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Cholinesterase Inhibition, Biological Activity and Characterization of *Chrysophyllum albidum* Leaf and Stem-Bark Chloroform Extract Using GC-MS: An *In Vitro* Study

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ABSTRACT

Chrysophyllum albidum is a perennial plant of enormous importance including pharmacological or medicinal potentials. The study was aimed at evaluating the cholinesterase inhibition and biological activity of *Chrysophyllum albidum* leaf and stem-bark chloroform extract. The biological activities (qualitative, quantitative) were carried out using standard methods. Antioxidant ability of the extracts was assessed using 2, 2-diphenylpicryl-1-hydrazyl (DPPH). The total flavonoids and phenolic contents were expressed as quercetin (QEq) and gallic acids equivalent (GAEq) respectively. Flavonoids, tannins, steroids and saponnins were detected in *C. albidum* leaf extract, while flavonoids, alkaloids, tannins, saponnins, cardiacglycosides and resins were detected in the stem-bark extract. 0.588 and 1.544 mg/QEq were total flavonoids content; 4.448 and 4.536 mg/GAEq were total phenolic content for leaf and stem-bark extracts respectively, the alkaloid content recorded was 1.490 and 3.86% respectively. Total tannins content quantified was 2.39 and 4.08 g/TAEq for the leaf and stem-bark extracts. The plant extracts exhibited *in vitro* antioxidant property by scavenging free radicals generated by DPPH. The leaf extract showed 90% of acetylcholinesterase and butylrylcholinesterase inhibition, with the stem-bark extract showing high acetylcholinesterase inhibition. The extracts are rich in volatile compounds as shown by GC/MS spectra of the stem-bark (9-octadecynoic acid methyl ester, 13-Docosenoic acid, erucic acid and squalene). The leaf extract shows Cyclopropaneoctanal, 9,12-Octadecadienoic acid, Oleic acid, Hexadecanoic acid, 9-Tetradecanal and 9-Octadecenoic acid. The *C. albidum* leaf extract GC/MS spectra showed 54.03% fatty acids with 22.93% hexadecanoic acid, while the stem-bark extract of *C. albidum* contained 50.85% of fatty acid comprising mainly of 9-octadecynoic acid.

Keywords: Biological activity, *Chrysophyllum albidum*,*In vitro* study, Cholinesterase inhibition, Medicinal plant

Introduction

Plants provide many resources, ranging from food, purification of the environment, aesthetic values and health management. They are primary sources of traditional medicines and the synthetic organic compounds. Antioxidants, antibacterial and antifungal are some of the important attributes to plant derived traditional medicine.[1]

Chrysophyllumalbidum is an important medicinal plant found mainly in the tropic (Nigeria) that belongs to the family Sapotaceae.[2] It is predominant in other countries, including Nigeria, Cameroun, Uganda, Cote d' Ivoire and Niger.[3]

It has been employed as a therapeutic agent against many diseases such as yellow and malaria fever, diarrhea and vaginal infections[4], stomachache and diarrhea[5]. The cotyledons are used as unguents for the treatment of vaginal infections[6] and as hypoglycemic and hypolipidemic agent[7], antibacterial[2]. *C. albidum* parts (root, bark and leaf) are employed in managing sprain, bruise and wound; also inhibit microbial growth of known wound contaminants.[3]

Despite the numerous medicinal or therapeutic benefits attributed to the plant, some individuals have reported weakness and anorexia after ingesting this plant extract, thereby limiting its usage. Also, there is increase of antibiotic resistant strains of pathogenic microbes, leading to the emergence of new multi-resistant bacterial strains due to negligence on the use of conventional antibiotics, inability to procure them due to high cost of the drugs and unavailability. There is need to seek for an alternative natural source of remedy for bacterial infection. One of the alternative sources of remedy is *Chrysophyllum albidum.* The study was designed to ascertain the cause of the side effects by characterizing the biological activities of leaf and stem of *Chrysophyllum albidum.*

Materials and Methods

Analytical chemicals used include, Acetylcholine and Butyrylcholine iodide, 2,2-diphenyl-1-picrylhydrazyl, Naphthylethylenediamine hydrochloride, Ascorbate, 1,10- Phenanthroline, Sulfanilamide, 5,5'-dithio-bis (2-nitrobenzoic acid) and Quercetin (Sigma Aldrich, Germany).

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Sample collection

Chrysophyllumalbidum fresh leaf and stem-bark were collected from Edem-ani village, Nsukka Local Government Area on 22nd November, 2019 and identified by Mr. Felix Nwafor of Pharmacognosy Department, university of Nigeria, Nsukkaas *ChrysophyllumalbidumG. Don*. (*Sapotaceae)* and deposited in their herbarium with voucher number PCG/UNN/0359.

Sample preparation

The leaf sample was dried at 25ºC (room temperature) for 7 days, milled into fine particles using electric blender and stored in a refrigerator (4ºC). 200 g *Chrysophyllumalbidum* leaf and stem-bark powder was soaked in 500 mL of 70 % chloroform for 48 h in an air tight glass container. After maceration, the mixture was filtered, concentrated and used for analysis.

Qualitative phytochemical analysis

Qualitative phytochemical determination of *C. albidum* leaf and stem-bark extract was determined as described by A. O. A. C. method[8], with little modification. The following phytochemicals were screened alkaloids, cardiac glycosides, tannins, saponnin, flavonoids, resins, steroids and terpenoids.

Quantitative phytochemical analyses of Chrysophyllumalbidum leaf and stem extract

The total phenolic content was evaluated as described by Nwiduet al.[9]. Total flavonoids, alkaloid and tannins concentration of *C. albidum* leaf and stem-bark extract was determined as described by Senguttuvan et al.[10] Total flavonoid (TFC) content was quantified as µg quercetin equivalent/gram of extract.

In-vitro antioxidant activity

1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging potential.

DPPH scavenging potential of *Chrysophyllum albidum* leaf and stem-bark extract was assessed using DPPH as described by Shen *et al*.[11] The radical scavenging potential was estimated as the percentage using the equation below;

$$\text{DPPH radical scavenging (\%)} = \frac{Control\ absorbance - Sample\ absorbance}{Control\ absorbance} \times 100$$

Acetylcholinesterase and Butrylcholinesterase inhibitory activity assay

Acetylcholinesterase and butrylcholinesterase inhibition were assayed using Ellman*et al*.[18] methods, using their standard substrates (acetylthiocholine and butrylcholine iodides). Each sample concentration was mixed with 500 µL enzyme solution, incubated at 37°C for about 45 min. Absorbance was read at 412 nm after adding 3.5 mL; 0.5 mM acetylcholine and butyrylthiocholine, 1 mM DTNB, in 0.05M phosphate buffer (pH 7.20) using JENWAY 6404 spectrophotometer. Assay reactions with plant sample were all performed in duplicate. The percentage enzyme inhibition was calculated as follows;

The acetylcholinesterase and butyrylylcholinesterase activities were calculated with the formula.

$$AChEactivity\ \% = \frac{Ao - A1}{Ao} \times 100$$

$$BChEactivity\ \% = \frac{Ao - A1}{Ao} \times 100$$

Where A_o is control absorbance and A_1 is sample absorbances

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis was used to detect the volatile compounds present in the leaf and stem-bark extracts. on GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (Schimadzu GCMS-QP2010). The oven temperature was programmed from 110°C with an increase of 10°C/minute, to 200°C, then 5°C/minute to 280°C, ending with a 9 minutes isothermal at 280°C.

Statistical analysis

Statistical analysis was done using the statistical program GLM. Mean of values were compared using independent t test of significance ($p < 0.05$).

Results and Discussion

Table 1 shows the phytochemicals (flavonoids, alkaloids, saponnins, cardiac glycosides, tannins, steroids, resins and terpenoids) present in the extracts of *C. albidum*. Alkaloids, glycosides, resins and terpenoids were not detected in the leaf extract, while terpenoids were not detected in the stem-bark extract.

The stem-bark extract contains more compounds than the leaf extract as shown (Table 1). In the work of Morufu*et al.*[13], tannins, saponins, flavonoids and steroids were also detected, with additional terpenoids. The additional terpenoid reported could due to difference in the extracting solvent used (methanol and water). Also, Akaneme[14] reported the presence of alkaloids, resins and terpernoids in the leaf extract of *C. albidum*, which were not detected in the present study. The difference in the phytochemicals detected may be as a result of the extraction method (boiling), that may have destroyed some other phytochemicals due to high temperatures. Some phytochemicals such as alkaloids, terpenoids, resins and cardiac glycosides were not detected in this study.

Table 2 shows the quantitative analysis of total flavonoids, phenolics, alkaloids and tannins contents of the leaf and stem-bark extracts as 1.544 mg/QEq and 4.536 mg/GAEq, 3.86 % and 4.08 mg TEq/g respectively, while total flavonoids, phenolics, alkaloid and tannin contents of the leaf extract were 0.588 QEq, 4.448 GAEq, 1.49 % and 2.39 mg TAEq/g respectively. Belinda *et al.*[15] reported a higher total phenolic content of 156 mg/QEq and total flavonoid content of 31.16 mg/GAEq. This is higher when compared to the concentration of total phenolic content of *C. albidum*obtained from different organic solvent of extracts of the seed, fruit pulp and skin that yielded 6.72, 1.28 and 12.66 and that of the flavonoids were 9.42, 2.69 and 11.66 respectively expressed as mg/GAEq.[16]

George *et al.*[2] also detected flavonoids and steroids but not saponnins and tannins. The presence of flavonoids and phenolic in the extracts could be responsible for some of the beneficial effects attributed to the plant. Flavonoids inhibit enzymes involved in free radical production such as cycloxygenase, lipoxygenase or inducible nitric oxide[17].

There are many methods to determine the antioxidant potentials of plant materials. Some common methods include reducing power and DPPH assay.[4]

The result of ferric cyanide reducing power assessment of the plant extract is shown in Figure 1. The absorbance of the extract increased with increasing concentration, indicating its antioxidant properties. This finding supports a report by Oloyede and Oloyede[18], which showed that the antioxidant activity of *C. albidum*fruit is very high (92.5%).

Table 1: Qualitative Phytochemical analysis of *Chrysophyllumalbidum* leaf and stem extract

Phytochemicals	Leaf	Stem
Flavonoid	detected	detected
Alkaloid	not detected	detected
Saponin	detected	detected
Glycosides	not detected	detected
Tannins	detected	detected
Resins	not detected	detected
Steroids	detected	detected
Terpenoids	not detected	not detected

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The high antioxidant activity may be as a result of high amount of phenolic compounds present in the fruit. Another study indicated that antioxidant activity of *C. albidum*exocarp was concentration dependent[19], which correlates well with our finding.
Similarly, the leaf extract also showed comparable significant antioxidant and free radical scavenging capacities as reported[3, 20].
Figure 2 shows the DPPH radical scavenging ability of the extracts exhibiting a concentration dependent potential and it was seen that as the concentration of the extracts increased, the inhibition decreased. This is in agreement with the work reported by Belinda et al.[15], where the antioxidant activities increased as the concentration decreased. The free radical scavenging ability of the plant extract could be due to the rich phytochemicals contained therein, including flavonoids, which contain phenolic hydroxyl groups, responsible for the radical scavenging and chelating ability of many plants used as food and medicine[21]. Such free radical scavenging potentials could prevent oxidative stress–related chronic diseases or disorders[22].
Morufu*et al.*[2] reported positive result of the plants leaf which showed 69-88% with increasing concentration of extract. George *et al.*[2] also reported similar increasing DPPH scavenging activity with concentration of the methanol and schnapps extract of Chrysophylumalbidum.
*C. albidum*stem-bark extract showed 92.31 and 23.07 % inhibition of actylcholinesterase and butyrylcholinesterase activity respectively, while 97.95 and 97.70% were the percentage inhibitions of actylcholinesterase and butyrylcholinesterase activities of *C. albidum*leaf extract respectively. *C. albidum*stem-bark exhibited high acetylcholinesterase inhibition, with low inhibition against butyrylcholinesterase. The cholinesterase inhibitory property of the extract could beresponsible for dizziness experienced by individuals who ingest it. Phytochemicals constituents of plant extracts are responsible for the inhibition of butyrylcholinesterase activity *in vitro*[28]. Obohet *al.*[23] obtained a lower acetylcholinesterase inhibition in their study on aqueous extract of C. *albidum*fruitpulp, seed coat and bark coat which ranged from 12-57%, indicating that the leaf of *C. albudum* has more acetylcholinesterase inhibitory potential.

Gas-chromatography-Mass spectroscopy (GC-MS).
Gas chromatography-mass spectrometry analysis is an important tool for the identification of organic compounds[24]. The spectra of the obtained corresponded with fifteen organic compounds present in the extract of the *C. albidum* leaf as follow: 9,12-tetradecadien-1-ol acetate (3.23 %), 11-octadecenoic acid methyl ester (7.87 %), octadecanoic acid-2-oxomethyl ester (5.31 %), tridecanoic acid methyl ester (5.36 %), n-hexadecanoic acid (7.60 %), 13,16-octadecadienoic acid methyl ester (5.15 %), 14-Methyl-14-(3-oxobutyryloxy)-hexadec-15-enoic acid methyl ester (4.95 %), 11-octadecenoic acid methyl ester (2.79 %), 9-octadecynoic acid methyl ester (17.96 %), 13-Docosenoic acid (2.97 %), erucic acid (1.15 %), with 2-Methyl-Z,Z-3,13-octadecadienol having the highest concentration (34.54 %) and squalene having the lowest concentration (1.12 %).
Similarly, Hexadecanoic acid (14.7 %) was the major compound detected. The concentration of Hexadecanoicacid (14.7 %) is high[25], when compared Hexadecanoic acid (7.60 %) reported in this study. Hexadecanoic acid is a fatty acids responsible for the anti inflammatory property of oils[26], which has added to the pharmacological potentials of the plant materials.
Also, the spectra of the obtained corresponded with thirteen organic compounds present in the extract of the *C. albidum* stem-bark extract are as follow: Propanoic acid (0.83 %), Ethanol (1.48 %), Butane (0.74 %), Ethanol (0.59 %), Pentadecanoic acid (1.10 %), Hexadecanoic acid (21.85 %), 9,12-Octadecadienoic acid(0.94 %), 11-Octadecenoic acid (3.60 %), Octadecanoic acid (0.89 %), Cyclopropaneoctanal (40.36 %), 9,12-Octadecadienoic acid (7.92 %), Oleic acid (15.12 %), Hexadecanoic acid (1.08 %), 9-Tetradecanal (1.97 %) and 9-Octadecenoic acid (1.53 %). Concentration of oleic acid (15.12 %) reported was higher when compared to the concentration (10.56%) detected in *Newbouldialaevis* reported by Iwu*et al.*[27].Iwu*et al.*[27] reported the presence of oleic acid, octadecanoic acid, and squalene, which were some of the volatile compounds suspected to be responsible for the biological activities reported in the work. Similarly, high concentrations (27.53 and 21.316 %) of oleic acid were detected and quantified in the leaf of *N. laevis* and *F. exasparata*[28]

Table 2: Quantitative phytochemical properties of *C. albidum* leaf and stem extract

Parameter	Stem	Leaf
Total flavonoid content (mg/QEq)	1.544 ± 0.02	0.588 ± 0.19
Total phenolic content (mg/GAEq)	4.536 ± 0.16	4.448 ± 0.72
Alkaloids content (%)	3.860 ± 1.04	1.490 ± 0.06
Tannins content (mg/TEq/g)	4.080 ± 0.08	2.390 ± 0.39

Table 3: The reducing power of *Chrysophyllumalbidum leaf and* stem extract

Conc. (mg/mL)	Stem (%)	Leaf (%)
50	81.03±0.04	83.72±0.58
100	84.4±0.53	88.10±0.14
150	76.70±0.01	92.30±0.14
200	54.40±0.80	94.81±0.23
250	30.90±0.02	96.28±0.19

Table 4: Cholinesterases inhibition properties of *C. albidum*leaf and stem extract

Parameter	Stem	Leaf
Acetylcholinesterase Inhibition (%)	92.31	97.95
Butylrylcholinesterase Inhibition (%)	23.07	97.70

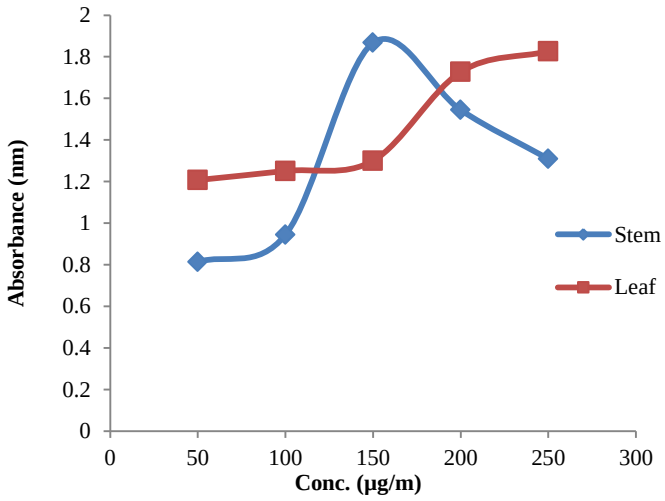

Figure 1: The reducing power of *Chrysophyllum albidum leaf and* stem extract

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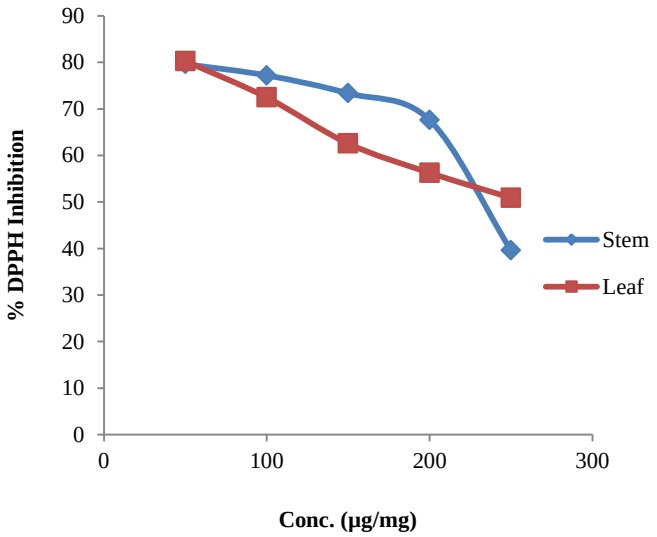

Figure 2: DPPH inhibition of *Chrysophyllumalbidum*leaf and stem extract.

Conclusion

Chrysophyllumalbidum(leaf and stem-bark) extracts were rich in flavonoids, tannins, phenolics and alkaloids that have exhibited antioxidant activities. The leaf extract showed high acetylcholinesterase and butyrylcholinesterase inhibition. This could be responsible for the side effects observed as side effect after its ingestion. Some volatile compounds identified were Hexadecanoic acid (22.93%), 9,12-Octadecadienoic acid (7.92%), Oleic acid (15.12%), Hexadecanoic acid (1.08%), 9-Tetradecanal (1.97%), tridecanoic acid methyl ester (5.36%), n-hexadecanoic acid (7.60%), 13,16-octadecadienoic with 2-Methyl-Z,Z-3,13-octadecadienol having the
highest concentration (34.54%) and squalene having the lowest concentration (1.12%).

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

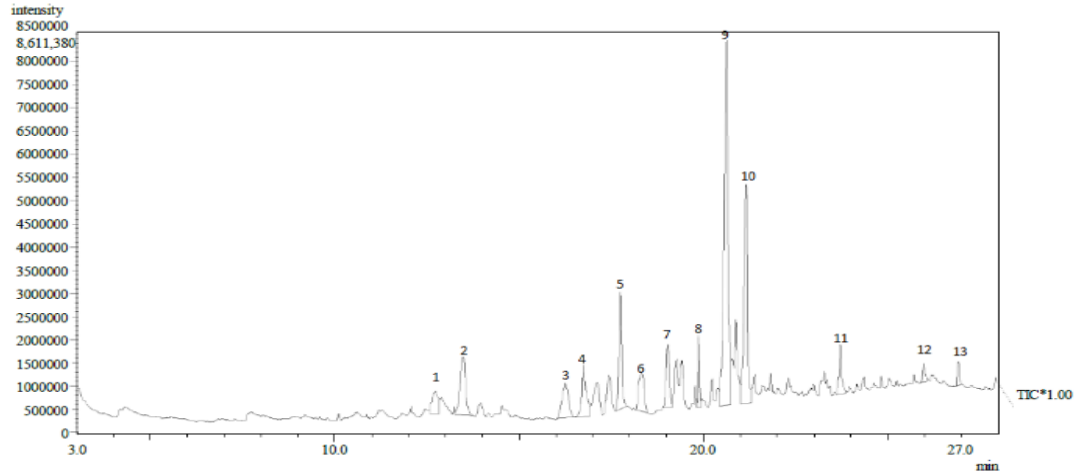

Figure 3: Gas chromatogram of*C. albidum* leaf extract

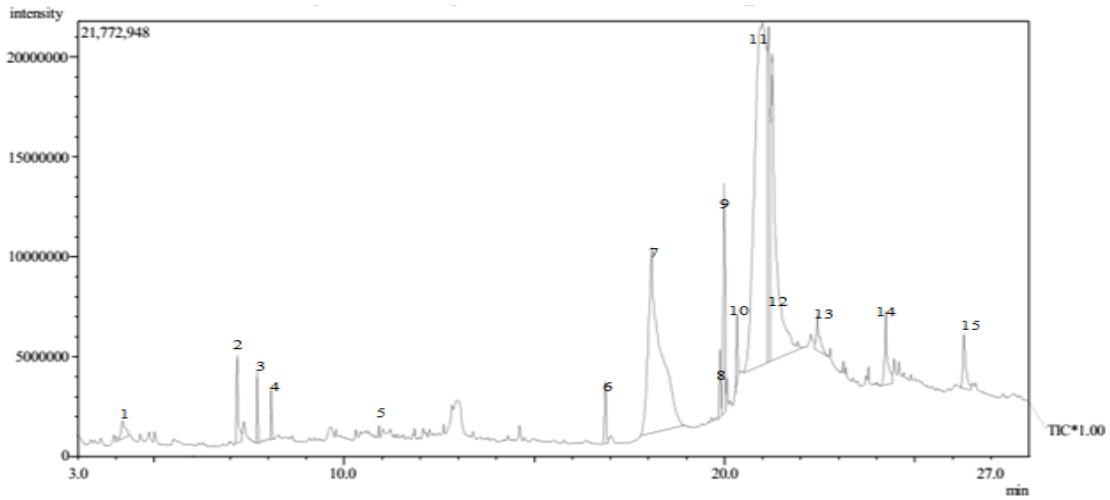

Figure 4: Gas chromatogram of *C. albidum* stem-bark extract

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