

Screening for Potential Antioxidants from *Chrozophora senegalensis* Crude Extract

Kwaji Andrew^{1*}, Saminu Yunana¹, Abubakar Samaila¹,
Atiko Rejoice¹ and Aliyu, U. Elnafaty²

¹Department of Chemistry
Faculty of Science
Gombe State University
PMB 127 Gombe.

²College of Medical Sciences
Gombe State University
PMB 127 Gombe

Email: andrewkwaji@yahoo.com.

Abstract

Numerous reports have indicated oxidative stress as the under-lying factor or associated with the onset or progression of numerous human ailments and disorders such as dementia, Alzheimer disease, diabetes, macular degeneration and aging. *Chrozophora senegalensis* (*C. senegalensis*) is a plant used in traditional medicine to cure both infectious and non-infectious diseases. This research was aimed to carry out the antioxidant activities guided isolation and characterization of phytochemicals from *C. senegalensis* methanol crude extract. The plant sample was extracted using maceration and concentrated on a rotary evaporator at 45°C. Three different but complementary methods; Ferric reducing antioxidant power (FRAP), Nitric oxide and hydrogen peroxide assays were used to evaluate the antioxidant activities of *C. senegalensis* crude extracts. Column chromatography was used to isolate and purify the compounds from the most active n-hexane fraction and coded as CSC-1, CSC-2 and CSC-3. The compounds were characterized using NMR (¹H & ¹³C), GC-MS, melting point determination and in comparison with literature. The percentage free radical scavenging activity for FRAP, nitric oxide and hydrogen peroxide assays were 94.51%, 92.38% and 91.67% respectively while that of ascorbic acid standard was 95.43±0.76%. The isolated compounds were identified as 1-hexacosanol (CSC-1), 1-triacontanol (CSC-2) and oleic acid (CSC-3). The isolation and characterization of these bioactive compounds from *C. senegalensis* crude extract validates its usefulness in traditional medicine practice.

Key words: Antioxidant activity, Characterization *C. senegalensis*, Compounds.

INTRODUCTION

Plants serve as a major source of antioxidants for the neutralization of free radicals generated during essential cellular activities in living systems. The two main forms of free radicals include the reactive oxygen species which occurs chiefly as hydroperoxyl ion (HO₂) and hydroxyl ion (HO·) and the reactive nitrogen species (RNS) such as nitric oxide (·N=O), nitrous acid (HNO₂) and nitrogen dioxide (NO₂). Although the generation of free radicals is necessary during metabolic processes and immune responses, excess cellular accumulation of

*Author for Correspondence

free radicals leads to oxidative stress which is regarded as the principal cause or under-laying factor for several human ailments such as rheumatism, arthritis, Alzheimer disease, cardiovascular diseases, diabetes and dementia (Kumar *et al.*, 2021). It is also associated with the progression of these disorders. This occurs when excess free radicals generated during normal body physiological activities causes the degradation or mutation of macromolecules such as DNA, lipids and proteins. This breakdown affects cell membrane integrity and normal cell homeostasis. This molecular level cell injury builds up and causes significant changes in cell physiology which in acute or chronic cases can lead to cell malfunction and ultimately manifests as a non-organic disease.

Studies had shown that the consumption of plant phytochemical substances can mitigate, cure or even prevent the onset or progression of diseases associated with oxidative stress (Ayoka *et al.*, 2022). Plant phytochemical substances such as phenolic acids, flavonoids, lignans, stilbenes, tannins and vitamins (A, C and E), coumarins and sterols are known to serve as useful antioxidants (Kumar *et al.*, 2021). *Chrozophora senegalensis* had been reported to contain such useful phytochemical substances with antioxidant and other medicinal properties (Kwaji *et al.*, 2023). The methanol leaf extract of *Chrozophora senegalensis* had been reported to contain several types of flavonoids whose detailed chemical structures had been elucidated and their antioxidant activities properly evaluated. Among the flavonoids isolated, quercetin derivatives and amenthoflavone demonstrated the highest antioxidant properties (Vassalo *et al.*, 2006). In order to characterize a substance as an antioxidant requires more than a single procedure to evaluate its antioxidant property (Oliviera-Silva *et al.*, 2023).

Consequently, the present study has adopted three procedures for the determination of the antioxidant properties of the crude extracts of *C. senegalensis* viz; ferric reducing antioxidant power, nitric oxide and hydrogen peroxide scavenging activities. In view of the lack of reports on the other classes of phytochemical constituents of *Chrozophora senegalensis* based on available literature, there is need to further screen the crude methanol extract fractions of *Chrozophora senegalensis* for the isolation and possible identification of other potential antioxidant compounds. Consequently, we report for the first time the isolation and characterization of 1-hexacosanol, 1-triacontanol and oleic acid from the hexane fraction that displayed the most potent antioxidant activity relative to the ethylacetate and n-butanol fractions of *Chrozophora senegalensis* crude extract. Previous reports had shown that these compounds possess significant antioxidant and antimicrobial properties (Fernandez-Arche *et al.*, 2009; Malarvizhi *et al.*, 2016; Rehan *et al.*, 2020).

MATERIALS AND METHODS

Collection and Plant sample identification

The plant sample (*C. senegalensis*) was collected in November from Yamaltu-Deba of Gombe State, Nigeria and subsequently identified at the Department of Botany of Gombe State University. The plant was compared with a deposited herbarium specimen.

Preparation, Extraction and Fractionation

The *C. senegalensis* sample was dried under shade and pulverized to powder. The powdered sample of *C. senegalensis* (1 Kg) was extracted by soaking in methanol with occasional agitation (maceration technique) for a week. The extract was filtered and clarified with Whatmann No.1 filter paper. The combined extract was concentrated on a rotary evaporator at 45°C to obtain a crude extract. The crude methanol extract of *C. senegalensis* was partitioned or fractionated with n-hexane, ethyl acetate and n-butanol successively. Each of the solvent-solvent extraction process was repeated six times with 250 mL portion of each solvent to obtain sufficient

quantity of a given fraction for column chromatography separation. The combined n-hexane fractions (NHF), ethyl acetate fractions (EAF) and n-butanol fractions (NBF) were each concentrated on a rotary evaporator at 45°C. This afforded three different fractions of the plant methanol extract.

Preparations of Antioxidant Test Reagents

Trichloroacetic acid solution (10%): Trichloroacetic acid (10 g) was transferred into a 100 mL volumetric flask and dissolved in a little amount of distilled water and the total volume was made up to mark with distilled water.

Ferric Chloride Solution (0.1%): Ferric chloride (0.1 g) was transferred in to 100 mL volumetric flask and dissolved in sufficient amount of distilled water. The total volume was made up to mark with distilled water.

Potassium ferricyanide Solution (1.0 %): Potassium Ferricyanide (1.0 g) dissolved in sufficient distilled water and transferred into 100 mL volumetric flask. The total volume was made up to mark with distilled water.

Test Sample stock and working solutions: Washed test tubes were sterilized in autoclave at a temperature of 121°C for 15 min. Extracts of n-hexane, ethyl acetate and n-butanol (0.5 g each) were weighed and dissolved in 0.5 mL of 10% DMSO and 4.5 mL of deionized water to obtain a concentration of 100 mg/mL. Working solutions were prepared from the 100 mg/mL stock solutions for all samples and standard. All other reagents used were prepared using appropriate dilutions of stock solution.

Antioxidant Activity Test Methods

The FRAP assay was performed according to the method reported by Dibala *et al.* (2014) with little modification. Five different concentrations of extract solution (0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL) were transferred into test tubes and 2.5 mL of phosphate buffer pH 6.8 and 2.5 mL of $K_3[Fe(CN)_6]$ solution were added into the test tubes. The mixtures were incubated for about 20 minutes at 50°C. Trichloroacetic acid solution (10%, 2.5 mL) was added to the test tubes. The mixtures were centrifuged at 3000 rpm for 10 min and 2.5 mL of the supernatant was withdrawn from the mixture and mixed with 2.5 mL of distilled water. Freshly prepared $FeCl_3$ solutions (0.1%, 0.5 mL) was added to the dilute mixture and the absorbance of the solution was read at 700 nm using spectrophotometer against blank. A blank solution contains the same solution mixture but without plant extract. An ascorbic acid was used to produce a calibration curve. All experiments were conducted in triplicates. Ferric reducing antioxidant power (FRAP) was calculated using the equation obtained from the linear regression of a standard curve by substituting change in absorbance at 700 nm (ΔA_{700nm}) values for each fraction.

Nitric Oxide scavenging Activity Assay

The nitric oxide scavenging activity assay of *C. senegalensis* was performed according to the method reported by Sreejayan and Rao, (1997). Five different solutions (100 μ L each) of the fractions at various concentrations (0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL) were taken in triplicates in test tubes for plant extracts and standards. Sodium nitroprusside (10 mM, 3 mL) was added to the extracts in each test tube and the mixture of the solutions were incubated at room temperature for about 2.5 hrs. Griess reagent (3 mL) was added to all test tubes. The reaction mixture without the sample served as the negative control. The absorbance of the solutions were measured at 546 nm using spectrophotometer against blank. A typical blank

solution contains the same solution mixture without plant extract and was used to produce calibration curve. The nitric oxide scavenging activity was calculated using the formula below.

$$\text{Scavenging activity (\%)} = [\text{Control OD} - \text{Sample OD} / \text{Control OD}] \times 100$$

Hydrogen Peroxide Scavenging Activity

The hydrogen peroxide scavenging activity of the plant extract was carried out according to the method described by Ruch *et al.* (1989) with slight modification. Five different extract and standard solutions at various concentrations (0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL) were transferred in test tubes. From each concentration, 0.10 mL of the plant extract and standards were taken in triplicates in different test tubes and 0.60 mL of H₂O₂ solution was added. The total volume of solution was made up to 4 mL by adding 3.30 mL phosphate buffer solution. An identical reaction mixture of the solution without the sample served as a negative control. The mixture of the solutions were incubated for about 10 min at room temperature. The absorbance of the mixture of solution was measured at 230 nm against the blank. The hydrogen peroxide scavenging activity was calculated using the formula below.

$$\text{Scavenging activity (\%)} = \frac{\text{OD}(\text{control}-\text{sample})}{\text{OD control}} \times 100. \text{ Where 'OD' is the optical density or absorbance of sample and standard.}$$

Evaluation of fifty percent effective/inhibitory concentration (EC₅₀).

The values of percentage antioxidant activities and concentrations were correlated using the Microsoft Excel window program to obtain a linear regression equation for the plant activity. EC₅₀'s were obtained from the equations generated simply by replacing 'y' by 50 and solving the resultant equation (Oliveira-Silva *et al.*, 2023).

Isolation of secondary metabolites hexane fraction

Column chromatography purification of hexane fraction (8.0 g) of *C. senegalensis* was performed using gradient elution of hexane (100%) followed by hexane/ethyl acetate (0-100%) and then ethyl acetate/methanol (0-20%) at 5% increase in volume of eluting solvents as described by Kwaji *et al.* (2018). A total volume of 300 mL was used for each batch of solvent combination, One hundred and twenty-two fractions of 50 mL were collected. The fractions were pooled together based on similarities in TLC profile to give 16 sub-fractions. Fractions 9-17 which showed one spot was recrystallized from methanol and designated as CSC-1 with R_f value 0.52 in hexane: Ethyl acetate (7:3). The melting point was found to be 79-81°C. A second compound which showed one spot was obtained from fractions 56-78 was washed and also recrystallized from methanol. Its R_f value was 0.58 in hexane: ethyl acetate (6:4) with melting point of 85-87°C and designated as CSC-2. The third compound was obtained from fractions 94-108 which showed two spots. A secondary column was packed and then eluted isocratically with hexane: ethyl acetate (4:6) to obtain a single spot with R_f value of 0.54 in hexane:ethyl acetate (4:6) solvent system and was designated as CSC-3 as shown in Table 5.

Spectroscopic Analysis of Isolated Compounds

Characterization of isolates was performed using nuclear magnetic resonance spectroscopy, NMR (¹H, ¹³C), GC-MS spectra data, melting point determination and in comparison with literature.

RESULTS AND DISCUSSION

The yield of crude extract obtained from *C. senegalensis* sample (1 kg) was 187.00 gram which translates to a percentage yield of 12.50%. while the successive fractionation of the crude

methanol extract (187 g) with n-hexane, ethyl acetate and n-butanol are presented in Table 1.

Table 1: Percentage recovery of fractions.

Extract	color	weight of extract	% yield
n-hexane	dark green	26.2 g	26.2
Ethyl acetate	dark green	14.4 g	14.4
n-butanol	reddish brown	28.5 g	28.5

Antioxidant Activities of *C. senegalensis* crude hexane extract.

Ferric Reducing Antioxidant Power: A linear increase in reducing power was observed over the concentration range 100 – 500 µg/mL for all fractions and the ascorbic acid standard. The n-hexane fraction (62.20%) showed a higher reducing power relative to those of ethyl acetate (9.76%) and n-butanol fraction (17.48%) at 100 µg/mL while that of the ascorbic acid was found to be 73.13% at the same concentration (Table 2). The observed high antioxidant activity of fractions may indicate the presence of antioxidant compounds. The 50% FRAP effective concentrations (EC₅₀) were also evaluated using regression analysis and found 67.61 µg/mL (309.03 µg/mL, 251.19 µg/mL and 23.71µg/mL for n-hexane, ethylacetate, n-butanol fractions and ascorbic acid respectively. This shows that the n-hexane fraction is about three times less active than the standard ascorbic acid while the ethylacetate and n-butanol fractions are about ten-fold less active than the standard. Rutin and 1,3,6-trigalloyl glucose out of five compounds isolated from *Chrozophora oblongifolia* displayed high FRAP values of 2.66 and 7.23 mM AAE/mg respectively. Even when the antioxidant activities were evaluated using DPPH and ORAC assays, the two compounds still retained their dominance over the rest (Abdallah *et al.*, 2022). This shows that the genus *Chrozophora* could be a potential source of useful antioxidant compounds.

Table 2: % FRAP of Fractions and Standard

S/No	Concentration (µg/mL)	n-hexane (%)	Ethyl acetate (%)	n-butanol (%)	Ascorbic acid (%)
1	100	62.20	9.76	17.48	73.14
2	200	66.26	33.74	38.62	76.34
3	300	74.39	49.19	50.00	84.88
4	400	87.40	55.28	66.67	92.20
5	500	94.31	72.76	86.18	96.10

Nitric oxide scavenging activity

The result of nitric oxide assay (Table 3) showed linear increase in nitric oxide activity over the concentration range of 100 – 500 µg/mL for all fractions and ascorbic acid standard. The n-hexane fraction showed the most potent nitric oxide scavenging activity relative to the ethyl acetate and n-butanol fractions. The values for ascorbic acid, hexane, ethyl acetate and n-butanol fraction are 64.97, 28.46, 14.68 and 21.04 % respectively. From regression analysis, the EC₅₀ nitric oxide scavenging activity for crude extract fractions of n-hexane, ethylacetate, n-butanol and ascorbic acid standard are 190.55 µg/mL, 295.12 µg/mL, 257.04 µg/mL and 56.23 µg/mL respectively. In a separate study, Zourgui *et al.* (2020) reported the EC₅₀ values of nitric oxide scavenging activities for the ethanol and aqueous extracts of *Opuntia streptacantha* as 0.02±0.003 mg/mL and 0.12±0.005 mg/mL. These results are in close agreement with those obtained in the current study. On the other hand, *Chrozophora prostata* extract fractions of methanol, ethanol and chloroform exhibited quite significant nitric oxide antioxidant activity with EC₅₀ values of 37.5, 12.0 and 38 µg/mL similar to that of ascorbic acid (32.0 µg/mL) standard (Mohammad *et al.*, 2015). These results underscores the importance of the genus *Chrozophora* as a potential source of antioxidant compounds.

Table 3: Nitric oxide Activity of *C. senegalensis* extract fractions.

S/No	Concentration (µg/mL)	n-hexane (%)	Ethyl acetate (%)	n-butanol (%)	Ascorbic acid (%)
1	100	28.46	14.68	21.04	64.97
2	200	48.60	25.81	36.94	69.16
3	300	64.49	45.95	49.66	80.29
4	400	77.21	62.37	64.49	90.99
5	500	92.58	77.74	79.33	94.91

Hydrogen Peroxide Activity

The result of hydrogen peroxide assay indicated a linear increase in hydrogen peroxide scavenging activity with increases in concentration (100 – 500 µg/mL) for all fractions and ascorbic acid. The hexane fraction (48.04%) showed the most potent hydrogen peroxide scavenging activity relative to the ethyl acetate and n-butanol fractions (Table 4). The EC₅₀ values were found to be 46.77, 114.82, 138.64 and 199.53 µg/mL respectively. The most active hexane fraction is about two and half times less active than the ascorbic acid standard. Mohammad *et al.* (2015) reported the hydrogen peroxide EC₅₀ values of *Chrozophora prostrata* methanol, ethanol and chloroform crude extract fractions as less-than 5 µg/mL with no significant difference relative to that of ascorbic acid standard. In living cells, hydrogen peroxide may decompose to yield hydroxyl radical which potentially leads to lipid peroxidation and deoxyribonucleic acid mutation. Natural antioxidants acting as chain breakers can mitigate such undesirable effects with concomitant significant reduction in oxidative stress (Ghosh *et al.*, 2019). The observed high antioxidant activity of the fractions may indicate the presence of useful antioxidant compounds.

Table 4: Hydrogen peroxide scavenging activity

S/No	Concentration (µg/mL)	n-hexane (%)	Ethylacetate (%)	n-butanol (%)	Ascorbic acid (%)
1	100	48.04	25.49	28.92	67.60
2	200	62.75	39.71	46.57	71.47
3	300	74.02	56.37	62.75	81.76
4	400	83.33	70.10	75.49	93.63
5	500	91.67	81.37	84.80	95.29

Table 5: *C. senegalensis* hexane fraction isolated compounds

SN	Compound	Rf value	Colour	Mass of Isolate	% Recovery
1	CSC-1	0.52	White crystal	82 mg	0.82%
2	CSC-2	0.58	White crystal	64 mg	0.64%
3	CSC-3	0.54	White crystal	96 mg	0.96%

Characterization of CSC-1

¹H and ¹³C NMR of CSC-1

¹H NMR spectrum of CSC-1 (400Hz, CDCl₃) (Fig.2) revealed typical signals from δ 0.95 ppm to δ 3.61 ppm, representing methyl (CH₃), methylenes (CH₂)₂₄ and a de-shielded methylene attached to a hydroxyl (OH) group. The oxygenated carbon C₁ (2H, t) indicated a signal at δ 3.61 ppm. The methylenes Carbons C₂-C₄ (2H, m) also indicated signals at δ 1.56 – 1.30 ppm, C₅-C₂₅ all showed signals at δ_H 1.26 ppm and the methyl protons of C₂₆ (3H, t) indicated a signal at δ 0.88 ppm (Table 6). These assignments strongly agree with literature and suggests the presence of a fatty alcohol (Ali and Sultana, 2016; Oscar *et al.*, 2018).

The ¹³C NMR spectrum of CSC-1 (Fig.3) showed a molecular compound with 26 carbon atoms consisting of 1 methyl (CH₃, 3H, t) and 25 methylene (CH₂, 2H, m) groups. From the ¹³C nmr, the signal at δ 63.09 ppm downfield (C-1) indicates the presence of hydroxylated carbon. The

methylenes (CH₂) from C₂₋₂₅ indicate a signal at δc 29.64 ppm. For the C-26, a terminal alkyl carbon, the signal appears at δc 14.10 ppm (Table 6). The isolated compound CSC-1 showed parent molecular ion [M⁺] peak at m/z 382 amu. Other fragments relative to the base peak are m/z 364(5%), 336,(5%) 181(5%), 167(10%), 153(10%), 125(40%), 111(65%), 97(100%), 83(90%) and 57(85%). The parent molecular ion m/z ratio corresponds with the molecular formula C₂₆H₅₄O and suggests that the compound is 1-hexacosanol (Fig.1.) The fragments at m/z 364 and 336 are due to the loss of H₂O (18 amu) and C₂H₄ (28 amu) respectively. The signal at m/z 181 is characteristic of 1-hexacosanol fragmentation due to the loss of C₁₁H₂₃ (155 amu). Fragments at m/z 97 (100%) base peak is due to loss of a methylene group from m/z 111 or ethylene molecule from m/z 125. The observed mass fragmentation pattern is consistent with literature (Shaaban *et al.*, 2018; Abouelela *et al.*, 2018).

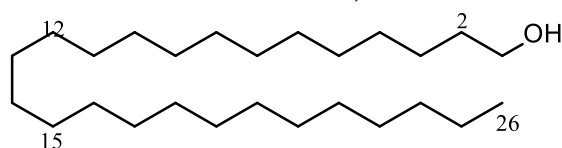


Fig.1: 1-hexacosanol structure

Table: 6: ¹H & ¹³C NMR data of CSC-1 compared with Oscar *et al.*, 2018).

Carbon atom	¹³ C NMR Experimental	¹³ C NMR Literature	¹ H NMR Experimental	¹ H NMR Literature
C-1	63.09	63.09	1.56	1.56
C2-25	29.64	29.65	1.24	1.25
C-26	14.10	14.11	0.95	0.94

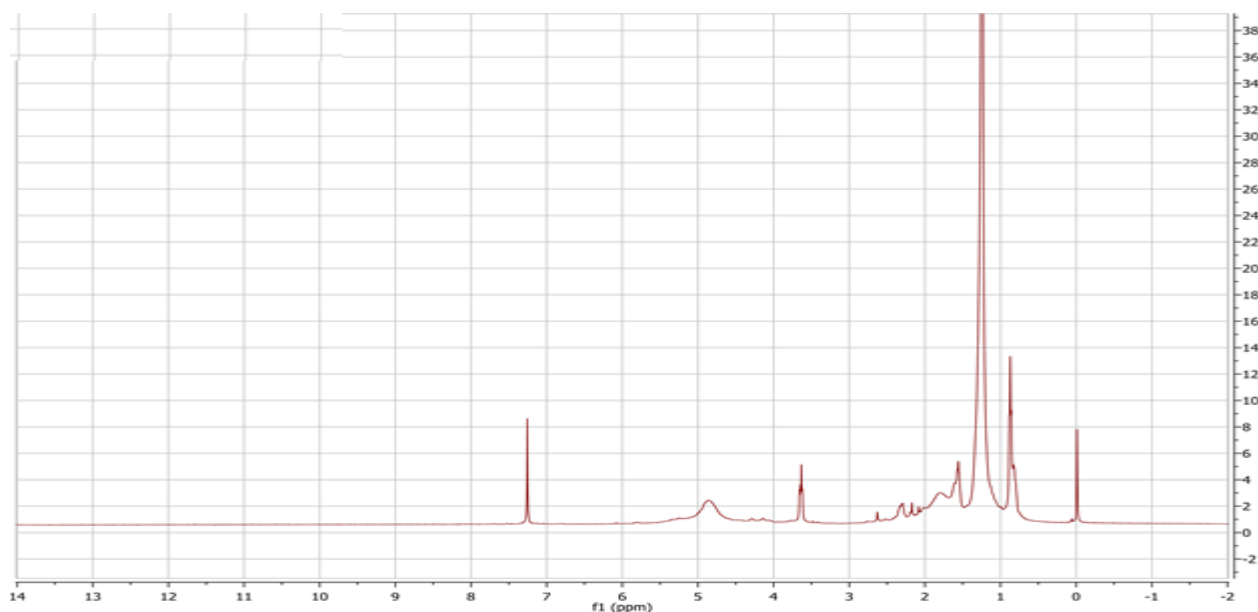
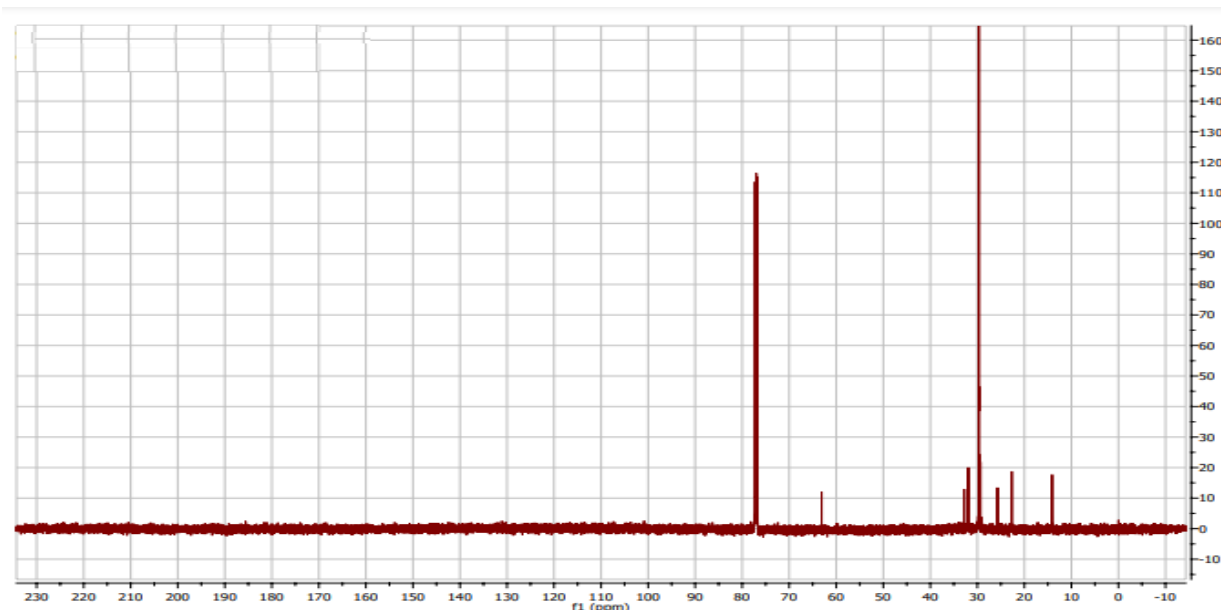


Fig. 2: ¹H NMR of CSC-1

Fig. 3: ^{13}C NMR of CSC-1

^1H and ^{13}C NMR of CSC-2

^1H NMR spectra of CSC-2 (400Hz, CDCl_3) (Fig.4) revealed signals from δ 0.83 ppm to δ 3.61 ppm and represents a methyl (CH_3), methylene (CH_2) groups and a hydroxyl group (OH) group. A signal at δ 1.57 ppm indicates a proton on an oxygenated carbon C-1 (2H, t). Methyl protons at C-30 (3H, t) is indicated by a signal at δ 0.83 ppm. The peak from the hydroxyl group (1H, s) is indicated by a signal at δ 3.61 ppm (Kumari *et al.*, 2017).

The ^{13}C NMR (Fig.5) spectrum showed a molecular compound consisting of 30 carbon atoms. The signal at δ 63.09 ppm downfield indicates C-1 for the presence of hydroxylated carbon. The methylene (CH_2) from C2-29 indicated signals at δ 22.68-33.67 ppm. The C-30 methyl carbon signal appears at δ 14.11 ppm.

GC-MS spectrum of CSC-2 showed parent molecular ion $[\text{M}^+]$ peak at m/z 438 (amu). The m/z of base peak is 57 (100%). The fragment of other peaks relative to the base peak are m/z 420, 392, 209, 195, 181, 167, 153, 139, 125, 111, 97, 83, 57 and 43. Fragments at m/z 420 and 392 may be due to the loss of H_2O (18 amu) and $\text{H}_2\text{C}=\text{CH}_2$ (28 amu) respectively. The signal at m/z 209 characteristic of 1-triacontanol is due to loss of $\text{C}_{13}\text{H}_{27}$ (183 amu). The Fragments at m/z 57(100 %) being the most intense peak results from the loss of C_4H_8 (56 amu) and C_7H_{13} (97 amu). The parent molecular ion m/z ratio corresponds with the molecular formula $\text{C}_{30}\text{H}_{62}\text{O}$ and suggests that the compound is 1-triacontanol (Fig.6). These results are consistent with literature and strongly suggests that CSC-2 is 1-triacontanol (Kumari *et al.*, 2017. and Mori *et al.*, 2020).

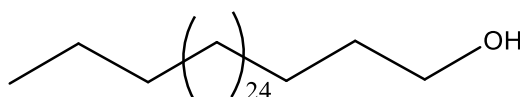


Fig.6: 1-triacontanol structure

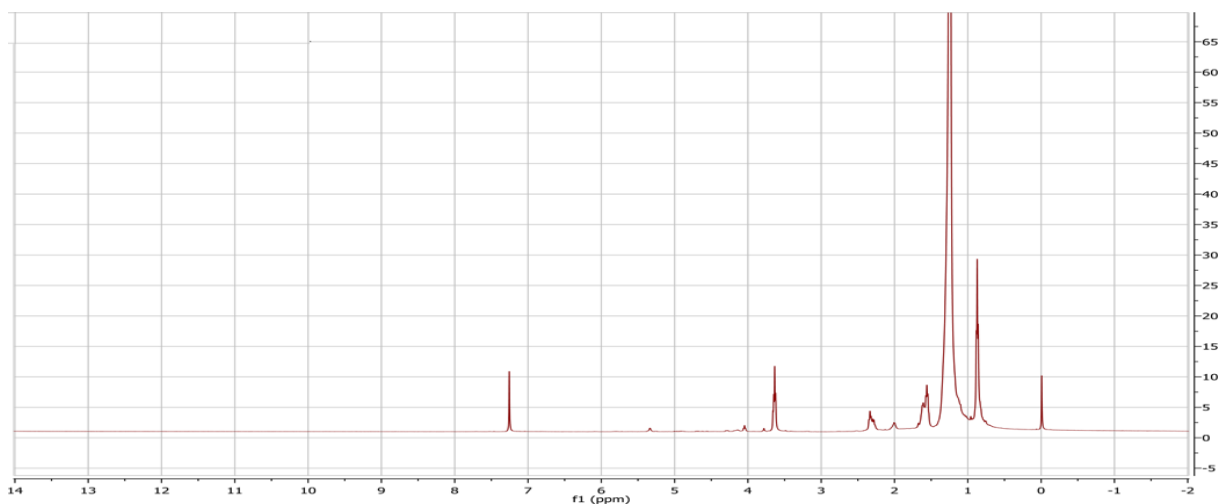


Fig. 4: ¹H NMR of CSC-2

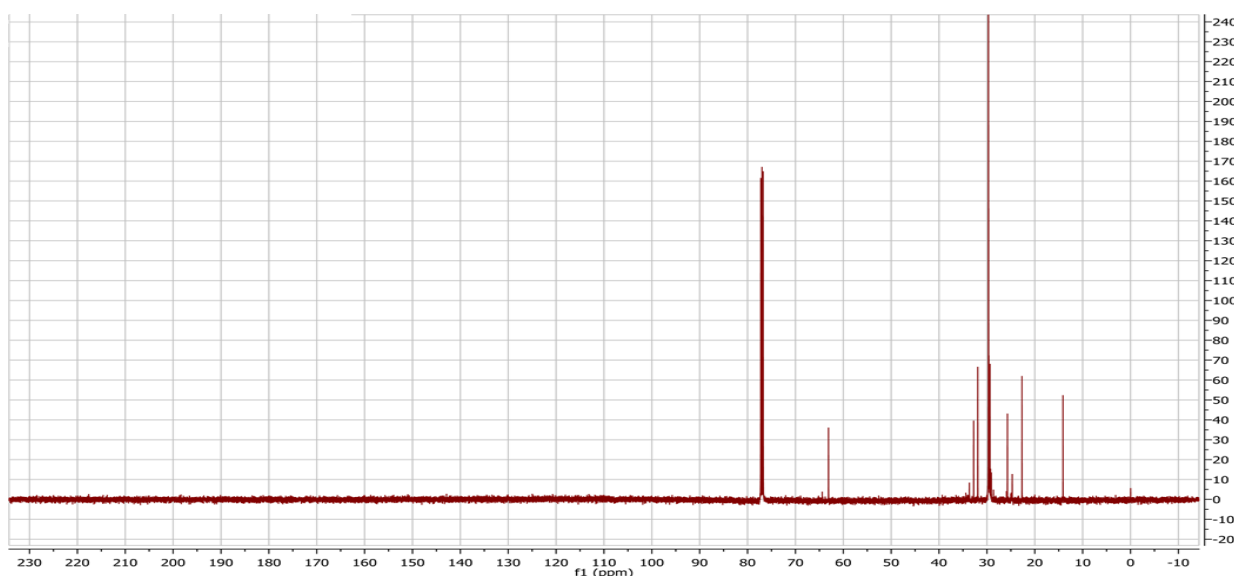


Fig. 5: ¹³C NMR of CSC-2

Table 7: ¹H & ¹³C-NMR data of CSC-2 compared with Kumar *et al.* (2017).

Carbon atom	¹³ C NMR Experimental	¹³ C NMR Literature	¹ H NMR Experimental	¹ H NMR Literature
C-1	63.09	63.11	1.57	1.56
C2-29	29.42	29.44	1.25	1.24
C-30	14.11	14.11	0.83	0.83

¹H and ¹³C NMR of CSC-3

¹H NMR spectrum of CSC-3 (400Hz, CDCl₃) (Fig.6) revealed proton signals at δ_H 0.83 ppm to 5.4 ppm representing methyl (CH₃) at δ_H 0.86, methylene groups (CH₂) at 1.25 - 2.35 ppm, methine carbon (CH) at δ 5.40 ppm and hydroxyl group (OH) at δ 3.7 ppm. The ¹³C NMR spectrum of CSC-3 (Fig.7) showed a signal at δ 178 ppm downfield C-1, indicates the presence of carboxylic acid group. The δ_C 22.68 - 65.57 ppm indicates a chain of methylene groups (CH₂) from C-2 indicate, methine carbons C9-10 (CH) is indicated by signals at δ 129.73 ppm and 130 ppm while the signal at δ 14.11 ppm up-field indicates the presence of methyl (CH₃) group (Table 9). The GC-MS spectrum revealed parent molecular ion [M⁺] peak at m/z 282 (amu). The base peak m/z is 55(100%). The fragments of other peaks relative to the base peak are m/z 264, 222, 165, 151, 137, 123, 111, 97, 83, 69, 55, and 43. The parent molecular ion m/z ratio

corresponds to the molecular formula $C_{18}H_{34}O_2$ and suggests that the compound is oleic acid (Fig.8). The loss of H_2O (18 amu) was indicated by the presence of a fragment at m/z 264 while the m/z 222 amu might be due to the loss of a H_2O (18 amu) and C_3H_6 (42 amu) respectively. The signal at m/z 165 which is characteristic of the oleic acid could be due to loss of C_4H_9 (57 amu). The fragmentation pattern is consistent with literature and confirms the presence of oleic acid (Malarvizhi *et al.*, 2016; Abdelrheem *et al.*, 2020).

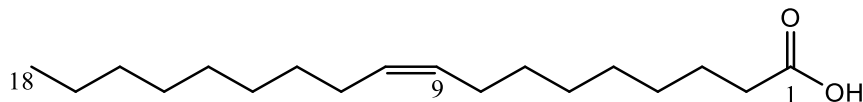


Fig.8: Oleic acid structure

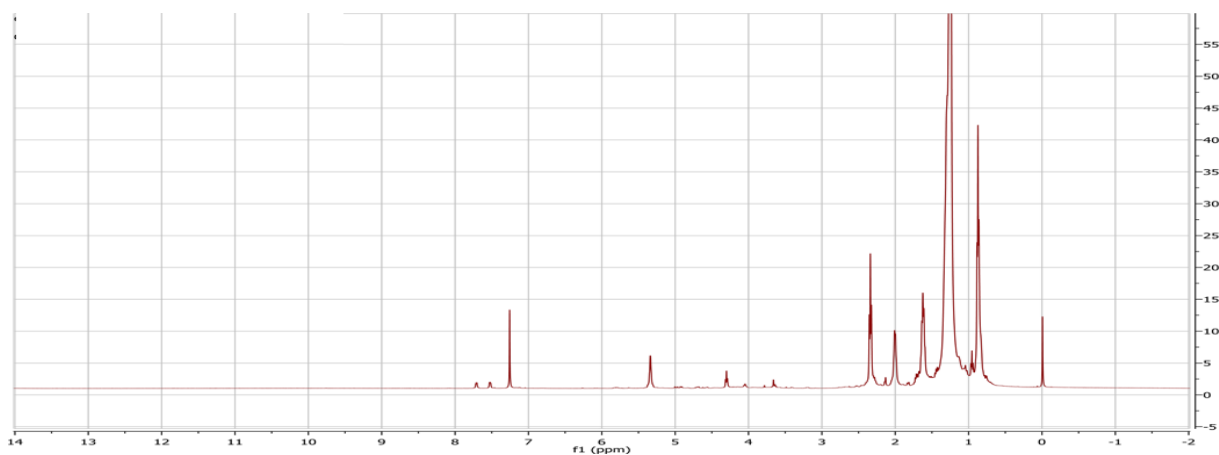


Fig.6: 1H NMR of CSC-3

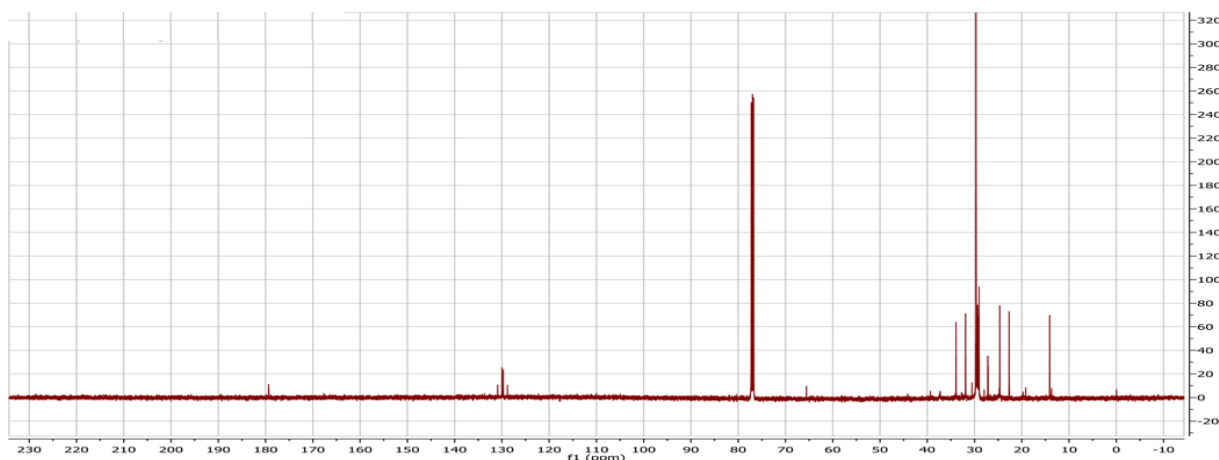


Fig.7: ^{13}C NMR of CSC-3

Table 8: 1H & ^{13}C NMR data of oleic acid compared with Malarvizhi *et al.*, (2016).

Carbon atom	^{13}C NMR Experimental	^{13}C NMR Literature	1H NMR Experimental	1H NMR Literature
C-1	65.57	65.57	4.30	4.31
C-2	29.30	29.30	2.30	2.30
C3-17	29.23	29.23	2.00	2.01
C9-10	129.70-130.1	129.73-130.3	5.40	5.40
C8-11	29.12	29.11	1.35	1.36
C-18	14.11	14.11	0.83	0.83

CONCLUSION

Three compounds were isolated namely, 1-hexacosanol, 1-triacontanol and oleic acid from *Chrozophora senegalensis* crude methanol extract hexane fraction which displayed the most potent antioxidant activity relative to the ethylacetate and n-butanol fractions. Studies had shown that the policosanols 1-hexacosanol and 1-triacontanol identified in this study has the potential to delay or prevent the onset of oxidative stress related diseases such as Alzheimer's, dementia and diabetes; likewise the oleic acid isolated and identified in this study. Additionally, these phytochemicals were reported to possess significant antimicrobial and hepatoprotective properties. The isolation and identification of these phytochemicals validates the ethnopharmacological relevance of *Chrozophora senegalensis*.

ACKNOWLEDGEMENT

We wish to thank the Management of Gombe State University for provision of research incentives. Similarly, our gratitude also goes to Malam Musa Muktar of Biochemistry for the cooperation accorded to us during the data acquisition period.

CONFLICT OF INTEREST.

The authors wish to declare that no conflict of interest exists.

REFERENCES

- Abdallah, H. M., Kashegari, A. T., Shalabi, A. A., Darwish, K.M., El-Halawany, A. M., Algandaby, M. M. *et al.* (2022). Phenolics from *Chrozophora oblongifolia* aerial parts as inhibitors of α -Glucosidases and advanced glycation end products; *In vitro* assessment, molecular docking and dynamics studies. <https://doi.org/10.3390/biology11050762>.
- Abdelrheem, A. A., Rahman, A. A., Elsayed, K. N. M., Abdelmageed, H. R., Mohamed, H. S. & Ahmed, S. A. (2020). Isolation, Characterization, *In-vitro* Anticancer Activity, DFT Calculations, Molecular Docking, Bioactivity Score, Drug-likeness and Admet Studies of eight Phytoconstituents from brown alga *Sargassum platycarpum*. *Journal of Molecular Structure*. <https://doi.org/10.1016/j.molstruc.2020.129245>.
- Aboulela, M. E., Orabi, M. A. A., Abdelhamid, R. A. & Abdelkader, M. S. A. (2018). Chemical and Cytotoxic Investigation of non-polar extract from *Ceiba pentandra* (L.) Gaertn. A Study supported by Computer based Screening. *Journal of Pharmaceutical Sciences*. 8(07): 057-064.
- Ali, M. & Sultana, S. (2016). Phytochemical Investigation of the aerial parts of *Protasparagus falcatus* (L.) Oberm. *Journal of Pharmacognosy and Photochemistry*. 5(5):184-188.
- Ayoka T. O., Ezema, B. O., Eze, E. C. & Nnadi, C. O. (2022). Antioxidants for the Prevention and Treatment of non-communicable diseases. *Journal of Exploratory Research in Pharmacology*. 7(3):178-188.
- Dibala, C. I., Konate, K., Diao, M., Ouedraogo, M. & Dicko, M. H. (2014). Phytoconstituents Analysis, Antioxidant Capacity and Antimicrobial Properties of Extracts from *Laggera aurita* L. (Asteraceae). *International Journal of Pharmacy and Pharmaceutical Sciences*, 6(7): 172-178.
- Fernandez-Arche, A., Martin, A. M., Vazquez, R. P., Perona, J. S., Terecio, C., Perez-Camino, C. *et al.* (2009). Long-chain fatty alcohols from Pomace Olive oil modulate the release of pro-inflammatory mediators. *Journal of Nutritional Biochemistry*. 20(3): 155-162; <https://doi.org/10.1016/j.jnutbio.2008.01.007>.
- Ghosh, P., Biswas, S., Dutta, A., Biswas, M., Das, S., Das, C. *et al.* (2019). Evaluation of Phytochemical Constituents and Antioxidant Property of the Leaf Acetone Extracts of

- Five herbaceous Medicinal Weeds. *Journal of Pharmaceutical Science and Research*. **11**(8):2806-2813.
- Kumari, S., Singh, R., Gurav, N. P. & Mehta, N. (2017). Isolation and Characterization of bioactive compounds from Stem of *Nyctanthes abortivus* Linn and effect of different fractions on Phytopathogens. *Asian Journal of Chemistry*. **29**(4): 787-791.
- Kumar, M., Pratap, V., Nigam, A. K., Sinha, B. K., Singh, M. K. & Gour, J. K. (2021). Plants as source of potential antioxidants and their effective nano-formulation. *Journal of Scientific Research*. **65**(3): 57-73.
- Kwaji, A., Adamu, H. M., Chindo, I. Y. & Atiko, R. (2018). Isolation, Characterization and Biological activities of Betulin from *Entada africana* Guill. and Perr. (Mimosaceae). **3**(1):28-31.
- Kwaji, A., Yunana, S., Abubakar, S. & Atiko, R. (2023). *Chrozophora senegalensis* A. Juss brine Shrimp Activity. *Journal of Pharmacognosy and Phytochemistry*. **12**(5):132-134.
- Malarvizhi, D., Anusooriya, P., Meenakshi, P., Sundaram, S., Oirere E. & Gopalakrishnan, V. K. (2016). Isolation, Structural Characterization of Oleic Acid from *Zaleya decandra* Root Extract, *Analytical Chemistry Letters*. DOI: 10.1080/22297928.2016.1238319
- Mohammad, S., Md.Shawkatul, I. B., Rumana, A. & Mohiuddin, A. B. (2015). *In vitro* antioxidant activities of the whole plant extract of *Chrozophora prostata* (dalz). *Annals of Biological Research*. **6**(4): 19-26.
- Mori, Y., Tanaka, A., Nakagawa, T., Amen, Y., Kuwano, Y., Tanizaki, Y. *et al.* (2020). Isolation and quantification of the plant growth regulator 1-triacontanol from Moso bamboo (*Phyllostachys pubescens*) shoot skin and its compost. *Agriculture and Forestry*. **66** (3): 81-93.
- Oliveira-Silva, S. K., Silva, K. W. L., Maior, L. P. S., Neri, T. S., Azevedo, P. V. M., Gomes D. C, S. *et al.* (2023). Phytochemical characterization, antioxidant potential and antibacterial activity of *Croton argyrophylloides* Muell. Arg (Euphorbiaceae). *Brazilian Journal of Biology*. <https://doi.org/10.1590/1519-6984.236649>.
- Oscar, N. D. Y., Desire, S., Oliver, S., Mala, O. M. T. G. & Barthelemy, N. (2018). Fatty alcohols isolated from *Prosopis africana* and Evaluation of antibacterial and antituberculosis activities. *Journal of Diseases and Medicinal Plants*. **4**(5): 128-132.
- Rehan, M., Shafiullah, Ansari, F. A. & Singh, O. (2020). Isolation, Identification, Antibacterial Activity and Docking of Fatty acid and Fatty alcohol from *Rumex dentatus* Leaf Extract. *International Journal of Pharmaceutical Science Review and Research*. **64**(1): 7-11.
- Ruch, R. J., Cheng, S. J. & Klaunig, J. E. (1989). Prevention of Cytotoxicity and Inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, **10**: 1003-1008.
- Shaaban, M., Ali, M., Tala, M. F., Hamed, A. & Hassan, A. Z. (2018). Ecological and Phytochemical Studies on *Euphorbia retusa* (Forssk) from Egyptian Habitat. *Journal of Analytical Methods in Chemistry*. <https://doi.org/10.1155/2018/9143683>.
- Sreejayan, N. & Rao, M. N. A. (1997). Nitric oxide scavenging by curcuminoids. *Journal of Pharmacy and Pharmacology*, **49**(1): 105-107.
- Vassalo, A., Cioffi, G., De Simone, F., Braca, A., Sanogo, R., Vanella, A. *et al.* (2006). New flavonoid from glycosides from *Chrozophora senegalensis* and their antioxidant activity. *Natural Product Communications*, **1**(12): 1080-1095.
- Zourgui, MN., Hfaiedh, M., Brahmi, D., Affi, W., Gharsallah, N., Zourgui, L. *et al.* (2020). Phytochemical Screening, Antioxidant and Antimicrobial activities of *Opuntia streptacantha* fruit skin. *Journal of Food Measurement and Characterization*. <https://doi.org/10.1007/s11694-020-00518-w>.