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Anticancer; antiviral activities and phytochemical studies of *Cleome droserifolia* (Forssk.) Delile in Nabq Protectorate, South Sinai, Egypt

Walaa A. El-Alem[1], Zeinab A. El-Swaify[1], Ahmed A. Mohamed[2], Ramadan Bedair[3], Om Mohamed A. Khafagi[1]
[1]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University (Girl's Branch), Cairo, Egypt
[2]Egyptian Environmental Affairs Agency, Cairo, Egypt
[3]Department of Botany and Microbiology, Faculty of Science, Al-Azhar University (Boy's Branch), Cairo, Egypt

The rising prevalence of different diseases, cancer, and multidrug-resistant species has presented a significant problem in the treatment of disorders. As a result, using plant extracts as sustainable antimicrobials and antitumors offers a promising treatment. The genus *Cleome* has many beneficial properties, and phytochemical components and has considerable potential as a novel therapeutic source. This study assessed the phytochemical composition, antiviral activity against HAV, anticancer effects against HepG2 (hepatocellular carcinoma cell line) and MCF7 (human cancer breast cell line), and antimicrobial efficacy of a 70% ethanol aerial part extract from *Cleome droserifolia* in vitro. In 70% ethanol extract of *Cleome droserifolia*, flavonoids, tannins, alkaloids, saponins, terpenes, and sterols were identified. The *Cleome droserifolia* extract showed great efficacy against the HAV, HepG2, and MCF-7 cell lines, with a selective index of 3.85, 2.253 and 1.18 respectively. The *Cleome droserifolia* extract was also effective against Gram-positive *Bacillus cereus* and Gram-negative *Enterobacter cloacae* bacteria and the fungus; *Candida albicans*. Gas Chromatography–Mass Spectrum (GC-MS) analysis revealed that benzene-1-pentyloctyl (5.77%), benzene-1-butylheptyl (5.06%), benzene-1-methyldecyl (4.93%), benzene-1-methyldecyl (4.77%), benzene-1-methylundecyl (4.66%) and benzene-1-pentylheptyl (4.38%) were the most abundant components, the extracts of *Cleome droserifolia* petroleum ether. Conversely, the percentages of indolizine, retinal, and retinol were 0.44%, 0.54%, and 0.06%, respectively. The findings of this study show that *Cleome droserifolia* is a promising antibacterial, antifungal, antiviral, and good breast and liver tumor suppressor, which may be an optimistic approach to fighting cancer.

Keywords: Anticancer, Antimicrobial, Antiviral, GC-MS, Phytochemical components

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CORRESPONDANCE TO
Walaa A. El-Alem,
Botany and Microbiology, Faculty of Science,
Al-Azhar University (Girl's Branch),
Cairo, Egypt
Email: w.elalem.azhar@gmail.com
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EDITOR
Prof. Sawsan Abd Ellatif,
City of Scientific Research and Technology Application, Alexandria, Egypt
Email: sabdellatif@srtacity.sci.eg

INTRODUCTION

Many different natural compounds with multiple therapeutic applications can be found in plants, and these products are constantly being identified to create new drugs (Singh et al., 2012). Over 85–90% of people worldwide depend on traditional medicine to resist various illnesses among against (Wangchuk, 2018). Medicinal plants include a wide range of classes of bioactive chemicals, including tocopherols, polyphenols, and alkaloids, as demonstrated by several studies (Singh et al., 2022; Alphonse et al., 2023; Thapliyal et al., 2023; Ahmed et al., 2023). The pharmacological properties of flavonoids and phenolic acids are highly needed due to their ability to exhibit antibacterial, anticarcinogenic, anti-inflammatory, and antiproliferative effects on tumor cells (Ćetković et al., 2004).

The Cleomaceae family is geographically distributed in Syria, Libya, Egypt, Palestine, and other semiarid and desert regions. It comprises approximately 180–200 species. In addition, the plants resemble aromatic cushions and are poor and evergreen (Moustafa et al.,2019 & El-Askary et al.,2019). *Cleome droserifolia* is an important species of *Cleome* due to its historical use in folk medicine that is becoming constantly more threatened. *Cleome droserifolia* is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used by the Bedouins of southern Sinai for the treatment of diabetes and used in the treatment of several disorders such as diarrhea, fever, and liver diseases and bronchitis, inflammation, malaria, and in the treatment of scabies and rheumatic fever (Rahman et al., 2004 & Aparadh et al., 2012 and Moustafa et al., 2019). The plant extract also has a hypoglycemic effect through the potentiation of peripheral, hepatic insulin sensitivity and diminishing intestinal glucose absorption (Nicola et al 1996). Numerous *Cleome* species, including *C. droserifolia*, have demonstrated potent antibacterial qualities due to the presence of sulfur- and nitrogen-containing compounds in their essential oils (Muhaidat et al., 2015 & Hashem and Shehata, 2021).

Numerous investigations revealed a connection between the presence of phenols, flavonoids, alkaloids, terpenoids, carotenoids, vitamins, and tannins and the biological activities of these plants, which include antimicrobial, antioxidant, anticancer, anti-inflammatory, and antidiabetic properties (Agbor et al., 2011 & Hashem and Shehata, 2021). *Cleome* genus is well-known in folk medicine for treating stomachache, skin allergies, and open wounds, as well as for exhibiting anticancer and hepatoprotective properties (Abdel-Kader et al., 2009; Maksoud et al., 2020).

The current study aimed to evaluate the biological activity of *Cleome droserifolia* plant as an antibacterial, antifungal, antiviral, and antitumor agent, in addition to studying the chemical compounds that may be responsible for these activities.

MATERIALS AND METHODS

Plant material: The aerial part of *C. droserifolia* was collected from Nabq Protectorate, South Sinai, Egypt in May 2021. The species was identified according to Boulose (1999) at the Department of Flora Research and Plant Taxonomy, Horticultural Institute, Agricultural Research Center. Fresh plant samples were air-dried at room temperature, grinded, and then stored until use.

Phytochemical analysis: Aerial parts ethanolic extract 70 % were screened for their phytochemical constituents (tannins, saponins, terpenes, sterols, flavonoids, and alkaloids) as described by Wall et al., (1954), & Woo et al., (1977) and Balbaa (1986) & Tiwari et al., (2011).

Chemicals: Streptozotocin was purchased from Sigma Chemical Company (St. Louis, MO 6, USA). All other chemicals used for this study were of analytical grade purity.

Preparation of extracts: The grinded air-dried plants (250 g) were subjected to extraction with petroleum ether (40–60 ^{0}C) and then extracted with 70% ethyl alcohol using a Soxhlet apparatus for 48h each. Whatman's filter paper (No. 1) was used to filter the extracts. A rotating evaporator was used to concentrate the filtrate at 46 ^{0}C. Each crude extract was dried, weighed, and then stored at 4 ^{0}C (Alkhatib et al., 2022). The crude petroleum ether extract was identified as Gas / Mass. The 70% ethyl alcohol crude extract was used for further experiments.

Gas chromatography-mass spectrometry (GC/MS)

A Thermo Scientific Trace 1310 gas chromatograph and an ISQLT single quadrupole mass spectrometer were used. Mode of ionization: EI; voltage of ionization: 70 eV; column: DB5-MS, 30 m, 0.25 mm ID (J & W Scientific). The following temperature program was used: 40 ^{0}C for three minutes, 280 ^{0}C for five minutes, 290 ^{0}C at a rate of 5 ^{0}C/min (kept for one minute), and finally, static at 7.5 ^{0}C/min. Helium served as the carrier gas at a flow rate of 1.0 ml/min, 300 ^{0}C detector temperature, and 200 ^{0}C injector temperature. NIST and WILEY Mass Spectral Database were searched for libraries (Youssef et al., 2023).

Identification of free phenolic acids using HPLC

High-performance liquid chromatography was the method of choice for separating and identifying free phenolic acids that were extracted and isolated from the aerial part of *C. droserifolia* according to (Danny et al. (2003). HPLC equipment was used for the analysis. An Eclipse C18 column (4.6 mm × 250 mm, or 5 µm), was used for the separation. Water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) were combined to produce the mobile phase, at a flow rate of 0.9 ml/min. The following programming sequences were used during the mobile phase: 0 min (82% A), 0–5 min (80% A), 5–8 min (60% A), 8–12 min (60% A), 12–15 min (82% A), 15–16 min (82% A), and 16–20 min (82% A). At 280 nm, the multiwavelength detector was used. (National Research Center, Dokki, Giza).

Biological Studies

Antibacterial and antifungal activity

The antibacterial and antifungal activity of *C. droserifolia* ethanol extract was determined by the agar diffusion method (using 100µl) at the Regional Center for Mycology and Biotechnology, Al-Azhar University The activity was determined as the diameter of the inhibition zone (mm/mg sample). Gentamycin was used as a positive control for bacteria, while 100 µg/ml of ketoconazole was used as a positive control for fungi. Ten milligrams per milliliter of the material were examined. The antibacterial efficacy of the *C. droserifolia* ethanol extract was assessed against a variety of different species of bacteria, including Gram-positive and Gram-negative bacteria (supplied by The Regional Center for Mycology and Biotechnology, Al-Azhar University). Gram-positive bacteria included *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* RCMB 027 (1) and Gram-negative bacteria included *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* RCMB 001 (1) (ATCC 23355). In addition to two species of fungi, *Aspergillus niger* (RCMB 002005) and *Candida albicans* (RCMB 005003(1) ATCC 10231). The activities of *C. droserifolia* ethanol extract was assessed by measuring growth inhibition zones to the closest millimeter (the combination of the inhibition zone diameter and the well diameter).

Antiviral activities of *C. droserifolia*. aerial parts ethanolic extract

Cells and viruses: Cercopithecus aethiops kidney epithelial cells, also known as the Vero cell line, were cultured in Roswell Park Memorial Institute media (RPMI 1640) (Gibco, Tunisia), supplemented with (2

mM) L-glutamine, (100 µg/mL) penicillin, (100 µg/mL) streptomycin, and 10% v/v fetal bovine serum (FBS). At 37 ^{0}C, the cells were incubated in a dry-out environment with 5% CO_2. It was determined what the 50% cytotoxicity concentration (CC50), 50% inhibition concentration (IC50), and maximum nontoxic concentration (MNTC) were. Moreover, the ratio of CC50 to IC50 was used to compute the selective index, or SI. Viruses that cause hepatitis A (HAV). The virus was obtained from (Science Way for scientific research and consultations).

Antiviral assay: The hepatitis A (HAV) virus was propagated separately in Vero cells. Determination of the maximum non-toxic concentration of each sample used of the MNTC on antiviral assay against HAV (All viruses were obtained from the Faculty of Medicine for Girls, Microbiology Department). 10,000 cells plated in 200ul media per well in a 96-well plate. Three wells should remain empty for blank controls. After an hour, the cells were allowed to adhere to the wells using an overnight (1:1 v/v) dilution of the non-lethal test sample. The virus/sample suspension was added 100 µl. After five minutes at 150 rpm, placed on a shaking table. The virus should be incubated for one day at 37^{o}C with 5% CO_2 to appear. In 96-well plates, prepare a minimum of 2 milliliters of MTT solution (5 mg/ml) in PBS. In each well, add the MTT solution (20 µl). The MTT was combined with the media, placed on a shaker, and spun at 150 rpm for 5 minutes to fully incorporate the MTT. The MTT was allowed to metabolize for one to five hours by incubating at 37 ^{0}C with 5% CO_2. The media was thrown away. The dish was dry to remove any remaining residue by using paper towels. DMSO was used to dissolve the formazan crystals that were formed (200 µl per well). The formazan and solvent were added on a shaking table for five minutes at 150 rpm to fully mix them. The optical density was measured at 560 nm and take the background out at 620 nm. The number of cells and optical density should be correlated.

Cytotoxic activities of *C. droserifolia*. aerial parts ethanolic extract

Examination of the cytotoxicity of the sample on cells (MTT protocol): For inoculation, the 96-well tissue culture plate was inoculated with 1 × 10^5 cells/ml (100 µl/ well) and incubated at 37^{o}C for 24 hours to develop a complete monolayer sheet. The growth medium was decanted from 96 well microtiter plates after a confluent sheet of cells was formed, and the cell monolayer was washed twice with wash media. Two-fold dilutions of the tested sample were made in RPMI medium with 2% serum (maintenance medium). 0.1 ml of each dilution was tested in different wells leaving 3 wells as control, receiving only maintenance medium. The plate was incubated at 37^{o}C and examined. Cells were checked for any physical signs of toxicity, e.g. partial or complete loss of the monolayer, rounding, shrinkage, or cell granulation. MTT solution was prepared (5 mg/ml in PBS) (BIO BASIC CANADA INC). 20 µl MTT solution was added to each well and placed on a shaking table at 150 rpm for 5 minutes, to thoroughly mix the MTT into the media. MTT was incubated (37^{0}C, 5% CO_2) for 4 hours to allow the MTT to be metabolized. The media was Dump off. The Plate was dried on paper towels to remove residue if necessary. Formazan (MTT metabolic product) was resuspended in 200µl DMSO and then placed on a shaking table at 150 rpm for 5 minutes, to thoroughly mix the formazan into the solvent. Optical density was measured at 560nm and subtracted background at 620 nm. Optical density should be correlated with cell quantity.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) was done. A two-tailed Student's t-test was used to compare the treatment means to the control means. We considered $P< 0.05$ to be statistically significant. In (San Diego, California, USA) GraphPad Prism software was utilized to calculate the IC50 and CC50.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical constituents, tannins, saponins, terpenes, sterols, flavonoids, and alkaloids were determined by the preliminary phytochemical screening of the ethanolic 70% extract of the aerial portions.

Chemical composition of the essential oil of *Cleome droserfolia* extract

The active compounds of *Cleome droserfolia* petroleum ether extract was identified by using GC-MS/MS. The results revealed the presence of sixty-nine compounds recorded in different amounts (Table 1 and Figure 1). Sterol and fatty acids are present. The major components are benzene-1-pentyloctyl 5.77%, benzene-1-butylheptyl 5.06%, benzene-1-methyldecyl 4.93%, benzene-1-methyldecyl 4.77%, benzene-1-methylundecyl 4.66% and benzene-1-pentylheptyl 4.38%. The lowest percentage was recorded for fenretinide (0.07%), whereas the

percentages of indolizine, retinol, and retinal were 0.44%, 0.54%, and 0.06%, respectively.

Identification of free phenolic acids using HPLC

Both qualitative and quantitative results of the Phenolic acid analysis of *C. droserifolia* using HPLC were tabulated and illustrated in Table 2. The results showed that *C. droserifolia* contains fourteen phenolic acids and rutin had the highest phenolic acid content (245.40 mg/ml) followed by ellagic acid and gallic acid (43.52 and 33.15 mg/ml), respectively. However, cinnamic acid and kaempferol had the lowest values (0.15 mg/ml and 0.25 mg/ml), respectively.

Biological Studies

Antifungal and antibacterial activity of *Cleome droserfolia*

The results of the antibacterial and antifungal activities of *Cleome droserfolia* (assessed in terms of the inhibition zone) are tabulated in Table 3. The 70% ethanol extract of *Cleome droserfolia* had an inhibitory effect on Gram-positive bacteria, specifically *Bacillus cereus*, with an inhibition zone of 8 mm. Moreover, there was no inhibitory effect on *Staphylococcus aureus*. On the other hand, the extract had no effect on *Escherichia coli*, however, had an antibacterial effect against Gram-negative *Enterobacter cloacae* with an inhibition zone of 11 mm. The growth of the fungus *Candida albicans* was inhibited and the inhibition zone recorded 10 mm whereas, *Aspergillus niger* growth was not affected.

Antiviral activity of *C. droserifolia*. aerial parts ethanolic extract

The maximum nontoxic concentration (MNTC) recorded 62.5 µg/ml. whereas, the 50% cytotoxicity concentration showed the value 157.3 ± 0.55 µg/ml. IC50(The 50% inhibition concentration) recorded 69.81 ± 4.6 µg/ml. On the other hand, selective index (SI) which was determined from CC50 and IC50 ratios showed the value 2.253 were tabulated in Table 4 and illustrated in Figures 3, 4. The cell line HAV A was rounded and shrink by ethanolic extract.

Cytotoxic activities of *Cleome droserifolia* aerial parts ethanolic extract

The results were tabulated and illusterated in Table 5 and Figures 5, 6. IC50 values of *C. droserifolia* ethanol extract against breast cancer MCF-7 and liver cancer HepG2 cell lines were 29.45 ± 0.41 and 96.01 ± 1.57µg/ml, respectively. Whereas the IC50 values of *C. droserifolia* ethanol extract against the normal WI-38 cell line was 113.41 ± 1 µg/ml Also, the selective index (SI) showed values of 3.85 and 1.18, respectively. The ethanol extract of this species showed variable toxic effects (Photos 2 and 3). Retinol and retinal were found to be present in *Cleome droserifolia* extract by GC/MS analysis, with corresponding values of 0.44% and 0.06%. Due to their strong receptor-binding affinities and capacity to regulate gene expression, retinoids (vitamin A) have been widely shown to have anticancer effects.

DISCUSSION

This study characterized the cytotoxic potential of an important medicinal plant of the Sinia Peninsula, *C. droserifolia*, growing in Egypt. The results presented here contradict those obtained by (Abd El-Gawad et al. 2018), who reported that sesquiterpenes, such as α-cadinol, δ-cadinene, and γ-muurolene, cis-nerolidol constitute the main class of essential oils. However, in their analysis of the composition of *C. droserifolia* ecospecies, iso-aromadendrene epoxide, a significant component in the current study was never noted. Variations in habitat could be the cause of this. The current results, however, concur with those of (Alkhatib et al. 2022). Benzene, (1-butylheptyl)-; benzene, (1-pentylhexyl)- and benzene, (1-butylnonyl)- are used for an antibacterial effect (Abu ElKhair et al., 2020). The present results of phenolic compounds agree with those of (Hashem & Shehata, 2021) who identified sixteen phenolic compounds, such as rutin, ellagic acid and naringenin, o-coumaric acid, ferulic acid, chlorogenic acid, and catechin. Gallic acid has anti-HCV activity, and immunomodulatory effect (Bai et al., 2021; Mashraqi et al., 2023), while chlorogenic acid has antioxidant activity, antibacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-viral, anti-microbial, anti-hypertension (Naveed et al., 2018)). Phenolic acid such as ferulic acid also has antimicrobial, anti-inflammatory, and antitumor properties (Stompor-Gorący et al., 2021). The current findings agree with the findings of (Muhaidat et al., 2015), who noted that *C. droserifolia* oils showed remarkable inhibitory effects on the *Bacillus cereus*. Additionally, the current results agree with those of (Hashem & Shehata., 2021) who reported that the methanolic extract of *Cleome droserifolia* exhibited unusual inhibitory actions against *Candida albicans*. Additionally, the present results agree with the results of (Alkhatib et al., 2022). The current results agree with those of (Samara et al. 2020), who reported that *Cleome droserifolia* exhibited antiviral efficacy against HAV.

Table 1. Bioactive compounds of *Cleome droserifolia* petroleum ether extract determined via GC/mass spectrometry.

No	Name of Compounds	RT	%	Formula	Mwt.
1	Nonanoyl chloride	4.39	1.05	$C_9H_{17}ClO$	176
2	1-Gala-1-ido-octonic lactone	6.62	0.10	$C_8H_{14}O_8$	238
3	2-Myristynoyl pantetheine	6.97	0.10	$C_{25}H_{44}N_2O_5S$	484
4	Indolizine	8.06	0.44	C_8H_7N	117
5	9,12,15-Octadecatrienoic Acid	9.27	0.11	$C_{28}H_{40}O_4$	440
6	2,2,3,3,4,4Hexaadeutero octadecane	9.47	0.26	$C_{18}H_{30}D_6O$	274
7	6-Aminohexanamide, N-[methyl-(N-D4-pyrrolidinyl)-2-butynyl]-N-[2-ominobutanoyl]	9.59	0.21	$C_{18}H_{32}N_4O_2$	336
8	D-Fructose, diethyl mercaptal, pentaacetate	10.59	0.27	$C_{20}H_{32}O_{10}S_2$	496
9	M-cymen-4-OL	12.47	0.57	$C_{10}H_{14}O$	150
10	2-(Acetyloxy)-1-(Hydroxymethyl)Ethyl acetate	13.15	0.08	$C_7H1_2O_5$	176
11	a-D-Glucopyranose,4-o-a-D-galactopyranosyl-	14.60	0.34	$C_{12}H_{22}O_{11}$	342
12	2-Acetyl-3-(2-Cinnamido) Ethyl-7-Methoxyindole	16.07	0.11	$C_{22}H_{22}N_2O_3$	362
13	3-Oxo-20-Methyl-11-a-Hydroxyconanine-1,4-Diene	16.66	0.39	$C_{22}H_{31}NO_2$	341
14	Phynol,2,6-Bis(1,1-Dimethylethyl)-4-Methyl	17.08	0.38	$C_{15}H_{24}O$	220
15	Falcarinol	17.49	0.09	$C_{17}H_{24}O$	244
16	Benzene, (1-butylhexyl)-	17.76	1.50	$C_{16}H_{26}$	218
17	Benzene, (1-propylheptyl)-	17.98	1.35	$C_{16}H_{26}$	218
18	Benzene, (1-ethylcotyl)-	18.42	1.21	$C_{16}H_{26}$	218
19	12,15-Octadecadiynoic acid, methyl ester	18.82	0.17	$C_{19}H_{30}O_2$	290
20	Cinnamic,4-hydroxy-3-methoxy-(5-hydroxy-2-hydroxymethyl-6-[2-(4-hydroxy-3-methoxyphenyl) ethoxy] -4-(6-methyl-3,4,5-trihydroxytetrahydropyran-2-yloxy) tetrahydropyran-3-yl) ester	19	0.19	$C_{31}H_{40}O_{15}$	652
21	Benzene, (1-methylonyl)-	19.30	1.81	$C_{16}H_{26}$	218
22	Retinal	19.60	0.06	$C_{20}H_{28}O$	284
23	Benzene, (1-pentylhexyl)-	19.95	2.01	$C_{17}H_{28}$	232
24	Benzene, (1-butylheptyl)-	20.03	5.06	$C_{17}H_{28}$	232
25	Benzene, (1-propylcotyl)-	20.27	3.81	$C_{17}H_{28}$	232
26	2-Naphthalenemethanol, decahydro-a, a,4a-trimethyl-8-methylene, [2R-(2aa,8aa)]-	20.57	0.84	$C_{15}H_{26}O$	222
27	Benzene, (1-ethylnonyl)-	20.74	3.71	$C_{17}H_{28}$	232
28	Azulene,1,4-dimethyl-7-(1-methylethyl)-	20.97	0.29	$C_{15}H_{18}$	198
29	3,20-Dioxo-11-a-hydroxyconanine-1,4-diene	21.19	0.10	$C_{21}H_{27}NO_3$	341
30	Benzene, (1-methyldecyl)-	21.59	4.77	$C_{17}H_{28}$	232
31	Cis-5,8,11,14,17-Eicosapentaenoic acid	21.94	0.07	$C_{20}H_{30}O_2$	302
32	Benzene, (1-pentylheptyl)-	22.12	4.38	$C_{18}H_{30}$	246
33	Benzene, (1-butyloctyl)-	22.21	4.93	$C_{18}H_{30}$	246
34	Benzene, (1-propylnonyl)-	22.49	3.75	$C_{18}H_{30}$	246
35	10,13- Octadecadiynoic acid, methyl ester	22.78	0.08	$C_{19}H_{30}O_2$	290
36	Benzene, (1-ethyldecyl)-	22.96	3.84	$C_{18}H_{30}$	246
37	Neoclovenoxid-alcohol	23.24	1.38	$C_{15}H_{24}O$	220
38	1-Heptatriacotanol	23.44	2.2	$C_{37}H_{76}O$	536
39	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butylnyl]-	23.53	0.19	$C_{11}H_{18}N_2O_2$	210
40	Androst-4-En-3-one,13-hydroxy-, (17a)-	23.59	0.21	$C_{19}H_{28}O_2$	288
41	Benzene, (1-methylundecyl)-	23.79	4.66	$C_{18}H_{30}$	246
42	Benzene, (1-pentyloctyl)-	24.20	5.77	$C_{19}H_{32}$	260
43	Benzene, (1-butylnonyl)-	24.34	3.49	$C_{19}H_{32}$	260
44	Benzene, (1-propyldecyl)-	24.60	3.13	$C_{19}H_{32}$	260
45	9,12,15-Octadecatrienoicacid, (2-phenyl-1,3-dioxolan-4-yl) methyl ester	24.88	0.96	$C_{28}H_{40}O_4$	440
46	Benzene, (1-ethylundecyl)-	25.08	3.31	$C_{19}H_{32}$	260
47	2-Naphthalenol,2,3,4,4a,5,6,7-octahydro-1,4a-dimethyl-7-(2-hydroxy-1-methylethyl)	25.36	0.38	$C_{15}H_{26}O_2$	238
48	Isoaromadendrene epoxide	25.63	0.56	$C_{15}H_{24}O$	220
49	Fenretinide	25.78	0.07	$C_{26}H_{33}NO_2$	391
50	Benzene, (1-methyldodecyl)-	25.90	3.80	$C_{19}H_{32}$	260
51	Cyclopropane butanoic acid,2- [[2-[[2-[(2-pentylcyclopropyl) methyl] cyclopropyl] methyl]-, methyl ester	26.26	0.50	$C_{25}H_{42}O_2$	374
52	3-Isopropyl-6,7-dimethyltricyclo [4.4.0.0(28)] decane-9,10-diol	26.77	2.08	$C_{15}H_{26}O_2$	238
53	Hexadecenoic acid	27.11	3.69	$C_{16}H_{32}O_2$	256
54	Hexadecenoic acid, ethyl ester	27.59	1.44	$C_{18}H_{36}O_2$	284
55	6-Methyl-11-propenyl-5-(toluene-4-suifonyloxy)-12,13-dioxartycyclo [7.3.1.0(1,6)] tridecane-8-carboxylic acid, methyl ester	27.91	0.22	$C_{24}H_{32}O_7S$	464
56	3-n-Heptyl-7-methyl-9-(2,6,6-trimethylcyclohex-1-enyl) nona-2,4,6,8-tetraenal	28.07	0.10	$C_{26}H_{40}O$	368
57	Cholestan-3-ol, methylene-, (3a,5a)-	28.64	0.23	$C_{28}H_{48}O$	400
58	9,12,15-Octadecatrienoic acid,2,3-dihydroxypropyl ester, (Z, Z, Z)-	29.40	0.76	$C_{21}H_{36}O_4$	352
59	Linoleic acid ethyl ester	29.58	1.58	$C_{20}H_{36}O_2$	308
60	9,12 Octadecadienoic acid (Z, Z)-	30.26	1.15	$C_{18}H_{32}O_2$	280
61	E, E, Z-1,3,12-Nonaadecatriene-5,14-diol	30.36	2.95	$C_{19}H_{34}O_2$	294
62	9-Octadecnoic acid (Z,)-	30.81	0.99	$C_{18}H_{34}O_2$	282
63	Octadecanoic acid, ethyl ester	31.26	0.53	$C_{20}H_{40}O_2$	312
64	Tricyclo [20.8.0.0(7,16)] triacontane,1(22),7(16)-diepoxy-	32.20	0.36	$C_{30}H_{52}O_2$	444
65	1H-2,8a-Methanocyclopenta[a] cyclopropa [e]cyclodecen-11-one,1a,2,5,5a,6,9,10,10a-octahydro-5,5a,6-trihydroxy-1,4-bis(hydroxymethyl)-1,7,9-trimethyl-, [1s-(1a,1aa,2a-5aa,6a,8aa,9a,10aa)]	32.42	1.62	$C_{20}H_{28}O_6$	364
66	Stigmast-5-En-3-ol, (3a, 24s)-	33.57	1.96	$C_{29}H_{50}O$	414
67	Retinol	34.46	0.54	$C_{20}H_{30}O$	286
68	4H-1-Benzopyran-4-One,2-(3,4-dihydroxyphenyl)-6,8-Di-a-D-Glucopyranosyl-5,7-Dihydroxy-	36.73	0.83	$C_{27}H_{30}O_{16}$	610
69	Pregnan-20-one,5,6-epoxy-3,17-dihydroxy-16-methyl-, (3a,5a,6a,16a)-	37.86	0.05	$C_{22}H_{34}O_4$	362

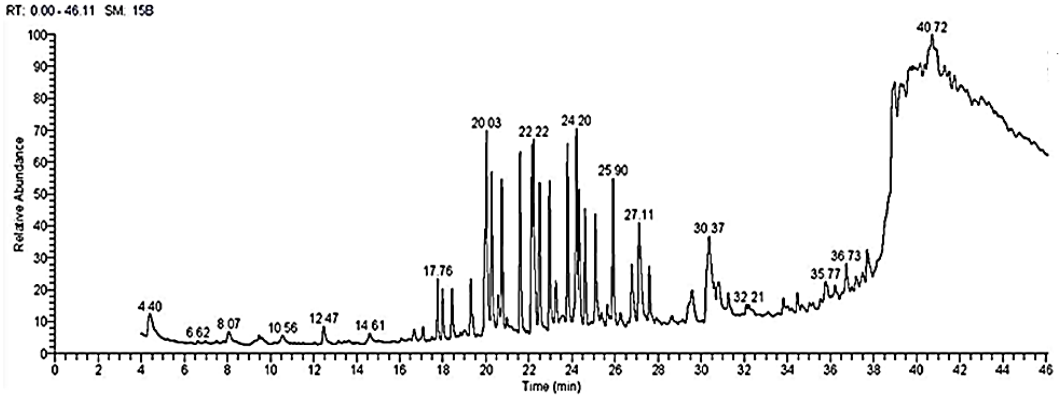

Figure 1. The spectra of active compounds in *Cleome droserifolia* petroleum ether extract via GC/ Mass spectrometry.

Table 2. Free phenolic acids in the *C. droserifolia* aerial portions were determined using HPLC.

No.	Name	mg/ml	Biological activity
1	Gallic acid	33.15	Anti-HCV activity, immunomodulatory effect (Bai et al., 2021; Mashraqi et al., 2023).
2	Chlorogenic acid	2.88	Antioxidant activity, anti-bacterial, hepatoprotective, cardioprotective, anti-inflammatory, antipyretic, neuroprotective, anti-viral, anti-microbial, and anti-hypertension (Naveed et al., 2018).
3	Catechin	0.89	Reducing skin damage, antioxidant and anticancer (Bae et al., 2020)
4	Methyl gallate	9.51	Anticancer, anti-inflammatory, antioxidant, neuroprotective, hepatoprotective and anti-microbial activities, and anti-diabetes (Liang et al., 2023)
5	Rutin	245.40	Anti-microbial, anti-fungal, and anti-allergic agent (Gullon et al., 2017)
6	Ellagic acid	43.52	Antioxidant, anti-hepatotoxic, anti-steatosic, anti-cholestatic, anti-fibrogenic, anti-hepatocarcinogenic and anti-viral properties (García-Niño et al., 2015)
7	Coumaric acid	1.89	Antioxidant and anticancer Antibacterial (Pei et al., 2016)
8	Vanillin	8.50	Anticancer, antidiabetic, antioxidant, antibacterial (Olatunde et al., 2022)
9	Ferulic acid	3.08	Antimicrobial, anti-inflammatory, and antitumor properties (Stompor-Gorący et al., 2021)
10	Naringenin	5.41	Antioxidant and anti-inflammatory activities (Uçar et al., 2023)
11	Daidzein	4.33	Anticancer, anti-cardiovascular, anti-diabetes, antiosteoporosis, and treatment skin disease, (Sun et al., 2016)
12	Quercetin	6.37	Antioxidant and it has anti-inflammatory, anti-cancer, and anti-cardiovascular disease effects (Wang et al., 2016)
13	Cinnamic acid	0.15	Antiangiogenic, antioxidant, and antitumorigenic effectiveness (Niero et al., 2013; Mashraqi et al., 2023)
14	Kaempferol	0.25	Anticancer (Lei et al., 2019), anti-inflammatory (Yeon et al., 2019), antioxidant (Wu et al., 2018)

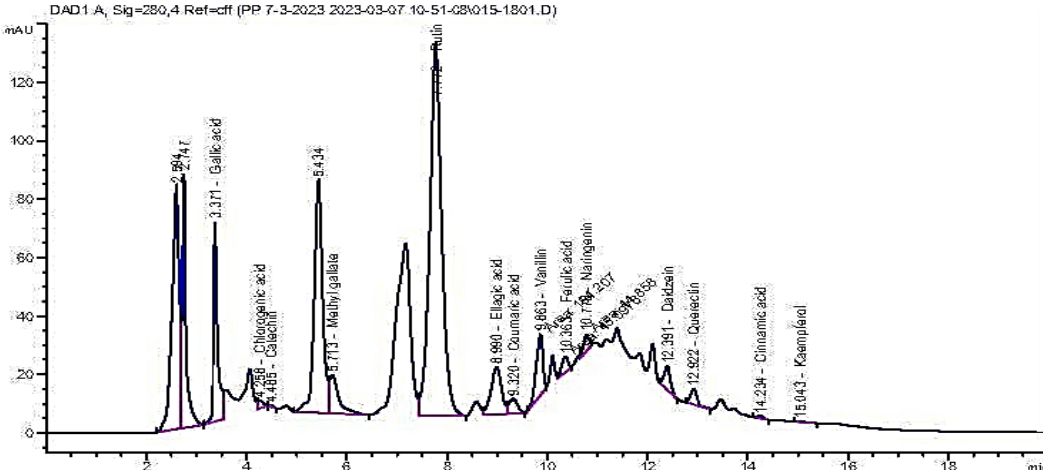

Figure 2. Free phenolic acids in the *Cleome droserifolia* aerial portions were determined using HPLC.

Table 3. Antimicrobial activities of *Cleome droserfolia* ethanol 70% extract.

Tested microorganisms	Diameter of nhibition zone mm*	
	Sample	Control
Fungi		*Ketoconazole*
Aspergillus niger (RCMB 002005)	NA	15
Candida albicans (RCMB 005003(1) ATCC 10231)	10	20
Gram-positive Bacteria		*Gentamycin*
Staphylococcus aureus (ATCC 25923)	NA	24
Bacillus cereus RCMB 027 (1)	8	25
Gram-negative Bacteria		*Gentamycin*
Escherichia coli (ATCC 25922)	NA	30
Enterobacter cloacae RCMB 001 (1) (ATCC 23355)	11	30

*The values represent the average for triplicate analyses, NA: not active.

Table 4. Antiviral activity of the *Cleome droserfolia* ethanol extract.

Cell line	MNTC µg/ml	CC_{50} µg/ml	IC_{50} µg/ml	SI
HAV	62.5	157.3 ± 0.55	69.81 ± 4.6	2.253

MNTC: maximum nontoxic concentration: CC_{50}: 50% cytotoxicity concentration. IC_{50}: The 50% inhibition concentration, and SI: selective index was calculated from the ratio between the CC_{50} and the IC_{50}.

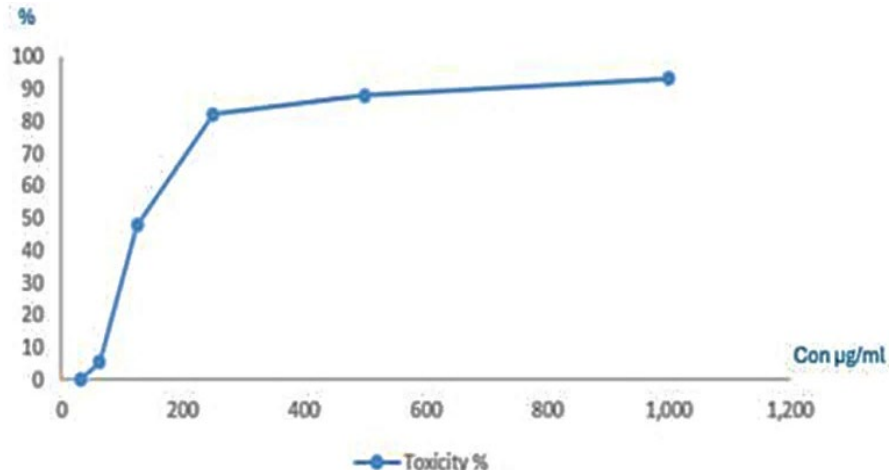

Figure 3. Determination of the maximum non-toxic concentration (MNTC) of *Cleome droserfolia* ethanol extract.

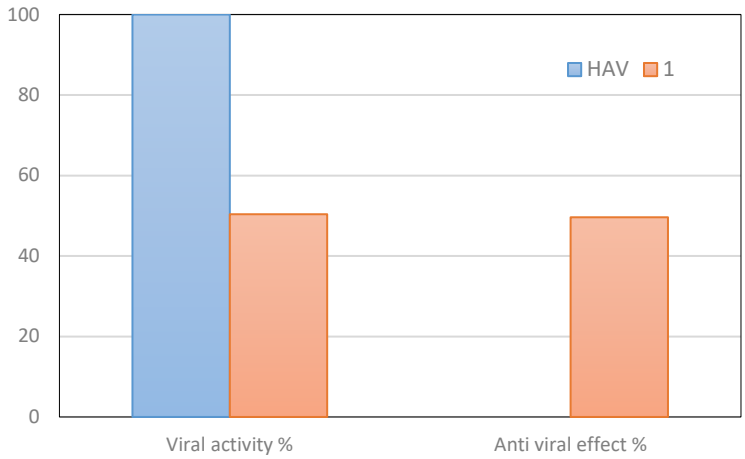

Figure 4. The antiviral effect of *Cleome droserfolia.* ethanol extract on Vero cells infected with HAV.

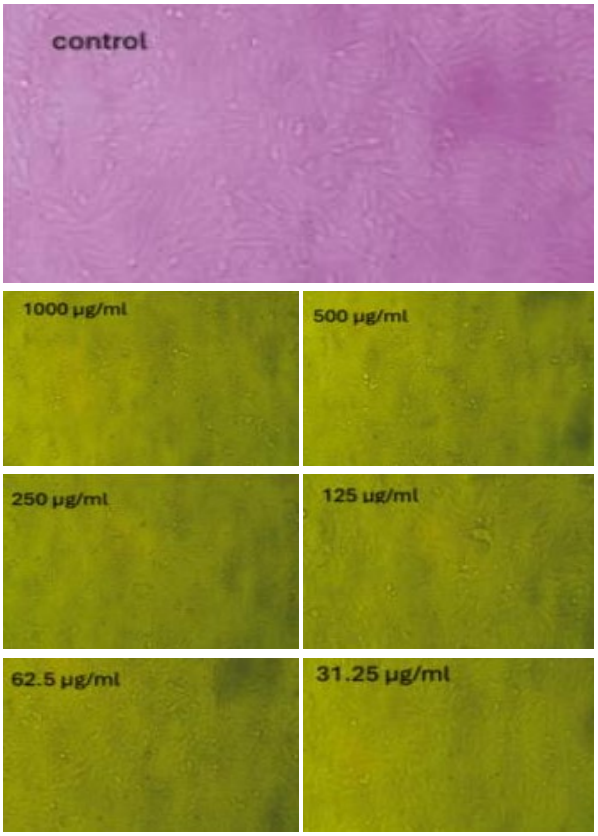

Photo 1. Morphological changes of Vero cells infected with HAV after treatment with *Cleome droserifolia* ethanol extract compared with control.

Table 5. Cytotoxic effects of *Cleome droserifolia* against cancer cell lines.

Cell Lines	IC_{50} (µg/ml)	SI
MCF-7	29.45 ± 0.41	3.85
HepG2	96.01 ± 1.57	1.18
WI-38	113.41 ± 1	

IC50: the half-maximal inhibitory concentration: human breast cancer (MCF-7), human liver cancer (HepG2) and WI-38: normal human fetal fibroblast .SI: The selectivity index is defined as the ratio of IC50 values for normal human fetal lung fibroblast (WI-38) divided by IC50 values for each cancer cell line.

Additionally, the current findings concur with those of (Chand et al. 2022), who stated that some chemical components found in cleome species possess a range of health-promoting characteristics, and the aerial portions may have traditional applications in both culinary and medical traditions. Strong substances with anti-cancer properties, including tannins, saponins, terpenes, sterols, flavonoids, and alkaloids, may be present in *Cleome* species. The HAV-infected Vero cells treated with 62.5 µg/ml plant extract were examined under a microscope. According to previous research, the HAV virus caused Vero cells to become round, shrivel, or granulate, as well as to lose some or

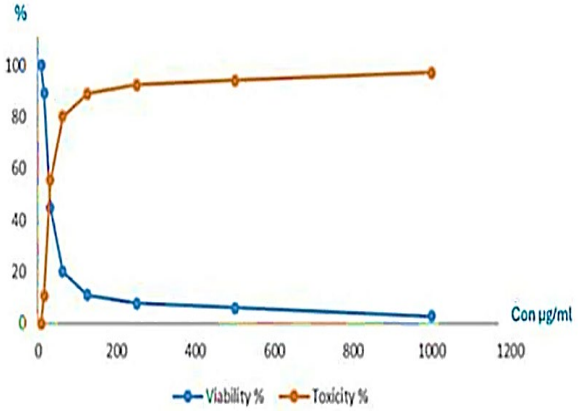

Figure 5. Determination of the half-maximal inhibitory concentration (IC_{50}) of *Cleome droserifolia* extract against breast cancer cell line (MCF-7).

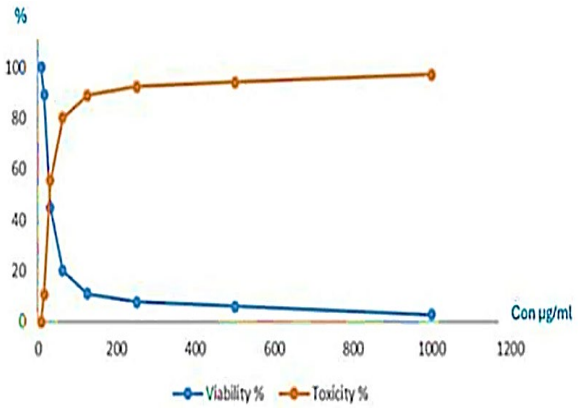

Figure 6. Determination of the half-maximal inhibitory concentration (IC_{50}) of *Cleome droserifolia* extract against Human liver cancer cell line (HepG2).

all their monolayers. The current results corroborate those of (Jin et al. 2022), who noted that retinoids may have anticancer effects against hepatocellular carcinoma in addition to potential anti-cancer effects on breast cancer. According to (Panicker et al., 2020), a methanolic extract of *Cleome droserifolia* can significantly kill cancerous and normal human cell lines and their results agree with the results of the present study. (Ezzat & Abdel Motaal, 2012) evaluated the cytotoxicity of both aqueous and ethanolic extracts of the aerial parts of *Cleome droserifolia*. The human breast cancer cell line (MCF7) was exposed to the extracts, which demonstrated anticancer effects, and the results agree with the present results (Figures 5, 6 and 7 & Photos 2 and 3). anticancer activities owing to the presence of active compounds as reported by Aboushoer et al., (2010) & Adewusi and Afolayan (2010) & and Abdullah et al., (2016).

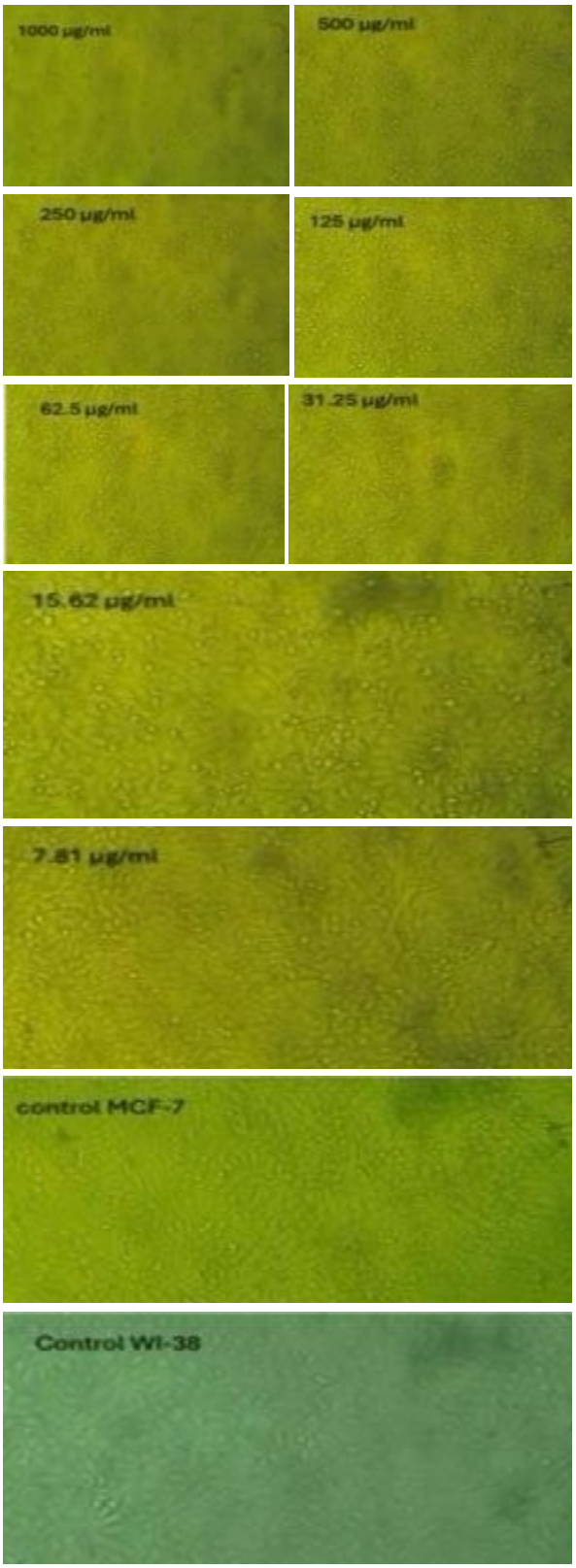

Photo 2. Morphological changes of breast cancer cell line (MCF-7) after treatment with *Cleome droserifolia* ethanol extract compared with control.

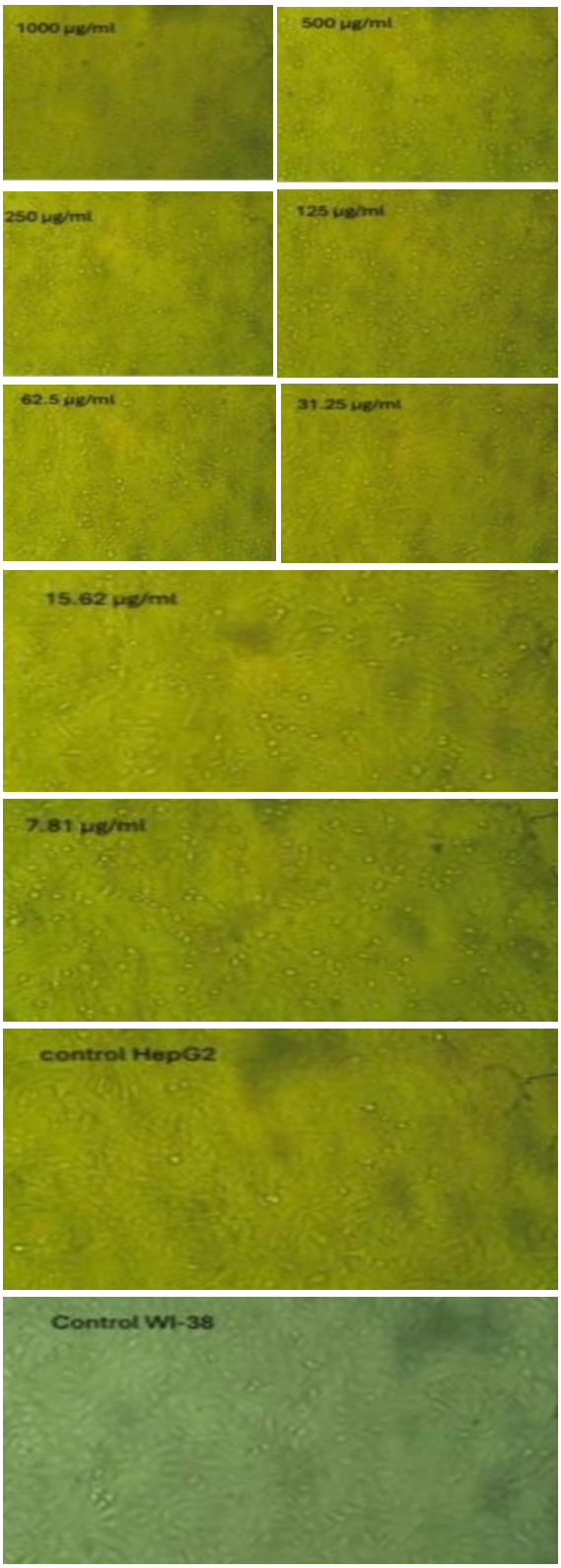

Photo 3. Morphological changes of liver cancer cell line (HepG2) after treatment with *Cleome droserifolia* ethanol extract compared with control.

CONCLUSION

This study reveals that *C. droserifolia* has effective cytotoxic potential against human cancer cells. The ethanol extract of *Cleome droserfolia* demonstrated the highest anticancer activity, with selective indices of 3.85 and 1.18 for MCF-7 and HepG2, respectively. The extract also demonstrates significant antibacterial activity. Gas chromatography-mass spectrometry (GC–MS) analysis exhibited the presence of several effective metabolites of compounds such as benzene, (1-pentylhexyl)-, benzene, (1-butylheptyl)-and benzene, (1-butylnonyl)-and certain fatty acids, the majority of which are responsible for the extract as an antibacterial agent. Consequently, to use *Cleome droserifolia* as a food preservative and in pharmaceutical research, it is imperative to extract its active ingredients. The current study recommends employing *Cleome droserifolia* as an antibacterial and anticancer agent.

ABBREVIATION

DMSO: Dimethyl sulfoxide
GC/MS: Gas chromatography-mass spectrometry
HAV: hepatitis A
HepG2: liver cancer
HPLC: High-performance liquid chromatography
MCF-7: breast carcinoma
MTT: [3-(4, 5-dimethylthiazol-2-yl)-2–5-diphenyltetrazolium bromide]
RCMB: The Regional Center for Mycology and Biotechnology
RPMI: Roswell Park Memorial Institute media
WI-38: lung fibroblast

CONFLICTS OF INTEREST

There are no conflicts to declare.

AUTHORS' CONTRIBUTIONS

Om Mohammed Khafagi proposed the idea and the study plan and participated in the data interpretation, manuscript writing, and revision. Zeinab A.S. El-Swaify proposed the idea and the study plan and participated in the data interpretation, manuscript writing, and revision. Walaa El-Alem participated in all the experiments, data interpretation, and manuscript writing, revision, and submission processes. Ahmed Abdallah Mohamed Mohamed guided the fieldwork team at Napq protectorate, located the stand, and participated in the chemical analysis of the plant extracts, data interpretation, manuscript writing, and revision. Ramadan Ibrahim Ramadan Bedair participated in sample collection of Napq protectrate, in statistical treatment and data management.

ETHICS APPROVAL

Not applicable

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