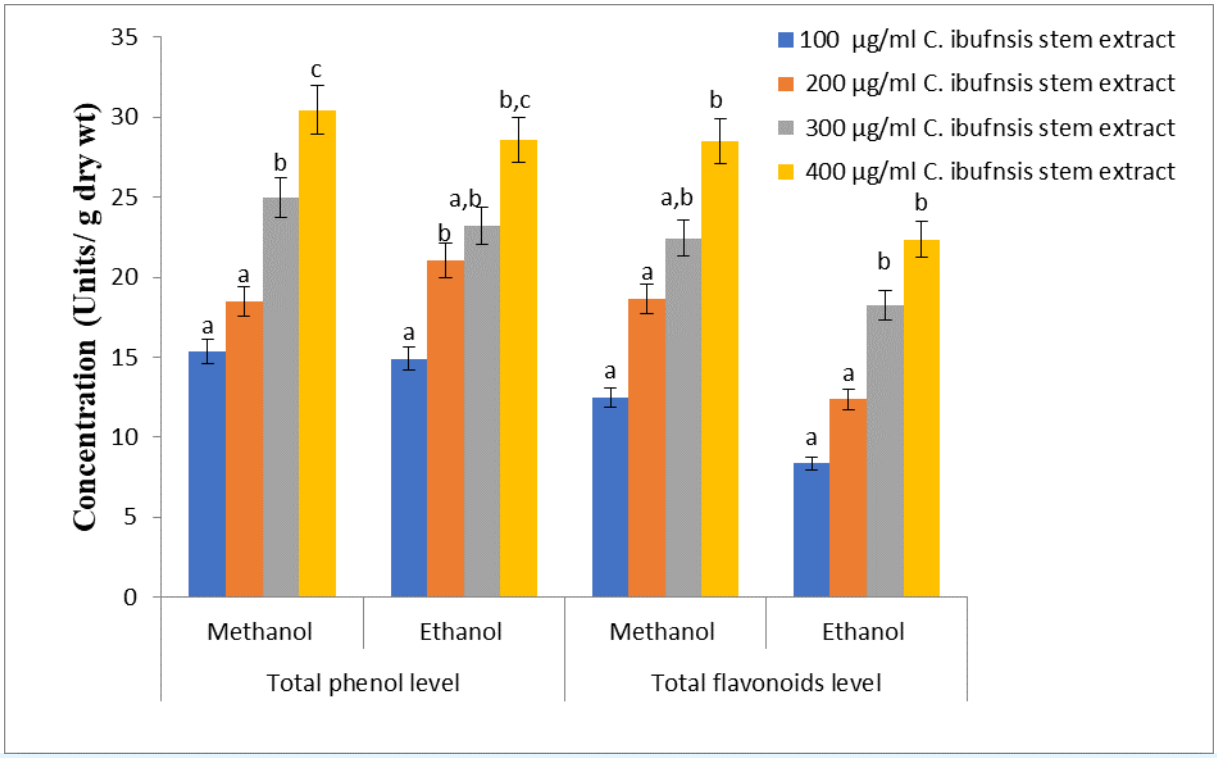


Fig. 1. Total flavonoids and total phenol level of *C. araloides* stem extracts
Triplicate values are represented inmean bar. Bars with different superscript letter (a, b, c) differ significantly at $p < 0.05$

found to be 46.08, 50.44, 56.89, 62.80 µg/ml while ethanol extract was 40.67, 45.35, 50.95 and 60.09 µg/ml. The IC_{50} of ABTS was89.19 µg/ml and 130.76 µg/ml for methanol and ethanol extracts respectively. ABTS assay has been employed as an index that reveals the antioxidant activity of test samples (Wu *et al.,* 2006). The scavenging of the ABTS radical by the stem extracts in this study was found to be high at the highest concentration of 400 µg/ml. This shows that extracts had good ability to scavenge ABTS radical. The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric iron (Fe^{3+}) complex and produce a coloured ferrous (Fe^{2+}) (Benzie and Strain JJ. 1996). Generally, the reducing properties are linked with the presence of compounds which exert their action in breaking free radical chain by donating a hydrogen atom (Duh et al. 1999). In this study, the absorbance of *C. araloides* stem extracts clearly increased, due to the formation of the Fe^{2+}- complex with increasing concentration. ABTS and FRAP was correlated positive (r^2 = 0.984). Hence, the *C. araloides* stem extracts should be able to donate electrons to free radicals stable in the actual biological and food systems (Zia-Ul-Haq et al. 2013).

Phenolic content and flavonoids level

The present study has revealed that the extracts of *C. araloides* stem contains substantial amount of phenolics and flavonoids and thus, it can be inferred that these phenolics and flavonoids are responsible for its marked antioxidant activity. The total phenolic and flavonoid contents in the examined stem of *C. araloides* methanol and ethanol extract at varied concentration were illustrated in **Fig. 1**. However, total flavonoid and total phenol content were positively correlated (r^2 = 0.9591). This is consistent with several reports that have shown close relationship between total phenolic contents and total flavonoids of *Cissus quadrangularis* extracts (Chidambara et al 2013; Bhujade et al. 2012). Therefore, *C. araloides* stem extracts have considerable antioxidant properties and may play a role in preventing human diseases (such as cardiovascular disease, genotoxicity and aging) in which free radicals are involved.

Table 4. Anti-genotoxicity property of *C. araloides* stem extracts

Group	DNA Fragmentation (%)
Control (*Allium cepa* roots growth in water)	3.35 ± 0.47[a]
Ethidium bromide control	3.41 ± 12.98[b]
Allium cepa roots growth in ethidium bromide and *C. Ibufnsis* stem ethanol extract	24.28 ± 5.19[c]
Allium cepa roots growth in ethidium bromide and *C. Ibufnsis* stem methanol extract.	15.28 ± 4.87[d]

Anti-genotoxicity property of *C. araloides* stem extracts

DNA damage of *C. araloides* stem was evaluated by measuring the level of fragmented DNA using diphenylamine (DPA).The results in **Table 4**, indicated that there were significant increase ($p<0.05$) in fragmented DNA level of *Allium cepa* roots growth in ethidium bromide only (ethidium bromide control) when compared with the *Allium cepa* roots growth in water. Significant decrease was observed in the fragmented DNA level of *Allium cepa* roots growth in ethidium bromide plus *C. araloides* stem ethanol extract and ethidium bromide plus *C. araloides* stem methanol extract when compared with ethidium bromide control at $p<0.05$. However, DNA fragmentation was significantly low in *Allium cepa* roots growth in methanol extract when compared with ethanol extract. Ethidium bromide is toxic and mutagenic compound that is used widely in electrophoretic procedures. The observed increase in DNA fragmentation in *A. cepa* roots growth in ethidium bromide may be associated with the down regulation of antioxidant gene expression. *C. araloides* stem extracts showed a significant decrease in fragmented DNA level with a clear indication that it has anti-genotoxic properties via decreasing the ROS generation by ethidium bromide (Sabini et al. 2013).

Infra-Red Spectroscopy (FTIR) and GCMS of *C. araloides* stem

Table 5 illustrates the chromatogram and infrared spectra of *C. araloides* stem methanol extract. Among the functional groups observed in the extract, OH group was found to be more present in the extract.. The presence of OH group in the extract probably indicates the higher antioxidant potential of the extract against ethidium bromide genotoxicity. A total of 25 peaks were observed from methanol extract of *C. araloides* stem when subjected to GC-MS. The presence of bioactive compounds justifies the use of the plant stem by

Table 5. Bioactive compounds of *C. araloides* stem methanol extract

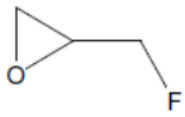
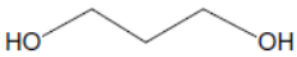
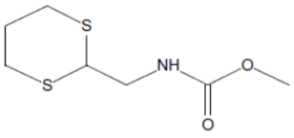
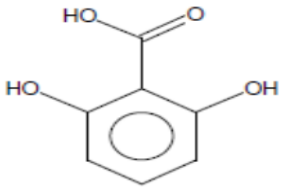
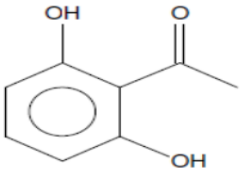
Peaks	R.T (Min.)	Name of compound	Molecular formula	MW	Structure
1	1.418	Oxirane, (fluoromethyl)-	C_3H_5FO	76	
2	1.546	1,3-Propanediol	$C_3H_8O_2$	76	
3	1.983	Carbamic acid, (1,3-dithian-2-ylmethyl)-, methyl ester	$C_7H_{13}NO_2S_2$	207	
4	2.431	Benzoic acid, 2,6-dihydroxy-	$C_7H_6O_4$	154	
5	3.793	Resorcinol, 2-acetyl-	$C_8H_8O_3$	152	

Table 5 (continued). Bioactive compounds of *C. araloides* stem methanol extract

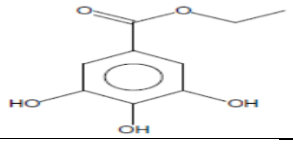
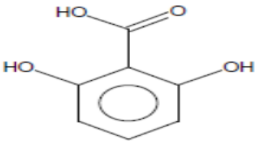
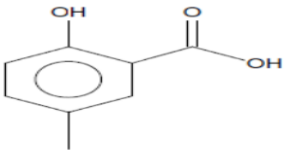
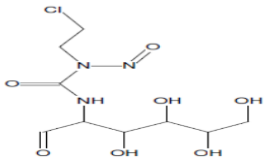
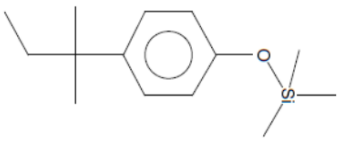
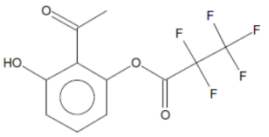
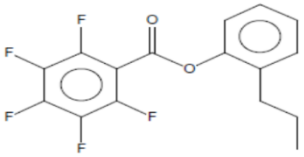
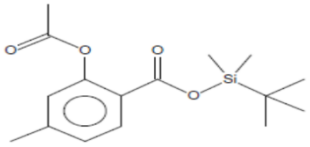
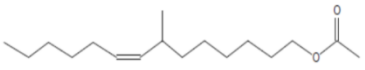
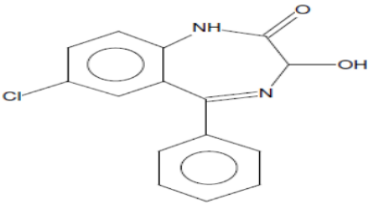
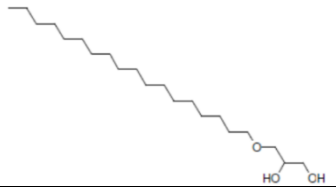
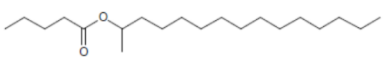
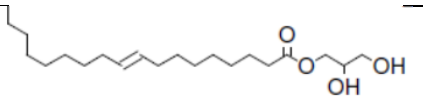
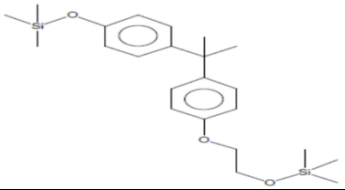
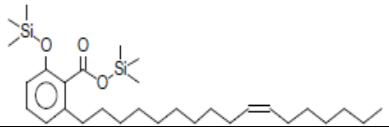
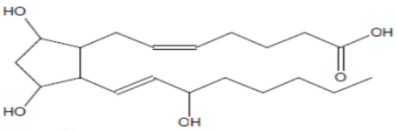
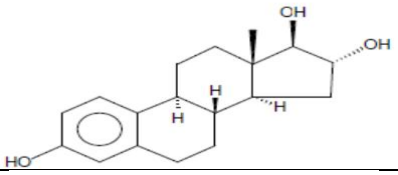
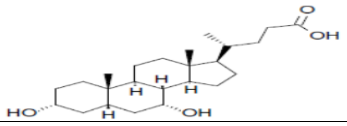
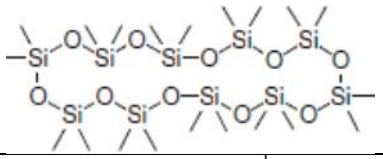
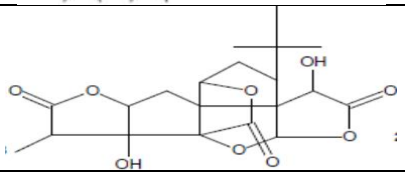
Peaks	R.T (Min.)	Name of compound	Molecular formula	MW	Structure
6	4.654	Ethyl gallate	$C_9H_{10}O_5$	198	
7	7.990	Benzoic acid, 2,6-dihydroxy-	$C_7H_6O_4$	154	
8	9.514	5-Methylsalicylic acid	$C_8H_8O_3$	152	
9	10.236	Chlorozotocin	$C_9H_{16}ClN_3O_7$	313	
10	12.430	4-tert-Amylphenol, TMS derivative	$C_{14}H_{24}OSi$	236	
11	15.730	Acetophenone, 2-hydroxy-6-pentafluoropropionyloxy-	$C_{11}H_7F_5O_4$	298	
12	17.063	Pentafluorobenzoic acid, 2-propylphenyl ester	$C_{16}H_{11}F_5O_2$	330	
13	18.564	tert-Butyldimethylsilyl 2-acetoxy-4-methylbenzoate	$C_{16}H_{24}O_4Si$	308	
14	19.775	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	268	
15	20.170	Oxazepam	$C_{15}H_{11}ClN_2O_2$	286	

Table 5 (continued). Bioactive compounds of *C. araloides* stem methanol extract

Peaks	R.T (Min.)	Name of compound	Molecular formula	MW	Structure
16	20.403	Batilol	$C_{21}H_{44}O_3$	344	
17	20.734	Valeric acid, 2-pentadecyl ester	$C_{20}H_{40}O_2$	312	
18	21.964	2,3-Dihydroxypropyl elaidate	$C_{21}H_{40}O_4$	356	
19	26.124	2-[(p-Trimethylsilyloxy)phenyl]-2-[(p-trimethylsilyloxyethylenoxy)phenyl]propae	$C_{23}H_{36}O_3Si_2$	416	
20	29.988	Ginkgolic acid 17:1 (2TMS)	$C_{30}H_{54}O_3Si_2$	518	
21	30.233	PGF2α	$C_{20}H_{34}O_5$	354	
22	31.397	Estriol	$C_{18}H_{24}O_3$	288	
23	32.497	Chenodiol	$C_{24}H_{40}O_4$	392	
24	33.545	Cyclodecasiloxane, eicosamethyl-	$C_{20}H_{60}O_{10}Si_{10}$	740	
25	34.692	Ginkgolide A	$C_{20}H_{24}O_9$	408	

traditional practitioners for the treatment of various diseases. Many plant parts are reportedly used traditionally and in homoeopathic medicines to treat several ailments, such as liver and spleen enlargement, hepatitis, renal disorders, asthma and whooping cough (Cock and Van Vuuren 2015; Wu et al. 2017).

CONCLUSION

The most commonly used drugs of modern medicine originated from natural sources. Instead of single active compound, conventional treatment options prefer whole plant or formulations of multi- targeted medicines for speedy recovery from any kinds of illness. The present study proved that the stem extracts of *C. araloides* holds excellent free radical scavenging ability and anti-genotoxic properties. It can be concluded that *C. araloides* stem investigation has opened up a new perspective in pharmaceutical research and development of novel antioxidant agents. It is hereby recommended that *C. araloides* be utilized in natural products to strengthen our health and antioxidant mechanism respectively.

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