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In-vitro Testing of Antioxidant, Anti-Parasite Activities, Cytotoxicity, and Chemical Evaluation of Abutilon Pannosum and Cassia Occidentalis Ethanolic Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AAE and ASK conducted the experiments and wrote the manuscript. Authors MEME and HHE reviewed the manuscript and supervised the experiments inside the laboratory. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study to detect Anti-giardia, antioxidant activities, cytotoxicity and evaluated the chemical constituent of ethanolic extracts of *Abutilon pannosum* and *Cassia occidentalis.*
Study Design: Various standard methods were used to detect of bioactivity for ethanolic extracts of plants used in this study.
Place and Duration of Study: This study was conducted in the laboratories of microbiology and

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parasitology and chemistry, the International University of Africa, Khartoum, Sudan, during May 2019.
Methodology: The ethanolic extract of *Abutilon pannosum* and *Cassia occidentalis* was used as an anti-giardia and anti-oxidant *in-vitro*, and toxicity tests were performed using brine shrimp and MTT assay. Also, the compounds of the plants used were detected by the GCMS apparatus.
Results: The ethanolic extracts of *Abutilon pannosum* showed high Anti-giardia activity (79%) in concentration (500 ppm) after 72 hours, whereas the activity of *Cassia occidentalis* extract showed (61%). The highest antioxidant activity of ethanolic extract of *Cassia occidentalis* was (68.7%), while it was weak in *Abutilon pannosum* ethanolic extract (45%) by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The results of cytotoxicity revealed that the ethanolic extracts are highly toxic to brine shrimp, but are not toxic to normal cell line (MTT). Chromatographic analysis using gas chromatography-mass spectroscopy (GCMS) showed good separation of compounds. GCMS detected 22 and14 important compounds in *Abutilon pannosum* and *Cassia occidentalis* extracts respectively. The common compound in both plant extracts is n-Hexadecanoic acid. This acid was reported as an antioxidant.
Conclusion: This study revealed that the biological activities of *Abutilon pannosum* extracts showed high activities of Anti-giardia and antioxidants. Non-cytotoxic in the normal cell line was shown. *Cassia occidentalis* showed high activity of Anti-giardia and weak activity antioxidant.

Keywords: In-vitro; antioxidant; anti-parasite; cytotoxicity; abutilon pannosum; cassia occidentalis.

1. INTRODUCTION

Traditional medicine is still used as the primary health care system for up to 80% of the world population, most of them are in developing countries. This popularity of traditional medicine is due to the better cultural acceptability, compatibility, and lesser side effects [1]. The frequent use of medicinal plants for the treatment of different diseases has encouraged several researchers to study their biological activities. Additionally, natural products can contribute to the discovery of novel antimicrobial and antioxidant components. Several pharmaceutical studies have demonstrated the antibacterial, antimalarial, antitrypanosomal, and antioxidant activities of Sudanese medicinal plants [2]. The use of plant and its products has a long history that began with folk medicine and through the years has been incorporated into traditional and allopathic medicine. Since antiquity, many plant species reported having pharmacological properties as they are known to possess various secondary metabolites like glycosides, saponins, flavonoids, steroids, tannins, alkaloids, tirpenes which is, therefore, should be utilized to combat the disease-causing pathogens [3]. *Abutilon pannosum* is one of the valuable medicinal plant burns of Malvaceae is tomentose under shrub widely distributed in India, North Africa, Asia, and Australia, and bears spherical fruits having about 25 carpels, each of which covers hairy plant widely distributed from tropical Africa to Australia through Asia. It grows to a height of 2 m and bears small, ovoid fruits, which contain tasteless seeds. It leaves have good medicinal activity for example antibacterial, antioxidant, antifungal [4]. *Cassia occidentalis* L belongs to the family Caesalpiniaceae. It is a plant with huge medicinal importance. Leaves of *C. occidentalis* plant have ethnomedicinal importance like a paste of leaves is externally applied on healing wounds, sores, itch, cutaneous diseases, bone fracture, fever, ringworm, skin diseases, and throat infection. Previous pharmacological investigations showed that C. occidentalis leaf extracts have antibacterial, antimalarial, antimutagenic, antimutagenic, antiplasmodial, anticarcinogenic, and hepatoprotective activity [5]. This study aimed to investigate in vitro antibacterial, antiparasite, antioxidant activities and cytotoxicity and chemical compounds of crude extracts for *Abutilon pannosum* and *Cassia occidentalis*.

2. MATERIALS AND METHODS

2.1 Plant Materials

The Whole part of *Abutilon pannosum* and *Cassia occidentalis* were collected from Khartoum University felid. The plant was identified and authenticated by Hamza Taj-Elsir in Herbarium of Botany Department, Faculty of Agriculture, Khartoum University. All plant parts were air-dried, under the shadow with good ventilation and then ground finely in a mill until their uses for extracts preparation.

2.2 Preparation of Crude Extracts

100 grams of both plant samples extracted with 80% methanol by soaking for 18 hours using a shaker (Stuart scientific, flash shaker, S F 1,

U K). The extracts were filtered and evaporated using a rotary evaporator (Buchi, 461, Switzerland) at 40°C.

2.3 Anti-Giardia Activity

2.3.1 Parasite isolate

Giardia lamblia used in the experiment was taken from patients of Ibrahim Malik Hospital (Khartoum). All taken samples were examined by wet mount preparation; the positive samples were transported to the laboratory in nutrient broth medium. Trophozoites of *E. histolytica* and *Giardia lamblia* were maintained in RPMI 1640 medium containing 5% bovine serum at 37 ± 1°C. The trophozoites were maintained for the assays and were employed in the log phase of growth.

2.3.2 Inoculums

E. histolytica and *Giardia lamblia* was inoculated in the RPMI 1640 medium and incubated at 37 ± 1°C for 48 h. parasites were counted under the microscope by the hemocytometer chamber.

2.3.3 *In Vitro* susceptibility assays

In vitro susceptibility assays used the sub-culture method of Cedillo-Rivera *et al.*, [6] which is being described as a highly stringent and sensitive method for assessing the antiprotozoal effects (gold standard) particularly in *E. histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis* Argüello-García *et al.*, [7]. 5 mg from each extract and compound was dissolved in 50 µl of dimethyl sulfoxide (DMSO) at Eppendorf tube containing 950 µl D. W to reach the concentration of 5 mg/ml (5000 ppm). The concentrates were stored at -20°C for further analysis. A sterile 96-well microtitre plate was used for different plant extracts, positive control and negative control. Three columns of a microtitre plate wells [8 columns (C) × 12 rows (R)] were chosen for each extract, 40 µl) of an extract solution (5mg/ml) were added to the first column wells C-1: On the other hand, 20 µl of complete RPMI medium were added to the other wells of the second column and third column (C-2 and C-3). Serial dilutions of the extract were obtained by taking 20 µl of extract to the second column wells and taking 20 µl out of the complete solution in C2 wells to C-3 wells and discarding 20 µl from the total solution of C-3 to the remaining 20 µl serial solutions in the successive columns. 80 µl of culture medium was complemented with the parasite and added to all wells. The final volume in the wells was 100 µl. In each test

$$\text{Mortality of parasites (\%)} = (\text{Negative control} - \text{Tested sample with extract}) / (\text{Negative control}) \times 100\%.$$

Only 100% inhibition of the parasite was considered when there was no motile parasite observed.

2.4 Antioxidant Activity

2.4.1 DPPH radical scavenging assay

The DPPH free radical scavenging activity Principle: The antioxidant activity was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH. Experiments were carried out according to using the method of Eltayeb et al. [8], with slight modification. Active samples can reduce the stable radical DPPH to the yellow-colored diphenyl- picrylhydrazine.

2.4.2 Assay

Test samples were allowed to react with 2.2 di (4-tretoctylphenyl)-1-picrylhydrazyl stable free radical (DPPH) for half an hour at 37oc in 96-wells plate. The concentration of DPPH was kept at (300 µM). The test sample was dissolved in DMSO while DPPH was prepared in ethanol. After incubation, a decrease in absorbance was measured at 517 nm using multiplate reader spectrophotometer. The percentage of radical scavenging activity of the sample was determined in comparison with a DMSO treated control. All tests were conducted in triplicate.

$$\text{DPPH radical scavenging (\%)} = 100 - \{(Ac - At)/Ac\} \times 100$$

Where At= Absorbance value of test compound; Ac=Absorbance value of the control.

2.5 *In-Vitro* Cytotoxicity

2.5.1 Cytotoxicity screening by brine shrimp

2.5.1.1 Storage of artemia salina eggs

Eggs of *Artemia salina* were stored at low temperatures (4ºC), they will remain viable for many years.

2.5.1.2 Hatching shrimp

Brine shrimp eggs, *Artemia salina* was hatched in artificial seawater prepared by dissolving 38 g of sea salt in one liter of distilled water. After 24-72h incubation at room temperature (37°C), the larvae were attracted to one side of the vessel with a light source and then collected with a pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing artificial seawater.

2.5.1.3 Brine shrimp assay

Bioactivity of the extract was monitored by the brine shrimp lethality test [9]. Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts. 50 mg of *Artemia salina* (Leach) eggs were added to a hatching chamber containing artificial Seawater (75 ml). The hatching chamber was kept under an inflorescent bulb for 48 h for the eggs to hatch into shrimp larvae. 20 mg of test extracts of the various plant species were separately dissolved in 2 ml of methanol, then 500, 50, and 5 µl of each solution were transferred into vials corresponding to 1000, 100, and 10 µg/ml, respectively. Each dosage was tested in triplicate. 10 larvae of *A. salina* Leach (taken 48 – 72 h after the initiation of hatching) were added to each vial (Fig. 4). The final volume of solution in each vial was adjusted to 5ml with Seawater immediately after adding the shrimps. One drop of dimethyl sulphoxide (DMSO) was added to the test and control vials before adding the shrimps to enhance the solubility of test materials. LD_{50} values were determined at 95% confidence intervals by analyzing the data on a computer loaded with a “Finney Programme.” The concentration at which it could kill 50% larvae (LD_{50}) was determined. LD_{50} values below 200 ppm are generally considered as significant according to Oladimeji et al. [10].

2.5.2 Cytotoxicity screening by micro-culture-tetrazolium MTT-assay

Micro-culture-tetrazolium MTT-assay was utilized to evaluate the cytotoxicity of plants. This colorimetric assay is based on the capacity of mitochondria-succinate dehydrogenase enzymes in living cells to reduce the yellow water-soluble substrate 3- (4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) into an insoluble, blue colored-formazan, a product measured spectrophotometrically. Since the reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells [11].

2.5.2.1 Cell Line and culture medium

Vero cell line (Normal cell line) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were subcultured twice a week.

2.5.2.2 Cell counting

Cells were counted using the improved Neubauer chamber. The coverslip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol, then dried. An aliquot of cell suspension was mixed with an equal volume of 0.4% trypan blue in a small tube. The chamber was charged with the cell suspension. After cells had settled, the chamber was placed under a light microscope. Using 40 X objective, cells in the 4 large corner squares (each containing 16 small squares) were counted. The following formula was used for calculating the cells:

$$Number\ of\ cells\ counted\ X\ Dilution\ factor\ X\ 10^4\ (Cells/ml).$$

2.5.2.3 Assay

Serial dilutions of extract were prepared in a 96 well flat bottomed plate (Nalge Nunc, Inter.). The outer walls of the plate were filled with 250 µl of in-complete culture medium except for the last row 6 middle wells (B - G), which were used for the negative control receiving 50 µl of culture medium and 2 µl of sterile 0.5% Triton X. 50 µl/wells complete culture medium (CCM) were added and 30 µl more was added to second column wells (B – G) that were used as first extract dilution wells. To the first dilution wells in the row, 500 µg of c suspension extract were added to the 80 µl. extract were then serially diluted by two-fold dilution from well B3 till B11 by transferring 250 µl to the next well after proper mixing. From the last dilution wells (B-11), 50 µl were discarded. Each compound was tested in triplicate. Cell suspension is a complete culture medium containing 2.5 X 10^5 /ml was properly mixed, and 150 µl of it were transferred into each well of the plate. The plate was covered

and placed in a 5% CO_2 incubator at 37°C for three-five days (72 hours-120 hours). On the third/ fifth day, the supernatant was removed from each well without detaching cells. MTT (3- (4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a yellow tetrazole) stock (5 mg/ml) was prepared earlier in 100 ml PBS (phosphate buffer saline). MTT suspension was vortexed and kept on a magnetic stirrer until all MTT dissolved. The clear suspension was filter sterilized with 0.2 μ Millipore filter and stored at 4°C or – 20 until use. MTT was diluted (1:3.5) in a culture medium and brought to room temperature. To each well of the 96 well plates, 50 μl of diluted MTT were added. The plate was incubated further at 37°C for 2 to 3 hours in CO_2 incubator. MTT was removed carefully without detaching cells, and 200 µl of DMSO was added to each well. The plate was agitated at room temperature for 15 minutes then read at 540 nm using microplate reader. The percentage growth inhibition was calculated using the formula below:

$$\% \text{ cell inhibition} = 100 - \{(Ac - At)/Ac\} \times 100$$

Where, At = Absorbance value of test compound; Ac =Absorbance value of the control.

2.6 Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

GC-MS technique was used in this study to identify the phyto-components present in the most active fractions. The tested extracts were analyzed by GC-MS using Shimadzu Mass Spectrometer-2010 series. 1 µL of the sample was injected in GC-MS equipped with a split injector. The MS was operated in the electron ionization (EI) mode (70 eV). Helium was employed as the carrier gas and its flow rate was adjusted to 1.2 mL/min. The analytical column connected to the system was an Rtx-5 capillary column (length-30 m × 0.25 mm i.d., 0.25 µm film thickness). The column head pressure was adjusted to 93.9 kPa. Column temperature programmed from 110°C (7 min) to 200°C at 10°C/min and from 200-280°C at 5°C/min withholds time 0 and 9 min respectively. A solvent delay of 4.50 min was selected. The injector temperature was set at 250°C. The GC-MS interface was maintained at 280°C. The MS was operated in the ACQ mode scanning from m/z 40 to 550.0. In the full scan mode, EI mass spectra in the range of 40–550 (m/z) were recorded at an electron energy of 70 eves. Compounds were identified by comparing mass spectra with the library of the National Institute of Standard and Technology (NIST), USA/Wiley.

2.7 Statistical Analysis

All data were presented as means ± S.D. Statistical analysis for all the assays results was done using Microsoft Excel program (2010).

3. RESULTS AND DISCUSSION

3.1 Antigiardial Activity

The anti-giardia potential of the ethanolic extract of different parts of candidate plants extracted by ethanol, with different concentrations (500, 250 and 125 ppm) and Metronidazole (the reference control) with concentration (312.5 µg/ml) was investigated against *Giardia lamblia* trophozoites *in vitro*. Ethanolic extract of *A. pannosum* showed (79%, 70%) inhibition at a concentration 500 µg/ml after 72 and 48h; this was compared with Metronidazole which gave 86% inhibition at concentration 312.5 µg/ml at the same time against *Giardia lamblia* (Fig. 1). *A. pannosum* showed with an inhibition concentrations (IC) more than 99.4 µg/ml after three days and gave an IC50 after three days (Fig. 2). *C. occidentalis* crude extract showed (61%, 60) inhibition at a concentration 500 µg/ml after 72 and 48h; this was compared with Metronidazole which gave 86% inhibition at concentration 312.5 µg/ml at the same time against *G. lamblia* (Fig. 3). Also crude extract showed with an inhibition concentration (IC) more than 240 µg/ml after three days and gave an IC50 after three days (Fig. 4). Protozoal diseases constitute major health problems worldwide, particularly in tropical developing countries. Among the protozoal parasites, *Giardia lamblia* and *Entamoeba histolytica* have the highest incidence of diarrheal diseases in developing countries. Chemotherapy is the first choice for the treatment of protozoal diseases; however, it has proven side effects. Therefore, there is a need for safe and effective treatment alternatives including plants as one of the readily available alternatives for the control and prevention of amoebiasis and giardiasis [12].

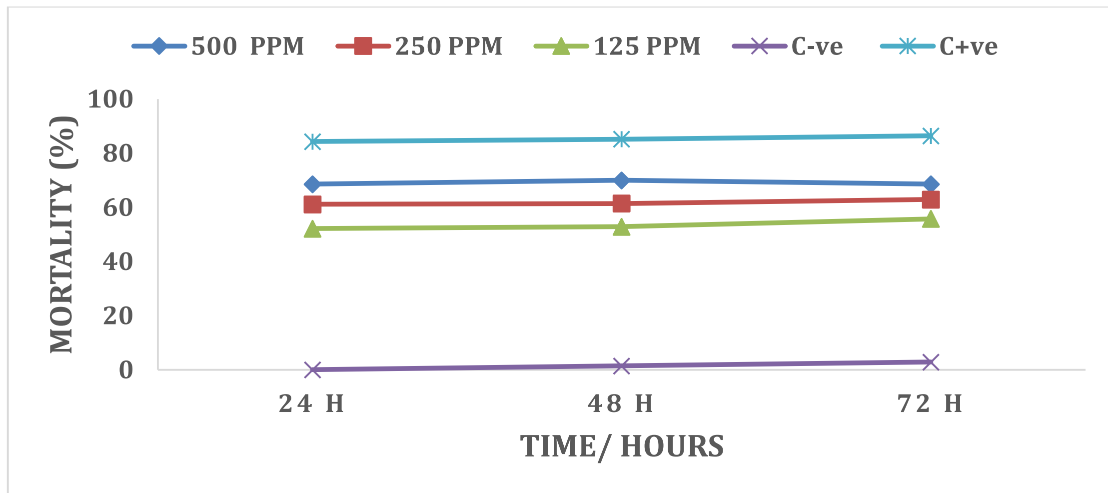

Fig. 1. ***In vitro*** **activity of** ***Abutilon pannosum*** **ethanol extract against** ***G. lamblia***

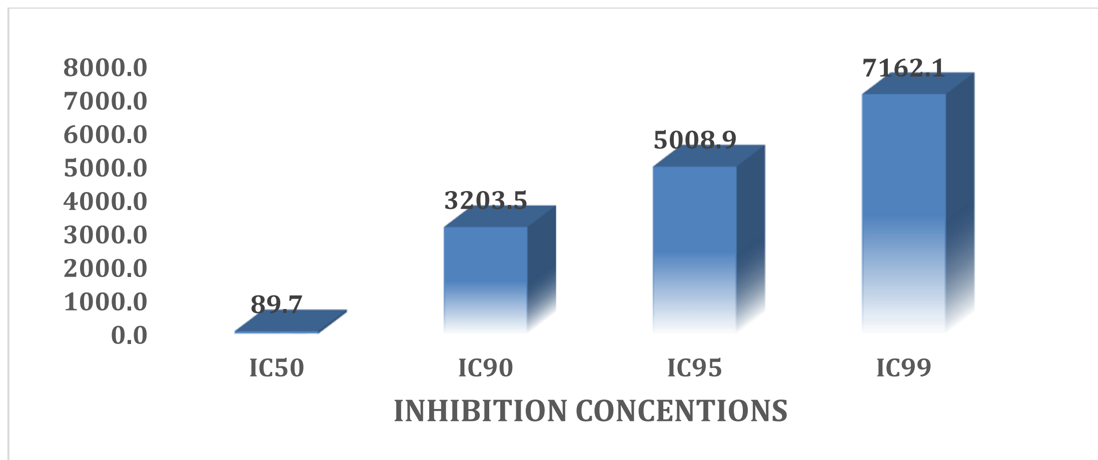

Fig. 2. Inhibition concentration (IC) ***Abutilon pannosum*** **ethanol extract against** ***G. lamblia***

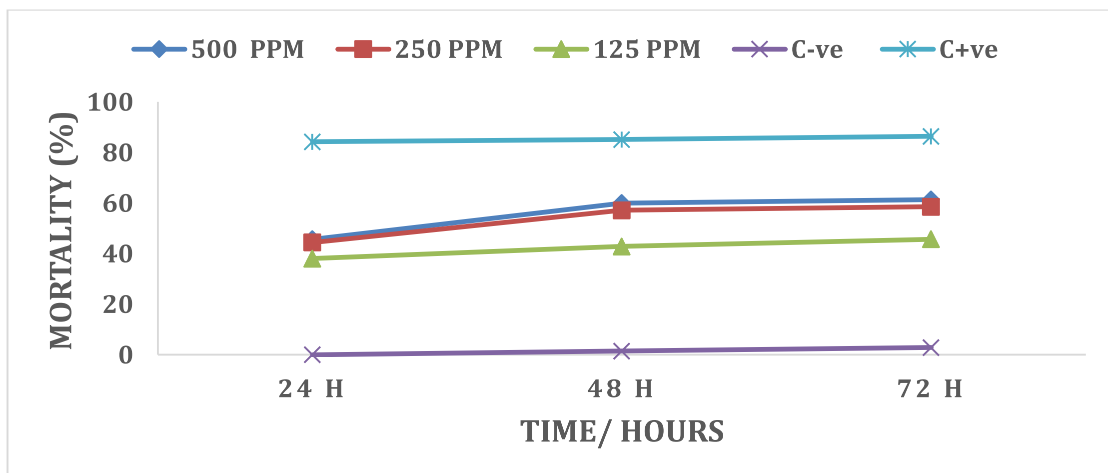

Fig. 3. ***In vitro*** **activity of** ***Cassia occidentalis*** **ethanol extract against** ***G. lamblia***

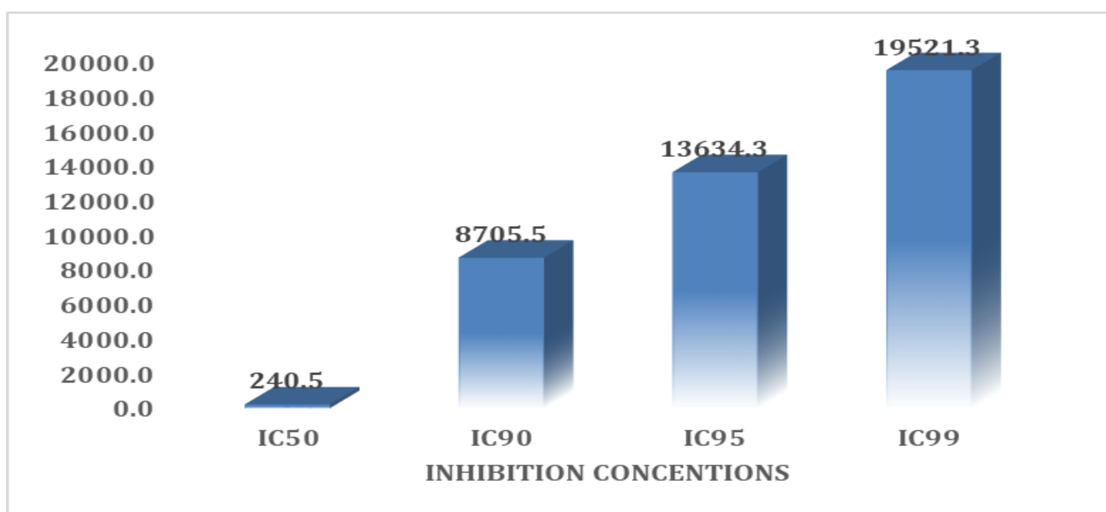

Fig. 4. Inhibition concentration (IC) *Cassia occidentalis* ethanol extract against *G. lamblia*

3.2 Antioxidant Activity

DPPH radical scavenging assay is the most common method used in the study of antioxidant activity of plant extracts Table 1. *Cassia occidentalis* crude extract showed moderate antioxidant activity (68.7±0.004), While ethanolic extract of *Abutilon pannosum* showed lower activity (45±0.024). The antioxidant activity may attribute to terpenoid content, as well as phenolic compounds (tannins) and (flavonoids) [13]. These results may be due to the moderate content of triterpenes, triterpenoids and fatty acids in the most ethanolic extract of Cassia occidentalis and low content of triterpenes in the ethanolic extract of *Abutilon pannosum*.

Table 1. Antioxidant activity of *Abutilon pannosum* and *Cassia occidentalis* crude extracts

Plants	Activity %S±/D
Abutilon pannosum	45±0.024
Cassia occidentalis	68.7±0.004

3.3 Cytotoxicity

Toxicity tests conducted using brine shrimp and normal cell lines (MTT) methods (Tables 2, 3). Ethanolic extracts of *Abutilon pannosum* and *Cassia occidentalis* were highly toxic in brine shrimp, whereas no toxicity was found when using MTT method.

Table 2. Cytotoxicity of plants extracts on brine shrimp

Samples	Total Number of shrimp	Concentrations (µg/ml)						ED (µg/ml)	The degree toxicity
		1000	100	10	1000	100	10		
		Number of dead			Number of survive				
Abutilon pannosum	10	10	03	02	00	07	08	100	High toxic
Cassia occidentalis	10	10	01	00	00	09	10	165.9	High toxic

Interpretation:Key: LD_{50}>249 µg/ml: High toxic; 250- 499 µg/ml: Medium toxicity; 500-1000 µg/ml Light toxicity; > 1000 µg/ml Non Toxic

Table 3. Cytotoxicity of plants extracts on normal cell lines (Vero cell line) as measured by the MTT assay

Name of Extracts	Concentration (µg/ml)			IC_{50} (µg/ml)	IC_{50}
	Inhibition (%) ± SD				
	500	250	125		
Abutilon pannosum	59.43± 0.01	46.57± 0.04	38.86± 0.02	279.94	> 100
Cassia occidentalis	66.28± 0.06	55.14± 0.02	44± 0.04	181.57	> 100
*Control		96.28± 0.01			

3.4 Gas Chromatography- Mass Spectrometry of Ethanolic Extract of *Abutilon pannosum*

The results for GC-MS analysis of the ethanolic extract of *Abutilon pannosum* to the identification of several compounds. Totally 22 compounds were identified which have been listed in (Table 4). The chromatograph showed 22 peaks with 22 individual compounds (Fig. 3). The major constituents were identified in the ethanolic extract were N,N-Dimethylglycine (54.94%), n-Hexadecanoic acid (8.90%), Phytol (6.63%), 1,3-Propanediol, 2-(hydroxymethyl)-2-nitro (4.93%) and many other compounds were identified as low level.

3.5 Gas Chromatography-Mass Spectrometry of Ethanolic Extract of *Cassia occidentalis*

The ethanolic extract of *Cassia occidentalis* revealed the presence of 11 compounds as shown in (Table 5). The chromatogram showed 11 peaks with 11 individual compounds (Fig. 6). The main compounds in *Cassia occidentalis* were n-Hexadecanoic acid (25.79%), 1,3-Propanediol, 2-ethyl-2-(hydroxmethyl)-2-nitro (14.18%), 9,12-Octadecadienoic acid (Z,Z)- (13.47%), Stigmasterol (11.73%), 9,12,15-Octadecatrienoic acid (Z,Z,Z)- (7.79%), and many other compounds were identified as low level

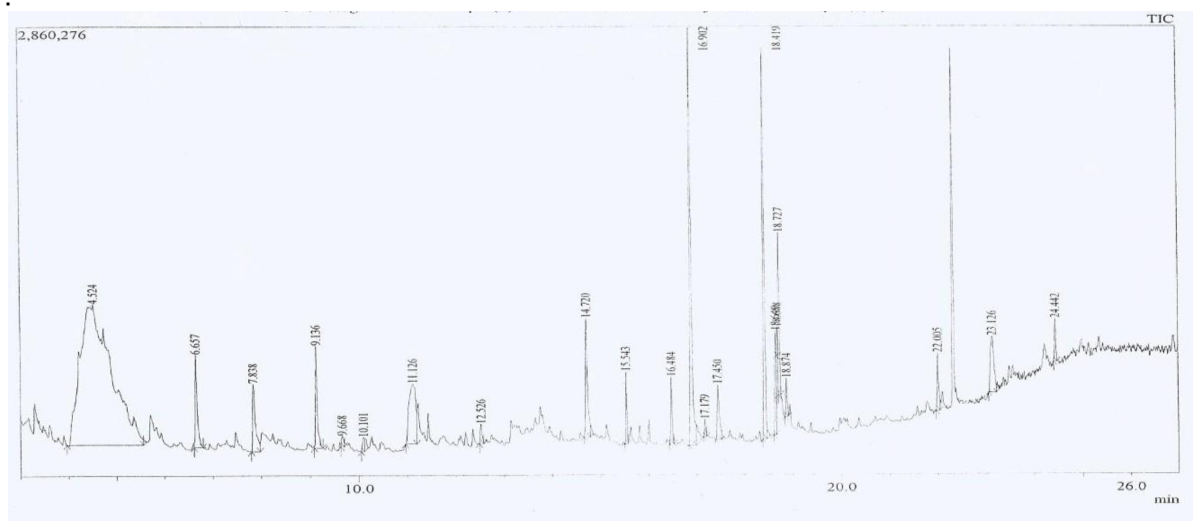

Fig. 5. GC-MS chromatogram of ethanolic extract of *Abutilon pannosum*

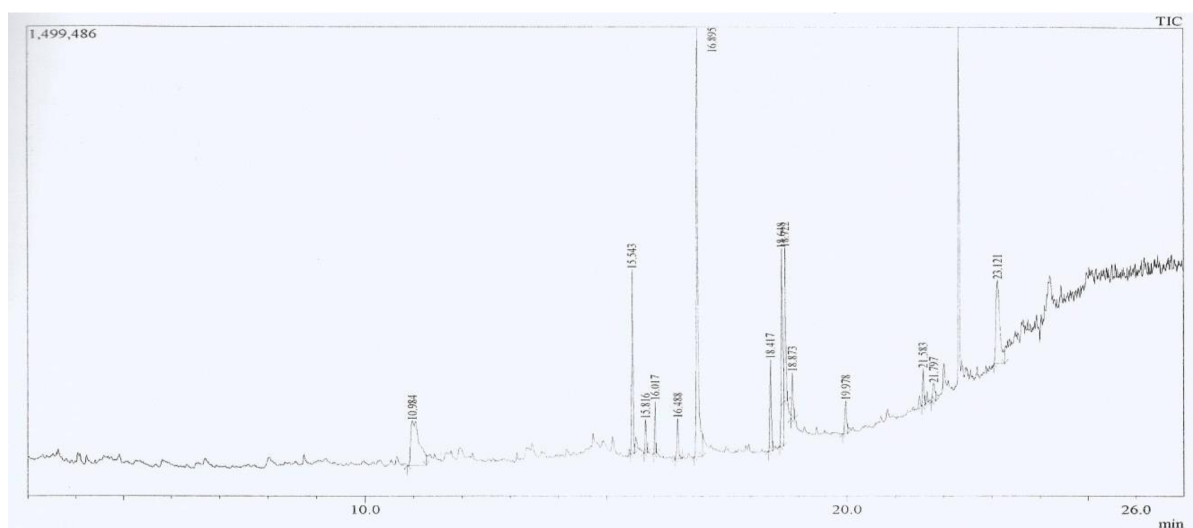

Fig. 6. GC-MS chromatogram of ethanolic extract of *Cassia occidentalis*

Table 4. Phytocomponents identified in the ethanol extract of the aerial part of *Abutilon pannosum* by GC-MS peak report TIC

Peak	R. Time	Area%	Compounds Names	Molecular Formula
1	4.524	54.94	N,N-Dimethylglycine	$C_4H_9NO_2$
2	6.657	2.33	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$
3	7.838	2.48	Benzofuran, 2,3-dihydro	C_8H_8O
4	9.136	2.18	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$
5	9.668	0.28	Phenol, 2,6-dimethoxy	$C_8H_{10}O_3$
6	10.101	0.36	DL-Proline, 5-oxo-,methyl ester	$C_6H_9NO_3$
7	11.126	4.93	1,3-Propanediol, 2-(hydroxymethyl)-2-nitro	$C_4H_9NO_5$
8	12.526	0.44	1,3-Isobenzofurandione, 4,5,6,7-tetrahydro-4,7-dimethyl	$C_8H_8O_3$
9	14.720	2.92	Tetradecanoic acid	$C_{14}H_{28}O_2$
10	15.543	1.05	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_{40}O$
11	16.484	1.18	Lidocaine	$C_{14}H_{22}N_2O$
12	16.902	8.90	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
13	17.179	0.25	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$
14	17.450	1.51	Benzenepropanoic acid, 2,5-dimethoxy	$C_{11}H_{14}O_4$
15	18.419	6.63	Phytol	$C_{20}H_{40}O$
16	18.649	1.94	9,12-Octadecadienoic acid, (Z,Z)	$C_{18}H_{32}O_2$
17	18.688	0.30	Oleic Acid	$C_{18}H_{34}O_2$
18	18.727	2.54	9,12,15-Octadecadienoic acid, (Z,Z,Z)	$C_{18}H_{30}O_2$
19	18.874	0.62	Octadecadienoic acid	$C_{18}H_{32}O_2$
20	22.005	1.11	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$
21	23.126	2.47	Stigmasterol	$C_{29}H_{48}O$
22	24.442	0.64	Squalene	$C_{30}H_{50}$

Table 5. Phytocomponents identified in the ethanol extract of the aerial part of *Cassia occidentalis* by GC-MS peak report TIC

Peak	R. Time	Area%	Compounds Names	Molecular Formula
1	10.984	14.18	1,3-Propanediol, 2-ethyl-2-(hydroxmethyl)-2-nitro	$C_6H_{14}O_3$
2	15.543	8.34	3,7,11,15-Tetramethyl-2-hexadeen-1-ol	$C_{20}H_{40}O$
3	15.816	1.61	9-Eicosyne	$C_{20}H_{38}$
4	16.017	2.24	Octadecene, 2-methyl-	$C_{19}H_{36}$
5	16.488	2.29	Lidocaine	$C_{14}H_{22}N_2O$
6	16.895	25.79	n-Hexadecanoic acid	$C_{16}H_{32}O_2$
7	18.417	4.67	Phytol	$C_{20}H_{40}O$
8	18.648	13.47	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$
9	18.722	7.79	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	$C_{18}H_{30}O_2$
10	18.873	2.41	Octadecanoic acid	$C_{18}H_{36}O_2$
11	19.978	2.16	2,6 Bis[2-(dimethylamino)ethoxy]pyridine	$C_{13}H_{23}N_3O_2$
12	21.583	1.73	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	$C_{12}H_{23}NO_2$
13	21.797	1.58	2-Ethylbutyric acid, eicosyl ester	$C_6H_{12}O_2$
14	23.121	11.73	Stigmasterol	$C_{29}H_{48}O$

4. CONCLUSION

This study revealed the biological activity of *Abutilon pannosum* and *Cassia occidentalis*, where it showed Anti-giardia and antioxidant activities and was non-cytotoxic in the normal cell line. The chemical content of these plants has also been identified.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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