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Phytochemical Investigation and Assessment of Antioxidant, Antimicrobial and Cytotoxic Activities of the Root Bark *Chrozophora oblongifolia* (Delile) Spreng. (Euphorbiaceae)

Mohamed R. Kamel[1], Alaa M. Nafady[1], Ahmed M. Hassanein[1], Reham Ragaey[2], Eman G. Haggag[2]*

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assuit branch, Assuit, Egypt*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt*

***Corresponding author:** *Eman G. Haggag, Department of Pharmacognosy, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt. Tel. +201000023022*
Email address: Eman.G.haggag@pharm.helwan.edu.eg.com

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ABSTRACT

Objectives: The present study aimed to investigate and isolate different phytochemical constituents and assess the antioxidant, antimicrobial and cytotoxic activities of the total methanolic extract and different fractions of *Chrozophora oblongifolia* root bark. **Methods:** Phytochemical screening of *Chrozophora oblongifolia* was performed using specific test for each class of compounds. Different chromatographic techniques were used to isolate and purify compounds and their structures were elucidated by using different 1D-NMR spectroscopy (^{1}H NMR and ^{13}C NMR). The antioxidant activity was evaluated using DPPH radical scavenging assay, antimicrobial activity was done by standard agar well diffusion assay and cytotoxic activity was performed by using MTT colorimetric assay method. **Results:** Phytochemical investigation of *chrozophora oblongifolia* revealed the presence of carbohydrates and / or glycosides, sterols and or triterpene, alkaloids, tannins and saponins and the absence of flavonoids and anthraquinones. After fractionation, methyl gallate **(1)**, gallic acid **(2)** and *β*-sitosterol-3-*O*-*β*-D- glucopyranoside **(3)** were isolated from ethyl acetate fractions. The result of the antioxidant activity showed that, the ethyl acetate fraction showed highest antioxidant activity followed by *n*-butanol and total methanolic extract. The result of the antimicrobial activity showed that, the total methanolic extract and ethyl acetate fraction showed moderate activity against *Staphylococcus aureus*, Vancomycin resistant *Staphylococcus aureus* and *Candida albicans*. The result of the cytotoxic activity showed that, the methylene chloride fraction followed by the total methanolic extract showed the highest cytotoxic activity against (MCF-7 and Huh-7) cancer cell lines. **Conclusion:** The root bark of *Chrozophora oblongifolia* is a rich source of different classes of active constituents as phenolics. The total extract and some fractions of *Chrozophora oblongifolia* showing antioxidant and antimicrobial and cytotoxic activities.

Keywords: *Antimicrobial; Antioxidant; Chrozophora oblongifolia; Cytotoxicity; Phytochemical screening*

INTRODUCTION

Natural products have been playing a vital role in health care for decades. Since ancient times, natural products represent the main source of compounds employed in drug discovery and development. Natural products have played central part in treatment and prevention of human diseases during thousands of years. Remedies based on natural substances come from different sources, among them terrestrial plants and microorganisms, sea macro and microorganisms, as well as terrestrial invertebrates and vertebrates. In the last century, several drugs from natural sources have been developed as Taxol from *T. brevifolia*, vincristine from *Vinca rosea* and morphine from *Papaver somniferum*[1]. The plants of family Euphorbiaceae offers economically and medicinally important plants, therefore the plant under investigation was selected to find pharmaceutical compounds and potential applications for the plant extract. Genus *Chrozophora* is a large genus belonging to family Euphorbiaceae with different species which possess different biological activities as *Chrozophora. oblongifolia* which possess antioxidant and antimicrobial activity[2], *Chrozophora hierosolymitana* which possess antifungal and antiyeast activities[3]. Also *Chrozophpra oblongifolia* aerial parts possess an antioxidant, hepatoprotective activities and increasing serum level of androgen in adult male rats[4]. The Phytochemical investigation of the root bark of *Chrozophora oblongifolia* is very limited, thus it was interested for the authors to investigate its secondary metabolites as well as assess its antioxidant, antimicrobial and cytotoxic activities.

MATERIAL AND METHODS

Plant materials

The roots of *Chrozophora oblongifolia* were collected in April 2016 from Saint Catherin, South Siniai, Egypt. The plant was identified and authenticated by Prof. Dr. Salah El-Naggar, Professor of Botany and Plant Taxonomy, Faculty of Science Assuit University, Assuit, Egyp. A voucher specimen (COE-002) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Assuit branch, Egypt.

Instruments

EI-MS was measured on JEOL JMS 600 Hz. ^{1}H NMR and^{13}C NMR spectra were measured on Bruker AMX- 600 and 400 spectrometers with standard pulse sequences operating at 600 and 400 MHz for ^{1}H NMR and 150 and 100 MHz in^{13}C NMR respectively. Chemical shifts are given in δ values (*ppm*) using tetramethylsilane as the internal standard. Column chromatography was carried out using glass columns of different sizes using silica gel (60-120, 70-230 and 230-400 mesh, E-Merck, Germany).

UV absorbances were acquired using the Schimadzu 1601, UV/Visible spectrophotometer (USA). Disposable cuvettes (1cm × l cm x 4.5cm) were used for visible absorbance measurements. Electric balance (Sartorius cpa 3245, Germany), automatic pipettes (AXYPet, Poland), autoclave (ALP, Japan), incubator (Thermo, Germany), laminar air flow cabinet (Nuarine, France), Vortex (Maxi mix II, Canada) and spectrophotometer (Jenway 6304, UK).

Chemicals

Molish's and Dragendorff's reagents were prepared fresh[5,6]. Ammonia, ferric chloride solution, conc. sulphuric acid, acetic anhydride, *n*-hexane, methylene chloride, ethyl acetate, methanol and *n*-butanol (were obtained from El-Nasr Pharmaceutical Chemical Co, Egypt). DPPH (2, 2-Diphenyl-1-picryl hydrazyl), ascorbic acid (were obtained from Sigma-Aldrich Chemicals Co, Germany). Dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Germany), McFarland standard (Sigma Aldrich, Germany), normal physiological saline (Otsuka, Egypt), Mueller-Hinton agar (Oxoid, UK), Amoxicillin (amx) as antibacterial standard (E.P.I.Co, Egypt), Cyclohexamide (CHX) as anti yeast standard (Bio basic ink, Canada) and (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) (MTT) (Biobasic / Canada). β-sitosterol-3-*O*-β-D-glucopyranoside (Department o Pharmacognosy, Al-Azhar University, Assiut branch, Assiut, Egypt).

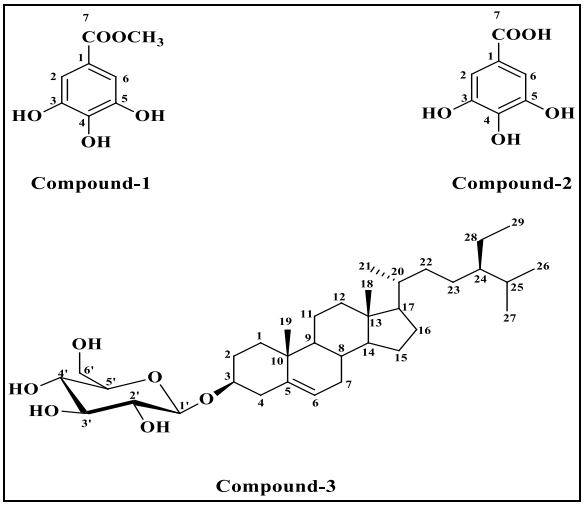

Figure 1. Structures of isolated compounds from *Chrozophora oblongifolia*

Reference pathogenic microbes

Staphylococcus aureus ATCC 25923, *Escherichia coli* ATCC 25922 *Pseudomonase aeruginosa* ATCC 27853, *Candida albicans* ATCC 10231 and clinical isolate of *VRSA* (Vancomycin resistant Staphylococcus aureus), which were used for assessment of antimicrobial activity were obtained from Egyptian Company for Production of Vaccines, Sera and Drugs (VACSERA), Egypt.

Cells lines

MCF-7 cell line (human breast cancer cells) and Huh-7cell line (human hepatocyte-derived carcinoma cells) are obtained from Department of Agro-Environmental Sciences, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

Experimental

Extraction and preliminary phytochemical screening

The air-dried root bark (2.2 Kg) of *Chrozophora oblongifolia* was extracted by maceration in 70% aqueous methanol till complete exhaustion [3 x 6 L, overnight]. The filtered methanolic extract was concentrated under reduced pressure to give a dark brown syrupy residue (400 g). A small part of the methanolic extract was subjected to preliminary phytochemical screening, while the rest was exposed to successive liquid/liquid fractionation.

Preliminary phytochemical screening was performed using 10 g of the total aqueous extract following the standard methods as stated below per each experiment.

a. Carbohydrates and / or glycoside: The aqueous solution of total extract of *Chrozophora oblongifolia* was combined with a few drops of Molisch's reagent (alcoholic solution of α-naphthol) in test tube. thereafter, 1ml of concentrated sulphuric acid was slowly added down the sides of the sloping test-tube without mixing to form a layer, a purple ring at the interface between the aqueous and organic layer indicating the presence of carbohydrates and / or glycoside [5].

b. Sterols and/or triterpenes: 5 mL of chloroform was added to the total extract, and then filtered. To the filterate, was added 2mL of acetic anhydride/Conc. H_2SO_4. The formation of a violet ring indicates the presence of sterols and or triterpenes[5].

c. Flavonoids: The total extract of *Chrozophora oblongifolia* was mixed with 2 ml of 2% NaOH solution, the development of an intensive yellow color, indicates the presence of flavonoids [5].

d. Tannins: The total extract of *Chrozophora oblongifolia* was mixed with 2 ml of 2% $FeCl_3$, the appearance of black or blue-green color indicates the presence of tannins[5].

e. Alkaloids and/or nitrogen bases: The acidic solution of total extract of *Chrozophora oblongifolia* was evaporated till dryness and the extract was dissolved in 2 ml of HCl, the formation of very faint brown precipitate with Wagner's reagent indicating the presence of alkaloids and / or nitrogen bases[6].

g. Anthraquinone: The alcoholic solution of the total extract of *Chrozophora oblongifolia* was boiled with 1ml dil. H_2 SO_4, then shaked with chloroform. The chloroform layer was transferred to another test tube and mixed with dil. NH_3. The development of rose red color indicates the presence of anthraquinone[6, 7].

f. Saponins: The alcoholic solution of total extract of *Chrozophora oblongifolia* was treated with equal volume of suspension of RBCs in normal saline and shaked gently, a clear red solution indicating the presence of saponins[8].

Main extract fractionation and phytoconstituents isolation

About 390 g of the total aqueous methanolic extract was subjected to successive liquid-liquid fractionation using *n*-hexane, methylene chloride, ethyl acetate and *n*-butanol till complete exhaustion for each fraction to give *n*-hexane (7 g), methylene chloride (11 g), ethyl acetate (17 g), *n*-butanol (140 g) soluble fractions and a residue (180 g). A part of the ethyl acetate soluble fraction (15 g) was chromatographed on silica gel (450 g), eluted with methylene chloride (100%), then with gradient addition of methanol till 100% methanol. Fractions were collected, each of about 300 mL. Similar fractions were pooled together, concentrated under reduced pressure to give nine subfractions labeled (Et-Co1-9). Firstly, subfraction (Et-Co3) was purified on silica gel and eluted with methylene chloride followed by methylene chloride-methanol gradient elution from (99:1) to (85:15), the fractions eluted with methylene chloride- methanol (94:6) afforded compound **1** (17 mg). Secondly, subfraction (Et-Co5) was purified on silica gel and eluted with methylene chloride- ethyl acetate-methanol- water (40:80:11:2) isocratic elution, affording compound **2** (13 mg). Thirdly, subfraction (Et-Co-8) was purified on silica gel column chromatography and eluted with methylene chloride-methanol gradient elution from (99:1) to (80:20), the fractions eluted with methylene chloride- methanol (85:15) afforded compound **3** (25 mg).

Biological investigation

Antioxidant activity

Antioxidant activity was determined by DPPH radical scavenging method[9]. DPPH 10×10^{-5} M solution was prepared by dissolving 40 mg of DPPH in 1000 ml ethanol. Ethanolic solution (0.2 mL) of each different fractions of *Chrozophora oblongifolia* of different concentrations (0.0625, 0.125, 0.25, 0.5, 1mg/ml) was mixed with 2 ml of ethanolic solution of DPPH (0.1mM). Similarly; 0.2 ml ethanolic solution of ascorbic acid of different concentrations (0.625, 0.125, 0.25, 0.5, 1mg/ml) were mixed with 2 ml of DPPH solution. A mixture of 0.2 ml of ethanol and 2 ml of ethanolic solution of DPPH (0.1 mM) served as control. Solutions were shaked and left for 30 min at room temperature. Absorbance was measured at λ_{max} 517 nm. The experiments were carried out in triplicate manner using ascorbic acid as a referance standard and antioxidant activity was expressed as percentage of DPPH radical scavenging relative to control using the following equation[10].

$$\%\ \text{DPPH radical scavinging activity} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} \text{X } 100$$

The scavenging effect of each tested sample was expressed as $SC_{50,}$ which is the concentration of the extract required for 50% scavenging of DPPH radicals compared with that of standard ascorbic acid. The decrease in the absorbance indicates an increase in DPPH radical scavenging activity.

Antimicrobial activity

Total methanolic extract, methylene chloride, ethyl acetate and *n*-butanol fractions dissolved in DMSO and adjusted at concentration 50 mg/ml[11]. Overnight cultures of standard pathogenic strains were prepared in nutrient agar at 37 ºC. Pure colonies of overnight plates were picked up and inoculated in 10 ml of 0.9 % normal saline, and the turbidity adjusted to be equivalent to 0.5 McFarland standards (prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate, with 9.95 mL of 1% sulphuric acid), then the optical densities (ODs) of cell suspensions were adjusted to obtain 0.45 absorbance units at λ_{max} 650 nm. The adjusted suspensions were used within 15 minutes. Using the standard well diffusion assay, 500 µl from each cell suspension was placed in sterile petri dish and 20- 25 ml of Mueller-Hinton medium (beef extract, acid hydrolysate of casein, starch, agar and distilled water) were poured into the plates for the preparation of seeded media. After solidification, two wells were made in agar using 0.7 cm cork porer, and each well was inoculated with 100 µl of the various tested extract. The effect of DMSO against all used pathogenic strains was tested as a negative control and the effect of standard antibiotics (Amoxicillin and Cyclohexamide, at concentrations 50 mg/ml), also tested as a positive control. All plates were incubated at 37 ºC for 24 hours. After incubation period, the diameters of inhibition zones around the wells were measured to assess the antimicrobial activity quantitatively[12].

The experiments were carried out in triplicate manner using Amoxicillin as a reference antibacterial standard and Cyclohexamide as a reference anti yeast standard.

Cytotoxic activity

Principle of assay

In this assay water soluble Yellow MTT [3-(4, 5- Dimethylthiazol-2-yl)- 2, 5-diphenyltetrazolium bromide, a tetrazole] is reduced to insoluble purple formazan by the mitochondria of the living cells. This reduction based on the mitochondria succinate dehydrogenase enzymes activity. The colored solution before and after reduction can be quantified by measuring its absorbance at a certain wavelength (usually between 500 and 600 nm). An increase in the viable cell number results in an increase in the amount of insoluble MTT formazan formed and subsequent increase in the measured absorbance[13].

Method of assay

The cell lines seeded in 96-well plates at a concentration of 1×10^4 cells/well were treated with two different concentrations of the total methanolic extract, *n*-hexane, methylene chloride and ethyl acetate subfractions (125 µg/mL and 250 µg/mL) and incubated in a humidified 5% CO_2 atmosphere at 37 °C.After 48 h incubation, 10 µL of 5 mg/ml MTT solution was added to each well and incubation continued for further 4 hrs. After removal of the supernatant, formazan crystals were dissolved in 100 ml DMSO. The reaction was stopped with acidified isopropanol solution, and the plate incubated in the darkness overnight at room temperature before reading the absorbance at 570 nm using ELISA reader. Triplicate experiments were performed. 5-Fluorouracil was used as a positive control. The percentage cell viability was determined as follows:

$$\%\ \text{Cell viability} = \frac{\text{Mean optical density(OD) of sample treated cell}}{\text{Mean optical density(OD) of control}} x\ 100$$

% cell inhibition (cytotoxic activity) = 100-cell survival

Statistical Analysis

Experimental results are expressed as mean ± standard error. Results were statistically analyzed using analysis of variance (one-way ANOVA) followed by

Tukey's t test for comparison between different groups. SPSS 20 version was used for the statistical analysis.

RESULTS AND DISCUSSION

Phytochemical screening

The total methanolic extract of the root bark of *Chrozophora oblongifolia* (Delile) Spreng was subjected to preliminary phytochemical screening to detect the presence or absence of the following constituents: carbohydrates and/or glycosides, sterols and/or triterpenes, flavonoids, tannins, alkaloids and/or nitrogenous bases, saponins, and anthraquinone. The results of phytochemical screening are compiled in the **Table 1**.

Table 1. Results of phytochemical screening of the root bark of *Chrozophora oblongiolia*

Constituents	Results
Carbohydrates and/or glycosides	+
Sterols and/or triterpenes	+
Flavonoids	-
Tannins	+
Alkaloids	+
Saponins	+
Anthraquinones	-

(+): present, (-): absent.

Compound 1: obtained as white amorphus powder (methanol), m.p. 200– 202 ºC. It is insoluble in *n*-hexane and chloroform, but soluble in methanol. It gave bluish green color with $FeCl_3$ indicating its phenolic nature[14]. It showed a single spot on precoated silica gel plates G_{60} F_{254} attained faint brown color after spraying with 10% *v/v* sulphuric acid and after heating the plate at 110°- 140°C till maximum color intensity. It showed R_f value of 0.55 (Methylene chloride-methanol 90:10). The 1D ^{1}H NMR and ^{13}C NMR (C_6D_5N) data are listed in **Table 2**.

Table 2. ^{1}H-NMR and ^{13}C NMR spectral data of compound 1 (C_6D_5N)

Atom No.	δ_H	δ_C
1	-	120.61
2	7.80, (1H, *s*)	109.91
3	-	147.27
4	-	140.74
5	-	147.27
6	7.80, (1H, *s*)	109.91
7	-	167.62
8	3.70, (3H, *s*)	51.50
Exchangable OH	5.81, (3H, *br. s*)	-

Compound 2: Obtained as white amorphus powder (methanol), m.p. 260 ºC. It is insoluble in *n*-hexane and chloroform, but soluble in methanol. It gave bluish green color with $FeCl_3$ indicating its phenolic nature[15].It showed a single spot on TLC which attained faint brown color after spraying with 10% *v/v* sulphuric acid using precoated silica gel plates G_{60} F_{254} after heating at 110°- 140°C till maximum spot intensity and showed R_f values of 0.32 (Methylene chloride-methanol 90:10) and 0.69 (Methylene chloride-ethyl acetate-methanol-water 8:15:4:1). ^{1}H NMR and ^{13}C NMR (CD_3OD) are listed in **Table 3**.

Table 3. ^{1}H-NMR and ^{13}C NMR spectral data of compound 2 (CD_3OD)

Atom No.	δ_H	δ_C
1	-	122.30
2	7.05, (1H, *s*)	110.34
3	-	146.42
4	-	139.54
5	-	146.42
6	7.05, (1H, *s*)	110.34
7	-	170.63

Compound 3: Obtained as white granular powder (methanol), m.p. 276 – 278 ºC. It is insoluble in *n*-hexane and chloroform, sparingly soluble in cold ethanol and methanol, soluble in hot methanol and ethanol. It gave red color with Salkowski`s test and violet ring superimposed by a layer of green color with Liebermann-Burchard`s test[16]. It attained a violet color after spraying with 10% *v/v* sulphuric acid and heating at 110-140ºC till maximum spot intensity. It showed R_f value 0.54 (Methylene chloride-Methanol 85-15). ^{1}H NMR and ^{13}C NMR (DMSO-d_6), 400 and 100 MHz respectively) are listed in **Table 4**.

Identification of isolated compounds

Compound 1: Chemical test of compound **1** indicates its phenolic[14]. Positive mode EI-MS showed the $[M+1]^+$ at *m/z* 185 calculated for molecular formula $C_8H_8O_5$. Examination of the ^{1}H-NMR revealed singlet aromatic signal at δ_H 7.80 (2H, H-2, 6) which, indicated the presence of tetra-substituted benzyl moiety[17, 18], furthermore the presence of singlet signal at δ_H 3.70, integrated to 3H which suggested the presence of aliphatic methyl functionality. This finding was confirmed from ^{13}C-NMR signals, which displayed signals at δ_C (167.62, C) attributed to carbonyl moiety (C-7) and up field shifted signal at δ_C (51.50, CH_3) (C-8) attributed to aliphatic CH_3 group. From the previous mentioned physical, chemical, chromatographic and spectral data (EI-MS, ^{1}H-NMR and ^{13}C-NMR) it was

concluded that compound **1** is methyl gallate[19], and this is the first report for its isolation from the root bark of *Chrozophora oblongifolia.*

Compound 2: Chemical test of compound **2** indicates its phenolic nature[14]. ^{1}H-NMR revealed downfield shifted aromatic signal at δ_H 7.80 (2H, s, H-2, H-6) which, indicated the presence of tetra-substituted benzyl moiety[17, 18]. This finding was confirmed from ^{13}C-NMR signals, which displayed signals at δ_C (122.30, C) assigned to (C-1), (110.34, CH) assigned to (C-2 and C-6), (146.42, C) assigned to (C-3 and C-5), (139.54, C) assigned to (C-4), in addition to downfield shifted signal at δ_C (170.63, C) attributed to carboxylic acid moiety (C-7)**.** From the previous mentioned physical, chemical, chromatographic and spectral data (^{1}H-NMR and ^{13}C-NMR) it was concluded that compound **2** is gallic acid and this is the first report for its isolation from the root bark of *Chrozophora oblongifolia.*

Table 4. ^{1}H-NMR and ^{13}C NMR spectral data of compound 3 (DMSO-d_6)

Atom No.	δ_H	δ_C	Atom No.	δ_H	δ_C
1	-	36.69	19	1.01, (3H, *s*)	19.41
2	-	29.74	20	-	36.69
3	3.4, (1H,*m*)	77.24	21	1.23, (3H, *d*, *J*= 6)	19.09
4	-	37.29	22	-	33.82
5	-	140.92	23	-	25.90
6	-	121.69	24	-	45.61
7	-	31.90	25	-	29.17
8	-	31.84	26	0.90, (3H, *d*, *J*= 7)	19.57
9	-	50.07	27	0.90, (3H, *d*, *J*= 7)	20.19
10	-	35.95	28	-	24.35
11	-	21.06	29	0.82, (3H, *t*)	12.15
12	-	38.78	Glc.		
13	-	42.33	1'	4.2, (1H, *d*, *J*= 4.2)	101.25
14	-	56.64	2'	-	73.94
15	-	23.08	3'	-	77.37
16	-	28.26	4'	-	70.58
17	-	55.93	5'	-	77.23
18	0.66, (3H, *s*)	12.26	6'	-	61.57

Compound 3: Chemical test of compound **3** indicates its steroidal or triterpenoidal nature[20]. ^{1}H-NMR spectrum showed the presence of a six methyl groups at δ_H 0.66 (3H, s), 0.82 (3H, t), 0.90 (3H, d), 0.90 (3H, d), 1.01(3H, s) and 1.23 (3H, d) could be assigned to H-18, 29, 26, 27, 19 and 21 respectively. Adoublet proton signal at δ_H 4.2 (1H, d, *J*= 7.6) could be assigned to anomeric sugar proton, the large coupling constant indicates its β configuration. Moreover a multiplet proton signal at δ_H 3.4 (1H, m) could be assigned to H-3 and finally the olefenic proton at δ_H 5.33(1H, br.s) could be assigned to H-6, suggesting that compound 3 is a β-sitosterolglucoside. The ^{13}C-NMR confirmed the presence of β-sitosterol nucleus as it showed 29 carbons distributed as six methyls, eleven methylenes, nine methines and three quaternary carbons.The carbon resonances at δc 101.25 (C-1`), 73.94 (C-2`), 77. 37 (C-3`), 70.58 (C-4`), 77.23 (C-5`) and 61.57 (C-6`) confirmed the presence of glucose moiety. From the previous physical, chemical, chromatographic and spectroscopic data, it could be concluded that compound **3** is identified as β-sitosterol-3-*O*-β-D-glucopyranoside; this was confirmed by authentication with authentic reference.

Antioxidant activity

The obtained results (**Table 5**) indicated that, the ethyl acetate fraction showed the highest antioxidant activity followed by *n*-butanol and methylene chloride fractions. The total aqueous methanolic extract and *n*-hexane fractions showed the lowest antioxidant activity. The highest antioxidant activity of ethyl acetate fraction and *n*-butanol fractions could be attributed to the presence of polyphenolic compounds in these fractions[21]. The antioxidant activity of polyphenolic natural substances is predominantly due to their redox properties, (the ability to act as reducing agents, hydrogen donors and singlet oxygen quenchers, and to some extent, could also be due to their metal chelating properties)[22].

Antimicrobial activity

The obtained results (**Table 6**) showed that the total methanolic extract and ethyl acetate fraction of *chrozophora oblongifolia* possess moderate antimicrobial activities against gram positive *S. aureus*, *VRSA* and *C. albicans* and no activity against *E. coli* and *P. aeruginosa*. The methylene chloride fraction and *n*-butanol fraction showed no activity against all tested microbes. The antimicrobial activity of the active fractions could be attributed to the presence of different classes of active ingredients as polyphenolics[23]. Most antimicrobial medicinal plants are effective against Gram positive bacteria, but few of them are effective against Gram negative bacteria, as the composition of these two types of bacteria differs. Gram negative bacteria have a lipopolysaccharide barrier on their outer membrane, while the Gram positive bacteria do not have this lipopolysaccharide barrier. Antimicrobial activity is one of the important properties of polyphenolic compounds. The antimicrobial activity of phenolics is due to their partially hydrophobic nature.

Table 5. Antioxidant activity of total extract and fractions of *Chrozophora oblongifolia* in comparison with standard antioxidant (Ascorbic acid)

Fraction	Concentration (mg/mL)					SC_{50} mg/mL
	1	0.5	0.25	0.125	0.0625	
	% DPPH radical scavenging activity					
Ascorbic acid	90.16±0.04	85.65±0.03	80.18±0.16	73.90±0.04	62.49±0.03	0.013±0.00009
Total extract	76.43±0.05	64.23±0.06	51.90±0.12	40.34±0.06	28.92±0.06	0.217±0.0033
n-hexane	39.27±0.10	26.12±0.04	16.67±0.11	12.87±0.05	6.93±0.04	3.510±0.1732
Methylene chloride	68.75±0.08	52.47±0.08	38.32±0.08	28.19±0.06	19.49±0.05	0.406±0.00333
Ethyl acetate	91.84±0.04	89.50±0.06	80.05±0.06	75.43±0.06	64.15±0.04	0.012±0.00067
n-butanol	89.90±0.02	84.03±0.06	76.81±0.05	69.04±0.06	59.08±0.05	0.023±0.00033

Values are expressed as mean ± SE M; n = 3

Table 6. Antimicrobial activity of total extract and fractions of *Chrozophora oblongifolia* in comparison with standard antimicrobials

Fraction	*Staph. aureus*	*VRSA*	*E. coli*	*P. aeruginosa*	*C. albicans*
Total extract	23.33±0.33	12.66±0.33	-	-	15.66±0.66
Methylene chloride	-	-	-	-	-
Ethyl acetate	31.00±0.57	27.33±0.88	-	-	20.66±0.88
n-butanol	-	-	-	-	-
Amoxicillin	36.00±1.00	14.66±0.33	22.00±0.57	25.33±0.66	-
Cyclohexamide	-	-	-	-	22.00±0.57

Values are expressed as mean ± SE M; n = 3

Table 7. Cytotoxic activity of total extract and fractions of *Chrozophora oblongifolia* in comparison with standard cytotoxic agent

Tested samples	Concentration	Cytotoxic activity (%)	
		MCF-7	Huh-7
5-Flurouracil	(10 µM)	44.38±0.211	56.59±0.38
Total methanolic extract	125 µg/mL	10.32±0.09	22.47±0.13
	250 µg/mL	15.82±0.66	31.28±0.68
n-hexane	125 µg/mL	37.31±0.14	38.49±0.53
	250 µg/mL	59.55±0.76	54.17±0.46
Methylene chloride	125 µg/mL	72.85±0.16	73.05±0.26
	250 µg/mL	83.83±0.37	82.79±0.55
Ethyl acetate	125 µg/mL	12.39±0.17	57.77±0.61
	250 µg/mL	22.25±0.13	72.52±0.23

Values are expressed as mean ± SE M; n = 3

They inhibit the hydrolytic enzymes, microbial adhesion and cell envelope transport proteins. Tannins are polyphenols that are known to bind and precipitate microbial proteins and render nutritional proteins unavailable for bacteria[24].

Cytotoxic activity

The obtained results (**Table 7**) showed that the methylene chloride fraction followed by *n*-hexane fraction posses the highest cytotoxic activity at concentration (125 and 250 µg/mL against MCF-7 and Huh-7 cell lines in comparison with 5-flurouracil as positive control at concentration (10 µM).

The cytotoxic activity of the active fractions attributed to the presence of polyphenolics[25], sterols[26] and triterpenes[27]. polyphenolics induce cell cycle arrest and apoptosis in different cancer cells and enhance the immunity and decrease inflammatory cytokine levels[28]. Sterols influence the programmed cell death in different cell lines and inhibiting tumor proliferation by promoting apoptosis[29]. Triterpenes can induce apoptosis in tumor cells by activation of caspases and modulation of pathways affecting cell proliferation and migration[30].

CONCLUSION

This study provides information on phytochemical constituents, antioxidant, antimicrobial and cytotoxic activity of the root bark of *Chrozophora oblongifolia* (Delile) Spreng. From the study the root bark considered as rich source of phytoconstituents as polyphenolics, also the total methanolic extract and some of its fractions showed considerable antioxidant, antimicrobial and cytotoxic activities making this part of the plant as potential source of antioxidant, antimicrobial and cytotoxic agents.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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