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Pharmacological Activities and Characterization of Phenolic and Flavonoid Compounds in Solenostemma argel Extract

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Abstract

Solenostemma argel is a desert medicinal plant indigenous to African countries. The goal of this work was to investigate the pharmacological properties of phenolic and flavonoid compounds present in the *Solenostemma argel*. Aerial parts (leaves and flowers) of *Solenostemma argel* were tested for antibacterial activity, antioxidant activity, cell viability and cytotoxic effects, and anti-inflammatory activity. Phenolic and flavonoid contents (HPLC), and volatile constituents (GC-MS) were also characterized. The antioxidant activity of *Solenostemma argel* extract increased from (34.47%) at a concentration of 10 µg/mL to (98.47%) at a concentration of 1280 µg/mL. The most sensitive organism was *S. aureus* with the highest inhibition zone and lowest MIC, with acetone extract. Whereas *C. tropicalis* was the most resistant, with the lowest inhibition zone. MTT assay indicated that the methanolic extract of *Solenostemma argel* had significant cytotoxic effects on the A549, Caco-2, and MDAMB-231 cell lines, respectively. The anti-inflammatory activity showed at lower concentrations of methanolic extract, and those effects were compared with indomethacin as a standard. Gallic was the most abundant phenolic acid, followed by syringic, p-coumaric, caffeic and ferulic acids, respectively. The Catechin and Quersestin, Luteolin and rutin flavonoids were also found in the methanolic extract. GC-mass analysis showed that aerial parts of *Solenostemma argel* were rich in 2-(5-methyl-5 vinyl tetrahydro-2-furanyl)-2-propanol (11.63%), hexanoic acid methyl ester (10.93%), 3-dioxolane,4-methyl-2-pentadecyl (9.69%), phenol, 2-(1,1-dimethylethyl) (8.50%). It can be concluded that methanolic extract of *Solenostemma argel* could be used as an available source of natural bioactive constituents with consequent health benefits such as antioxidants, anti-inflammation, anticancer and antimicrobial effects.

1. Introduction

The use of medicinal plants for therapeutic purposes is an ancient form of medication, which is universal among most non-western countries and predates modern medicine (Marrelli, 2021). It is thought that a significant percentage (~ 75%) of the human population uses herbs and other forms of traditional medicines to treat diseases **(**Pandey and Madhuri, 2009**).** In addition, a substantial number of current pharmaceuticals such as aspirin, codeine and quinine used for disease therapeutics in modern medicine are derived from historical herbal remedies and medicinal plants (Pandey and Madhuri, 2009; Akinyemi et al., 2018). Therefore, medicinal plants are important sources of highly effective pharmaceuticals for disease treatment and sustainable human health **(**Akinyemi et al., 2018).

Most common human diseases are caused by microbial infections and cellular damages caused by oxidative stress related to imbalances between the formation and neutralization of pro-oxidants (**Braca, and Sortino 2002;** Fan et al., 2019). Oxidative stress caused by free radicals alongside lipid peroxidation, are contributory factors to multiple diseases including cardiovascular diseases, atherosclerosis, cancer, and inflammatory diseases **(**Lobo et al., 2010; Lushchak 2014**).** Anand et al., 2019 **and** Marrelli, 2021**)** showed that some medicinal plants can be used to treat diseases linked to pathogenic micro-organisms and cellular damages such as inflammatory and immune disorders. Medicinal plants contain primary or secondary metabolites such as lignans, flavonoids, terpenoids, tannins, phenolic acids, alkaloids, quinones and coumarins which exhibit significant antioxidant, antimicrobial and anti-cancer activities (Kaur et al., 2011; Anand et al., 2019; Marrelli, 2021).

Cancer is one of the main causes of morbidity and mortality worldwide. Cancer is caused by unregulated cell proliferation which leads to the disruption in the function and structure of surrounding tissues **(**Gennari et al., 2007; **Gezici and Sekeroglu, 2019).** There are more than 100 forms of cancer with prostate cancer (in men) and breast cancer (in women) being the major forms of cancer in humans (Kelloff, 2008; Soliman et al., 2006). Despite the availability of treatments such as radiation, chemotherapy, immune and hormone therapy, cancer remains one of the leading causes of death worldwide aside from cardiovascular and infectious diseases (**Gezici and Sekeroglu, 2019).** While some of these cancer treatment approaches are moderately effective, their long-term use and success rates are hampered by associated chronic toxic effects (**Gezici and Sekeroglu, 2019).** Given that free radical activities and inflammation are associated with the cancer development, drug candidates which can scavenge free radicals and inhibit inflammation with limited toxic effects are better suited for use as anticancer agents.

Therefore, medicinal plants, continue to be investigated as a resource to treat different cancers such as colon cancer (Aiello et al., 2019; Ghorat 2020), breast cancer (**Kamble et al., 2019**) and prostate cancer (Asadi-Samani, et al., 2018). Nevertheless, no silver bullet is yet to be found from medicinal plants for the complete treatment and cure of cancer despite the evaluation of many candidates. Hence, there is a need to continue the evaluation of more plants for their anti-cancer and anti-inflammatory properties that would inhibit or prevent the development of cancer. One of the promising candidates is a plant called argel.

Argel (*Solenostemma argel* Hayne) is a wild herbal plant widely found in different countries such as Sudan, Saudi Arabia, and Egypt. Different parts of this plants (leaves, stems and bark) are used for pain relief and treating infections affecting the respiratory, urinary and gastrointestinal systems (Awad et al., 2006). These parts have also been used to treat liver, kidney, diabetes and cardiovascular diseases in these countries (Awad et al., 2006). Pregnane and phenolic glycosides extracts from the plant's aerial and root parts have been shown to possess antioxidant properties in human tumor cell lines (Ounaissia et al., 2016). Acetone extracts of this plant have also been shown to have antioxidant and anti-inflammatory properties in laboratory rats (Benmaarouf et al., 2020). However, despite its widespread use and anecdotal evidence of its anti-cancer properties, very little research has been carried out to evaluate its therapeutic properties. Therefore, this research work aims to evaluate the antimicrobial, antioxidants, anti-inflammation and anticancer of *Solenostemma argel* extracts.

2. Materials And Methods

2.1. Plant materials and sample preparation

The plant materials, *Solenostemma argel* (Family Apocynaceae), used in this study were collected from the Halaib and Shalatin (Gabal Elba) region in Egypt. The collected plant materials were washed using distilled water. The aerial parts were cut and air-dried in the shade. These air-dried plant materials were ground into a fine powder using Moulinex Dpa241 Choppers, Blender 1000, France, and stored at -20˚C in screwed in brown bottles until needed for further investigations.

Extraction process

One hundred grams of the powdered *Solenostemma argel* (aerial parts) was extracted by shaking at 150 rpm for 24 h at 25°C with 1 L of solvent (water, methanol, acetone, ethanol, chloroform, ether, ethyl acetate, and methylene chloride). The extracts were subsequently filtered using a Buchner funnel containing a Whatman No. 1 filter paper. The obtained residue was re-extracted with 500 mL of solvent, filtered, after which the filtrates (extracts) were pooled together and concentrated using a rotary evaporator at reduced pressure (Heidolph VV 2000). The concentrated extract was dried in a desiccator under vacuum until a consistent weight was obtained. The weight of the triplicate extracts (samples) was recorded, and each extract was re-suspended in the smallest amount of solvent possible to achieve a concentration of 10 mg/mL. The extracts were stored at -4˚C until use.

2.2. Antioxidant capacity assay

Antioxidant activity was determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay as described by Yen and Duh (1994). The assay reaction mixture contained 40 µL of *Solenostemma argel* extract from different solvent extractions at different concentrations, ranging from 10 to 1280-µg/mL, which had been prepared by diluting the extract with the extraction solvent and 3 mL of methanolic solution of 0.1 mM DPPH radical. The mixture was vigorously agitated and incubated at 37˚C for 30 min. The absorbance values were measured at 515 nm using a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). Ascorbic acid was used as a positive control. The absorbance value of the reaction mixture was calculated using the following equation:

DPPH scavenging activity (%) = 100 x (A_0 - A_1) / (A_0)

The absorbances of the control and sample, respectively, are A0 and A1. The results are presented as the average of three replicate analyses, with the major values as well as the standard deviation (SD) provided.

The 50% inhibitory concentration (IC50), the concentration required to 50% DPPH radical scavenging activity was estimated from graphic plots of the dose response curve using Graphpad Prism software (San Diego, CA, USA).

2.3. Antimicrobial activity

2.3.1. Microorganism

Eight clinical microbial isolates were used in the experiments (Gram-negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, and Acinetobacter baumannii*; Gram-positive bacteria: *Staphylococcus aureus, Streptococcus epidermidis, and Enterococcus faecalis* and yeast: *Candida tropicalis*. The isolates were collected and identified from clinical specimens (sputum, end tracheal tube (ETT), nasal swab, and laryngeal swab (from respiratory tract infections patients) from Giessen University Clinic, Germany (Hassan et al., 2017).

2.3.2. Paper disc diffusion assay

For disc diffusion assay, 100 µL of cell suspension was uniformly spread onto Mueller Hinton agar. The 6 mm diameter filter paper discs (Whatman No. 41) were positioned to contact the surface of infected agar and were impregnated with 25 µL extract made at a concentration of 10 mg/mL. The plates were incubated

for 24 h at 37 ◦C. The diameter of the zone of inhibition (ZOI) was then measured precisely, and the means of the triplicates were determined (Cheesbrough, 2005).

2.3.3. Determination of minimal inhibitory concentration (MIC)

Each of the *Solenostemma argel* extracts (acetone, chloroform, and ethyl acetate) was tested against the test isolates as a quantitative experiment to determine the minimum inhibitory concentrations (MIC) of the extracts. The bifold dilution method (Koo et al., 2002) was employed using a series of 6 tubes, each holding 100 µL of Mueller Hinton broth (Accumix–Verna, India) to achieve final dilutions of 3.12, 6.25, 12.5, 25, 50 and 100% (v/v). The test bacterial inoculum load was $5x10^5$ cfu/ml. Each test microbial isolate was inoculated into all the dilutions and the inoculated tubes were incubated overnight at 37°C. The MIC value of these fractions against the tested isolates was calculated using the greatest dilution of the tested different extracts of *E. cuneata* which inhibited growth (no turbidity in the tube) (Aamer et al., 2014).

2.4. Cell viability and cytotoxic effects

2.4.1. Mammalian cell lines

Three mammalian cell lines, the human lung cancer cell line (A549), the human colon adenocarcinoma cell line (Caco-2), and the human breast cancer cell line (MDA-MB-231) were used to assess the cytotoxic effects of the different extracts. These cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD) through personal communication with Prof. Dr. Mahmoud Al-Aeser, the Regional Center for Mycology and Biotechnology (RCMB) at Al Azhar University, Egypt.

2.4.2. Propagation of cell lines

Dulbecco's modified Eagle's medium (DMEM) was used to propagate the cells. The media was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, HEPES buffer, and 50 µg/mL gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub-cultured two times a week.

2.4.3. Cytotoxicity assay

The cytotoxicity assay was evaluated as described by Mosmann (1983). Briefly, the cells were seeded in a 96-well plate at a cell concentration of $1×10^4$ cells per well in 100 µL of growth medium using triplicate samples. A fresh medium containing different concentrations of the methanolic extract was added 24 h after seeding. Two-fold diluents of the methanolic extract were also added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 24 h. Three wells were used for each concentration of the test sample. Control cells were incubated without extract and with or without DMSO. The DMSO concentration in each well was too low (maximal 0.1%) to affect the experiment.

After incubating the cells at 37°C for 24 h, the viable cell yield was determined by a colorimetric method. After the end of the incubation period, the media in the well were aspirated and crystal violet solution (1%) was added to each well for at least 30 min. The plates were then rinsed with tap water until all the excess stains

had been removed. Glacial acetic acid (30%) was subsequently added to all wells, mixed thoroughly and the plates placed into the microplate reader (SunRise, TECAN, Inc, USA) for absorbance measurements at a test wavelength of 490 nm. All the results were corrected for the background absorbance detected in wells without added stain Treated samples were compared with the control cell in the absence of the tested compounds. The cytotoxic effects of different concentrations of the methanolic extracts on cells were calculated as described by Mosmann (1983**).** The obtained absorbance values were used to determine the number of viable cells and the % of viability was calculated using the formula (OD_T/OD_C)] x 100%.

OD_T = the mean optical density of wells treated with the tested sample

OD_C = the mean optical density of untreated cells.

The relationship between surviving cells and drug concentration was plotted to get the survival curve of each tumor cell line after treatment with the specified compound. The 50% inhibitory concentration (IC_{50}), the concentration required to cause toxic effects in 50% of intact cells, was estimated from graphic plots of the dose-response curve for each concentration. using GraphPad Prism software (San Diego, CA. USA).

2.5. In vitro anti-inflammatory activity

2.5.1. Erythrocyte suspension preparation

The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged for 10 min at 3000 g to generate an erythrocyte suspension.

2.5.2. Hypotonic solution-induced erythrocyte hemolysis

Membrane stabilizing activities of the methanolic extract (with high cytotoxic activity) and antioxidants was assessed using hypotonic solution-induced erythrocyte hemolysis. The sample used consisted of a stock erythrocyte (RBCs) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate-buffered saline (pH 7.4) containing the methanolic extracts (7.81–1000 µg/mL) or indomethacin (as positive control). The negative control sample consisted of 0.5 mL of RBCs mixed with hypotonic-buffered saline solution alone. The mixtures were incubated in 96 well plates for 10 min at room temperature and centrifuged for 10 min at 3000 × g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of hemolysis or membrane stabilization was calculated according to the modified method described by Shinde et al., (1999).

Inhibition of hemolysis (membrane stabilization %) = OD_1-OD_2/OD_1 x 100

OD_1 = Optical density of the hypotonic-buffered saline solution alone

OD_2 = Optical density of extract in the hypotonic solution

The IC_{50} value was defined as the concentration of the sample to inhibit 50% RBCs hemolysis under the assay conditions

2.6. Determination of the phenolic acids and flavonoids of *Solenostemma argel* methanolic extract

HPLC (Agilent 1100) which was used for the detection of phenolic acids and flavonoids consisted of two LC pumps, a UV/V detector, and a C18 column (150 x 4.60 mm, 5 µm particle size). The Agilent ChemStation was used to obtain and analyze chromatograms. Phenolic acids were separated using a gradient mobile phase consisting of two solvents: solvent A (methanol) and solvent B (acetic acid in ultrapure water, 1:25). Elution from the column was achieved with the following gradients: 0 to 3 min of solvent B, followed by 50% eluent A for the next 5 min, after which the concentration of A was increased to 80% for the next 2 min and then reduced to 50% again for the following 5 min. Flavonoids were separated using a gradient mobile phase consisting of two solvents: solvent A (acetonitrile) and solvent B (0.2%, v/v aqueous formic acid) with an isocratic elution (70: 30) program. The detection wavelength was set between 200 and 450 nm, with specific monitoring conducted at 220 nm. Identification of the phenolic and flavonoid compounds was performed by comparing the retention times of the analyses with reference standards according to the methods described by Mattila et al., (2000)

2.7. Determination of the volatile components of *Solenostemma argel* methanolic extract

GC-MS analysis of the methanolic extract was carried out using a Thermo Scientific TRACE 1310 Gas Chromatograph coupled with an ISQ LT (single quadrupole Mass Spectrometer). The column was DB5-MS, 30 m, 0.25 mm ID (J&W Scientific). Helium at a flow rate of 1.0 mL/min was used as carrier gas. The temperature program was as follows: started at 40°C, sample held at 40°C for 3 min; increasing to 280°C with 5.0°C/min heating rate ramp and maintained at this temperature for 5 min and before an increase to 290°C with 7.5°C/min heating rate and samples then maintained at this temperature for 1 min. The injection and detector temperatures were 200 and 300°C, respectively. Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of m/z 40–450. The compounds were identified by WILEY & NIST mass spectral data base.

2.9. Statistical analysis

An analysis of variance (ANOVA)was performed on the data to examine any significant difference between the samples used in his study. The means of triplicate samples were calculated as well as their standard deviation (SD). Duncan's multiple range tests ($p \leq 0.05$) were used to examine the significance of the variable mean differences. All statistical analyses were carried out using IBM SPSS version 16.

3. Results And Discussion

3.1. DPPH radical scavenging activity of Solenostemma argel extracts

To investigate the potential health- properties of *Solenostemma argel*, we carried out a screening of the antioxidant abilities of different extracts of kumquat peel. **Table (1)** shows that all *Solenostemma argel*

extracts scavenged the DPPH radical in a dose-dependent manner. In terms of the effect of the extraction solvent on antioxidant activity, methanol extract had the highest scavenging potency (79.36%), followed by ethanol (66.17%) and acetone (61. 77%) respectively. While chloroform showed the lowest scavenging potency (40.34%).

Data also showed that the scavenging potency increased with increasing *Solenostemma argel* concentration. The highest scavenging percentage (89.94%) was observed using the concentration of 1280 µg/mL that was followed by 640 µg/mL (85.86%) at concentration of 640 µg/mL (71.26%) and (71.29%) at concentration of 320 µg/mL respectively. On the other hand, the concentration of 10µg/mL gave the lowest scavenging potency with an average of 7.76%. The lowest IC50 value (16.8 µg/mL) was recorded with methanolic extract, while the highest value (129.6 µg/mL) was recorded with water extract (Table 2).

Table 1
Radical scavenging activity of Solenostemma argel extracts **at different concentrations toward DPPH**

Conc. (µg/mL	[a]Radical scavenging activities of extracts							
	Water	Acetone	Chloroform	Methylene chloride	Ether	Methanol	Ethanol	Ethyl acetate
1280	96.53 ± 0.79	94.78 ± 0.86	93.84 ± 0.92	96.82 ± 0.64	94.59 ± 1.07	98.47 ± 0.59	95.06 ± 0.62	89.94 ± 0.78
640	91.65 ± 1.23	91.36 ± 0.72	82.59 ± 1.37	90.96 ± 1.32	86.24 ± 1.83	96.29 ± 0.63	92.82 ± 0.74	85.86 ± 1.32
320	76.24 ± 1.48	85.16 ± 1.34	57.06 ± 2.86	82.24 ± 1.78	78.47 ± 1.59	94.65 ± 0.41	89.41 ± 0.65	71.29 ± 1.53
160	56.98 ± 1.76	76.35 ± 1.97	37.88 ± 3.94	67.06 ± 1.92	60.59 ± 2.65	92.12 ± 0.86	78.57 ± 1.33	55.53 ± 2.71
80	38.62 ± 3.44	55.43 ± 2.81	21.76 ± 1.83	50.76 ± 1.89	42.71 ± 3.13	87.35 ± 0.97	67.75 ± 1.44	43.18 ± 2.84
40	25.76 ± 1.28	40.88 ± 2.76	16.24 ± 1.62	31.88 ± 2.36	29.70 ± 2.34	74.06 ± 1.42	53.62 ± 1.94	27.65 ± 1.97
20	18.56 ± .73	28.41 ± 2.93	10.43 ± 0.81	20.82 ± 1.42	13.94 ± 1.52	57.18 ± 2.98	31.48 ± 2.36	19.42 ± 1.06
10	7.65 ± 0.19	15.82 ± 1.74	2.94 ± 0.54	14.58 ± 0.78	4.35 ± 0.61	34.47 ± 3.15	19.76 ± 1.42	7.76 ± 0.82
Group mean ± SE	51.49 ± 1.24	61. 77 ± 1.77	40.34 ± 1.54	56.81 ± 1.54	51.32 ± 2.23	79.36 ± 1.12	66.17 ± 1.52	50.1 ± 1.75

[a]Radical scavenging activity given as percentage inhibition

The percentage inhibition value of the standard compound ascorbic acid was 99.23% at a concentration of 1280 µg/mL

Table 2
IC_{50} values of Solenostemma argel extracts **extracts toward DPPH**

Type of extract	IC_{50} (µg/ml)
	Solenostemma argel extracts
Water	129.60 ± 5.48
Acetone	65.10 ± 3.1
Chloroform	26.10 ± 17.4
Methylene cholide	78.40 ± 2.84
Ether	112.60 ± 4.6
Methanol	16.80 ± 0.62
Ethanol	36.70 ± 3.2
Ethyl acetate	124.20 ± 6.4
The IC_{50} values for Solenostemma argel extracts were calculated using Quest Graph™ IC_{50} Calculator (AAT Bioquest, Inc, Sunnyvale, California, CA, USA). IC_{50} was determined with a non-linear model.	

The obtained results agreed with the results obtained by **(Kebbab-Massime et al.,2017**) who stated that the methanolic extract of *Solenostemma argel* had better radical scavenging activity than the aqueous extract at all doses tested using the DPPH assay.

Our study is unique because of evaluating the antioxidant activity of several argel extracts from different polarity solvents and related the antioxidant activity of these extracts to mainly phenolic acids and flavonoid **(Ahmed et al.,2020).**

3.2. Antimicrobial activity of *Solenostemma argel* extract

The antimicrobial activity of different *Solenostemma argel* extracts (water, methanol, acetone, ethanol, chloroform, ether, ethyl acetate, and methylene chloride) were tested against clinically isolated microorganisms that cause nosocomial respiratory tract infections (gram- negative bacteria: *Escherichia coli, Pseudomonas aeruginosa, Enterobacter cloacae, and Acineto- bacter baumannii*; gram-positive bacteria: *Staphylococcus aureus, Streptococcus epidermidis, and Enterococcus faecalis; and yeast: Candida tropicalis*). Antimicrobial activity was determined qualitatively and quantitatively based on the existence of inhibitory zones, MIC, and MBC values.

The most active solvents on most isolates were acetone, chloroform, and ethyl acetate, whereas methylene chloride and methanol had modest activity on these isolates. On the other hand, water, ether and ethanol extracts had no inhibitory effect on isolated bacteria **(**Table 3**).** Based on earlier findings, several extracts of *Solenostemma argel* (acetone, chloroform, and ethyl acetate) showed the best activity on respiratory tract infections isolated bacteria, therefore we chose it for MIC and MBC testing. The best extract with a powerful MIC for *Solenostemma argel* was ethyl acetate, followed by acetone, and finally chloroform. Conversely,

water, ethanol, and ether fractions have not acted against isolated microbes as given in Table (3). The data that obtained indicated that the extracts of (acetone, chloroform, methanol, methylene chloride, and ethyl acetate) of *Solenostemma argel*, recorded powerful antimicrobial action on eight respiratory tract infections isolates. Ethyl acetate and acetone extract have shown significant activity toward isolated microbes, where the most significant action into *C. tropicalis* is 26 and *E. cloacae* 24.5 mm with of the clear zone (each) and the weakest action on *S. epidermidis* 19 and *E. faecalis* 13.5 mm respectively. Chloroform and methanol extract giving mild activity against tested isolates where *S. aureus* and *E. coli* show high sensitivity about 22.5 mm and 19.5 (diameter of inhibition zone each), and while *E. cloacae, E. faecalis* shown resistance 17.5, 14.00 mm (inhibition zone) for each. Methylene chloride extract showing low activity against isolated microbes, the highest activity of the extracts against *A. baumannii*, *C. tropicalis*, and *S. aureus* 15 mm of clear zone, while *P. aeruginosa* exhibits resistance 13.50 mm (diameter of inhibition zone). *P. aeruginosa, S. aureus*, and *C. tropicalis* were more sensitive microorganisms for all tested extracts. On another hand, *E. faecalis* was no sense to all examine extracts. Chloroform and acetone extracts exhibit the most significant value of MIC and MBC action. On the other hand, ethyl acetate extract gives the most under results of MIC and MBC activity. *A. baumannii* gives high results of MIC and MBC activity, while *E. faecalis* shows no significant value of MIC as seen in the table (4). Form previous outcomes a various extracts acetone, chloroform, and ethyl acetate of *Solenostemma argel* had owned the most excellent activity on nosocomial respiratory tract infections isolated microorganisms, so we selected it for assaying MIC and MBC. *Solenostemma argel* extracta have more important MIC and MBC as given in table (3 and 4). Gram-negative bacteria: *Enterobacter cloacae*, and *Acinetobacter baumannii*, gram-positive bacteria: *Staphylococcus aureus* and *Enterococcus faecalis* and yeast: *Candida tropicalis* were most microorganisms have the great values of MIC and MBC of extracts (acetone, chloroform, and ethyl acetate) of *Solenostemma argel*, shown the powerful value of MIC toward all isolates microbes, while MBC of all extracts moderates excepting chloroform, which showed the greatest rate of MBC as existing in a table (4).

Table 3
Effect of various extracts of *Solenostemma argel* on respiratory tract infections microbes.

Isolates	Diameter of inhibition zone of extracts (mm)							
	Water	Methanol	Acetone	Ethanol	Chloroform	Ether	Ethyl acetate	Methylene chloride
	Gram-positive bacteria							
S. aureus	-	16.5 ± 2.1	22.5 ± 3.5	-	22.5 ± 0.7	-	24.5 0.7	15 ± 0
S. epidermidis	-	16 ± 1.4	18.5 ± 2.1	-	20 ± 0	-	19 ± 0	-
E. faecalis	-	14 ± 1.4	13.5 ± 0.7	-	-	-	-	-
	Gram-negative bacteria							
E. coli	-	19.5 ± 3.5	23 ± 0	-	21.5 ± 2.1	-	19.5 ± 3.5	14 ± 1.4
E. cloacae	-	18.5 ± 2.1	23.5 ± 2.1	-	17.5 ± 0.7	-	23.5 ± 0.7	14.5 ± 0.7
P. aeruginosa	-	17.5 ± 0.7	23 ± 2.8	-	22.5 ± 2.1	-	24.5 ± 0.7	13.5 ± 0.7
A. baumannii	-	16 ± 1.4	23 ± 0	-	21 ± 1.4	-	22.5 ± 3.5	15 ± 0
	Fungi							
C. tropicalis	-	17.5 ± 3.5	23 ± 1.4	-	19.5 ± 0.7	-	26 ± 1.4	15 ± 0

Table 4
Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) for different extracts of *Solenostemma argel* against respiratory tract infections microbes.

Isolates	Extracts					
	Minimum Inhibitory Concentration MIC))			Minimum bactericidal concentration (MBC)		
	Acetone mg/ml	chloroform mg/ml	Ethyl acetate mg/ml	Acetone mg/ml	chloroform mg/ml	Ethyl acetate mg/ml
	Gram-positive bacteria					
S. aureus	12.5	25	25	50	100	100
S. epidermidis	12.5	6.25	25	50	12.5	25
E. faecalis	25	N	N	100	N	N
	Gram-negative bacteria					
E. coli	25	25	25	100	25	100
E. cloacae	25	25	25	100	25	100
P. aeruginosa	25	25	12.5	100	25	50
A. baumannii	25	25	25	25	25	25
	Fungi					
C. tropicalis	25	25	25	25	100	100

Table 5
Sensitivity microbes toward standard antibiotics.

Isolates	Diameter of inhibition zone of control (antibiotics (mm))			
	Am 10 µg/ml	CAZ 30 µg/ml	CN 10 µg/ml	E 15 µg/ml
	Gram-positive bacteria			
S. aureus	N	N	26	30
S. epidermidis	N	N	25	R
E. faecalis	N	N	25	R
	Gram-negative bacteria			
E. coli	R	20	N	N
E. cloacae	22	R	N	N
P. aeruginosa	R	22	N	N
A. baumannii	21	R	N	N
	Fungi			
C. tropicalis	N	N	N	N

The standard antibiotics (amoxicillin, ceftazidime, erythromycin, and gentamicin were applied commercial drugs to the healing of RTIs. Unique *S. aureus* was sensitive for all standard antibiotics, while all microbes were of moderate sensitivity for tested antibiotics as given in the **Table (5).** Our results establish with several researchers, for example, **Zain**, et al., **(2011)**, a record that *Euphorbia cuneata* Vahl., and *Solenostemma argel* methanolic extracts have great antimicrobial action toward Gram-positive, Gram-negative bacteria, and fungi, on another side, The minimum inhibitory concentration (MIC) of plants fractions toward the examined microorganisms differed from one plant to the other. Also, Kirbag et al., (2013, **and (Soliman et al., 2021)** reported that reported that antimicrobic effects of some *Euphorbia* methanolic extracts (8 species of *Euphorbia*) gave the most powerful antimicrobial result against tested microbes (*Staphylococcus aureus* COWAN 1, *Bacillus megaterium* DSM 32, *Proteus vulgaris* FMC 1, *Klebsiella pneumonia* FMC 5, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50071, *Candida albicans* FMC 17, *Candida glabrata* ATCC 66032, *Candida tropicalis* ATCC 13803, *Epidermophyton sp*. and *Trichophyton sp*.) and this is because the wide spectrum of antimicrobic compounds in the plants, *Staphylococcus aureus* COWAN 1, and *Candida tropicalis* ATCC 13803 were the more sensitive microbes for all plants extracts. MIC of *Euphorbia* extracts (8 species of *Euphorbia*) toward the examined microorganisms varied from 3.12 mg/ml to 100 mg/ml, and this confirms our result. El-Zayat et al., (2021) explain that the antimicrobic action of different extracts of *S. argel* has a broader spectrum against *Bacillus cereus* (ATCC 11778TM), *Bacillus subtilis* (ATCC 19659TM), *Escherichia coli* (ATCC 10536TM), *Klebsiella pneumonia* (ATCC 10031TM), *Listeria innocua* (ATCC 33090TM), *Listeria monocytogenes* (ATCC 19115TM), *Pseudomonas aeruginosa* (ATCC 9027TM), *Salmonella enterica* (ATCC 15479TM) and *Salmonella typhimurium* (ATCC 14028TM) strains. The MIC of the *S. argel* extracts was estimated using broth dilution assay extended from 0.049 to 1.56 mg/ml. The results

that obtained were also agreement with the results that obtained by AL-Faifi (2019) who found that *E. cuneata* has antibacterial and anticancer properties. However, the findings were consistent with those of Li et al., (2016) who found that *E. cuneata* contained many phytochemicals, including quercetin, kaempferol glycoside, and naringenin. The obtained results were also agreement with the rescent results that publishes by Perera et al., 2022) who indicated that the *Syncarpia hillii* leaves are comprised of bioactive compounds that are bactericidal against several Gram-positive and Gram-negative bacteria. The antimicrobial activity of *Solenostemma argel* give the most powerful outcomes of antimicrobial action toward all isolated microorganisms. Also, the result of MIC and MBC action of *Solenostemma argel* extracts can support as sources for therapeutic compounds action.

3.3. Anti-proliferative Effects on A549, Caco-2, and MDA-MB-231 Cells

The micro culture tetrazolium (MTT) assay was used to measure the degree of cytotoxicity of the methanolic extract towards the cell lines. Three different cancer cell lines (A549, Caco-2, and MDA-MB-231) were used to test the cytotoxicity of the methanolic *Solenostemma argel* extract only depending on the highly effect as antioxidant and antimicrobial. In comparison to the control, cytotoxic activity was expressed as a percentage of cell viability in A549, Caco-2, and MDA-MB-231 cell lines. The multiple concentrations of methanolic extract from *Solenostemma argel* were used and effective doses were calculated from dose-response curve. The dose-response curve was used to calculate effective doses using several concentrations of methanolic extract from *Solenostemma argel* .

Table (6) presented the results of a cytotoxicity test of the *Solenostemma argel* extract against numerous cell lines. The cytotoxicity of *Solenostemma argel* methanolic extract against lung carcinoma cell line (A549) was investigated. Doxorubicin (reference standard) has an IC_{50} of 0.95 µg/mL, while *Solenostemma argel* methanol extract has an IC_{50} of 179 µg/mL (Table 6). The cytotoxicity of *Solenostemma argel* methanolic extract against the Caco-2 cell line was investigated. The IC_{50} value of Doxorubicin was 1.93µg/mL while the IC_{50} value of *Solenostemma argel* methanol extract was 243µg/mL (Table 6). On the other hand, the cytotoxicity of methanolic extract of *Solenostemma argel* against MDA-MB-231 cell line was also evaluated. IC_{50} value of Doxorubicin was 0.60µg/mL and IC_{50} value of methanol extract of *Solenostemma argel* was 731µg/mL (Table 6). Winning treatment with methanol extract of *Solenostemma argel*, the A549 cells showed an increased rate of cell death when compared to that in the Caco-2 and MDA-MB-231 cell lines at the same concentration of the extract used. Earlier studies that achieved by (Dai and Mumper, 2010) had found an association between the antioxidant activity and anticancer properties of plant extracts as well as evidence to suggest that the active constituents of Euphorbia species may have anticancer effects (Lin et al., 2012). The *E. terracina* polar and polar fractions, as well as the *E. paralias* polar fraction were very active against human acute myeloid leukaemia (THP1) cells, according to Ben Jannet et al. (2017) with no cytotoxicity found against normal cells (CD14 + monocytes). Similar results were obtained by **Soliman et al., (2021)** who analyzed the phytochemicals of different extracts of *E. cunatea* and investigated their anticancer activity against a panel of different cancer cell lines. He found that the methanol extract of *E. cuneata* had significant cytotoxic effects on the A549, Caco-2, and MDAMB- 231 cell lines, respectively and found a significant selective concentration-dependent cytotoxicity in the colon cancer cell line (Caco-2) compared to

normal cells (WI 38). This research studied the differential selectivity in anticancer activity between different extract of *Solenostemma argel* and holds significant promise for the development of new cancer therapy.

Table 6
Anticancer activity of *Solenostemma argel methanol extract* at different concentrations toward different cancer cell lines

Samples Conc (µg/mL)	***Solenostemma argel* methanol extract**								
	Cancer cell lines								
	#A-549			**##$CACO_2$**			**MDA-MB-231 ###**		
	Viability (%)	**Inhibitory (%)**	**S.D. (±)**	**Viability (%)**	**Inhibitory (%)**	**S.D. (±)**	**Viability (%)**	**Inhibitory (%)**	**S.D. (±)**
1000	7.83	92.17	1.09	13.95	86.05	1.09	34.59	65.41	2.37
500	21.67	78.33	1.75	32.76	67.24	3.42	63.73	36.73	3.91
250	37.85	62.15	3.49	48.52	51.48	2.86	80.95	19.05	2.83
125	59.21	40.79	2.37	76.31	23.96	3.97	93.83	6.17	1.95
62.5	78.04	21.96	1.78	88.42	11.58	2.64	99.56	0.44	0.48
31.25	95.26	4.74	0.82	97.16	2.84	1.32	100	0	-
15.6	99.13	0.78	0.51	100	0	0	100	0	-
7.8	100	0	0	100	0	0	100	0	-
0	100	0	0	100	0	0	100	0	-
#A549 cell line: lung carcinoma cell, doxorubicin (reference standard) has an IC_{50} of 0.95 µg/mL, the IC_{50} value of Solenostemma argel **methanol extract was 179 ± 15.2 µg/mL**									
##$CACO_2$: intestinal carcinoma cell line, the IC_{50} value of Doxorubicin was 1.93 ± 0.24 µg/mL, the IC_{50} value of Solenostemma argel **methanol extract was 243 ± 19.4 µg/mL ###MDA-MB-231; breast cancer cell line, the IC_{50} value of Doxorubicin was 0.6 ± 0.02 µg/mL, the IC_{50} value of** Solenostemma argel **methanol extract was 741 ± 49.7 µg/mL**									

3.4. In vitro anti-inflammatory activity (memberane stabilization %) of Solenostemma argel methanolic extract.

Many of recently studies reported that plants represent the main source of chemical compound for the development of new drugs, which intensifies the interest of transnational industries in searching for substances obtained from plant sources, especially that the vast majority of species have not yet been studied chemically or biologically, particularly concerning anti-inflammatory action **(Soliman et al., 2021**, Reis Nunes et al., 2020). Anti-inflammatory drugs can interfere in the pathophysiological process of inflammation, to minimize tissue damage and provide greater comfort to the patient. Therefore, it is important to note that

due to the existence of a large number of species available for research, the successful development of new naturally occurring anti-inflammatory drugs depends mainly on a multidisciplinary effort to find new molecules.

Data in (Table 7**)** demonstrated the anti-inflammatory activity (memberane stabilization %) of *Solenostemma argel* methanolic extract The methanolic extract of *Solenostemma argel* showed considerable stabilization of RBCs membranes at various concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.63, and 7.81µg g/mL). The percentage protection of methanolic extract at concentration 1000 µg g/mL was higher than that of concentrations (**24.40**). However, at lower concentrations, the percentage protection was observed to be reduced. The results were tabulated in Table 7. The percentage protection of methanolic extract at concentration 1000 µg/mL was higher than that of concentrations (24.4). However, at lower concentrations, the percentage protection was observed to be reduced. The IC_{50} value of indomethacin was 17.02 ± 0.91 µg/mL; however, the IC_{50} value for the methanolic extract was 24.4 ± 0.96 µg/mL. The date that obtained indicated that the *Solenostemma argel* methanolic extract has higher anti-inflomatry activitiy ethanolic extract.

Table 7
Anti-inflommatory activity (memberane stabilization %) of *and Solenostemma argel methanolic extract* at different concentrations.

Samples Concentration (µg/mL)	**Anti-inflommatory activity (memberane stabilization %)**			
	Solenostemma argel methanolic extract		**Indomethacin (as positive control)**	
	Membrane stabilization (%)	**S.D**	**Membrane stabilization (%)**	**S.D**
1000	**82.73**	**2.1**	**100**	**-**
500	**76.44**	**0.63**	**95.35**	**0.63**
250	**71.35**	**0.68**	**78.34**	**2.1**
125	**68.47**	**0.58**	**72.35**	**0.58**
62.5	**62.95**	**2.1**	**68.35**	**1.5**
31.25	**57.95**	**1.6**	**56.38**	**1.3**
15.6	**39.96**	**1.4**	**49.38**	**0.72**
7.8	**28.12**	**0.94**	**41.18**	**1.3**
0	**0**	**0**	**0**	**0**
IC50	**24.4 ± 0.96**		**17.02 ± 0.91**	
All determination were carried out triplicate manner and values are expressed as mean ± The IC50 value is defined as the concentration of inhibitor 50% of its activity under the assayed conditions				

The results that obtained were agreement with the results that achieved by Abdallah et al., (2020)who indicated that *E. cuneata* is traditionally used to relieve pain and inflammation *E. cuneata* has a substantial

protective effect against lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. This may be due to its antioxidant and anti-inflammatory properties. The presence of flavones, glucosides, and tannins in *E. hirta* extract contributes to its anti-infammatory function by inhibiting NO, according to **Shih et al., (2011)**; and various researchers have shown that increased NO production causes oxidative stress, DNA damage, and cell injury **(**Murphy, 1999**).** On the other hand, the results that obtained were agreement with the results obtained by **El-Agamy et al., (2020)** who study the protective anti-inflammatory activity of to vophyllin A against acute lung injury and its potential cytotoxicity to epithelial lung and breast carcinomas and found that to vophylin tovophyllin A ameliorates LPS-induced ALI through the suppression of oxidative stress and inflammation. These findings suggest the potential use of this compound as a future treatment for ALI. Similar results were reported by Abdallah et al., (2020) who studied the *Euphorbia cuneata* Represses LPS-Induced acute lung injury in Mice *via* Its antioxidative and anti-Inflammatory activities and concluded that the anti-inflammatory activity of *Euphurobia Cuneata* was obvious through its ability to suppress the activation of nuclear factor-κB (NF-κB), and hence its reduction of the levels of downstream inflammatory mediators. Similar results reported by Ibrahim et al., (2015) who investigatethe potency of *Solenostemma argel* as antiinflammatory and antioxidant effects and concluded that flavonoids and related polyphenols present in *Solenostemma argel* extract may be responsible for the anti-inflammatory and antioxidant activities which may be attributed to the radical scavenging and anti-inflammatory effects of some of its active constituents and *S. argel* is a very promising source of bioactive compounds. More similar results were achieved by EL-shiekh et al., (2021) who investigated the anti-inflammatory activity of *J. grandiflorum* L. total extract (JTME) against experimental ulcerative colitis and in rats and found that *J. grandiflorum* L. total extract (JIME) is highly recommended for the management of chronic inflammatory disorders it was successfully reduced the expression of pro-inflammatory cytokines and inflammatory mediators. Additionally, it had a potent antioxidant potential. (JIME) and its fractions showed inhibitory activity on cyclooxygenases and lipoxygenases as detected *in vitro*.

3.5. Identification of Phenolic Acids and Flavonoids of *Solenostemma argel* methanolic extract by HPLC.

Date in Table 8 and figure (1-A) presented the different phenolic compounds that obtained from the analyzed of *Solenostemma argel* methanolic extract by HPLC. Data indicated that the phenolic compounds that were investigated were consisting of five phenolic acids (syringic acid, p-coumaric acid, caffeic acid, gallic acid and ferulic acid). Data indicated that gallic acid, caffeic acid and syringic acid were the most abundant phenolic compounds was gallic (71.70%) among all the phenolic acids, then caffeic acid and ferulic acid were (16.75%) and (11%). Data in the same table and figure (2B) presented also the results of the analyzed flavonoids of *Solenostemma argel* methanolic extract by HPLC. Data indicated that there were five flavonoids were found five flavonoids (rutin, quercetin, kaempferol, luteolin, and 7 hydroxyflavone). The most abundant flavonoids compounds was catechin (47.32%) among all the flavonoids, then quersestin (18.47%) then luteolin (17.59%) then kamperol (14.36%) and the lowest amount from flavonoids was rutin (2.24%).

Table 8
Phenolic acids and flavonoids composition of methanolic extract of *Solenostemma argel*

NO.	Retention Time (min)	Compound Name	Peak Area%
1	4.8	Syringic acid	5.22
2	6.0	P-coumaric acid	5.14
3	8.0	Caffeic acid	4.69
4	10.0	Gallic acid	10.42
5	10.9	Ferulic acid	3.08
6	4.8	Rutin	0.59
7	6.8	Quercetin	4.86
8	8.0	Kaempferol	3.87
9	9.1	Luteolin	4.63
10	11.0	Catechin	12.45

The results that obtained were agreement with the results that obtained by Gopi et al. (2015) who found that ellagic acid, gallic acid, and quinic acid are the primary phenolics when used UHPLC-SRM/MS, whereas GC-MS analysis identified pyrogallol as the major constituent among the volatile components of the *E. hirta* methanolic extract. Flavonoids are plant-produced natural compounds with different phenolic structures. **Soliman et al., (2021)** observed that the methanolic extract of *E. cuneata* the pyrogallol was the most abundant phenolic acid, followed by caffeic, p-coumaric, ferulic, syringic, and gallic acids, respectively. The 7-hydroxyflavone and rutin flavonoids were also found in the methanolic extract. Four flavonoidal substances isolated and identified from the alcohol extracts of *E. cuneate* Vahl. as naringenin, aromadendrin, apigenin, and 4'-O-methoxy-luteolin-7-Orhamnoglucoside show antiulcerogenic potential, according to **Awaad et al.,((2013).** Similar results were obtained by Bahar et al., (2005) who found naringenin, isoaromadendrin, dihydroquercetin, and isosinensin have all been isolated from *E. cuneata*. From the results hat obtained we could be concluded that the *Solenostemma argel* methanolic extract included the flavonoid components (rutin, quercetin, kaempferol, luteolin, and 7 hydroxyflavone and phenolic acids (syringic acid, p-coumaric acid, caffeic acid, gallic acid and ferulic acid).

3.6. Determination of the volatile components of *Solenostemma argel* methanolic extract

The volatile components of a methanolic extract of *Solenostemma argel* were determined using GC-MS based on their retention time and peak area (Table 9 and Fig. 2). Data in the Table 9 and figure (2) indicated that twenty bioactive substances were identified and categorized based on their chemical structures using GC-MS analysis. Cis-2,6-Dimethyl-2,6-Octadiene (8.95%), 2,6-Octadiene, 2,6 Dimethyl (4.19%), Cyclohexene, 1-Methyl-4-(1 Methylethenyl)-,(S) (2.70%), 1,3-Dioxolane,4-Methyl-2-Pentadecyl (9.69%), Bicyclo[3.1.0]Hexane, 4-Methylene-1-(1-Methylethyl) (1.64%), N,N'-Bis(3-Aminopropyl) Ethylenediamine (3.33%), 2-Furanmethanol,

5-Ethenyltetrahydro-À,À,5-Trimethyl-, Cis (5.01%) 2-Furanmethanol,5-Ethenyltetrahydro-À,À,5-Trimethyl-, Cis (4.41%), Linalool (2.87%) Nonanoic Acid, 9-Oxo-, Methyl Ester (3.33%), Methyl 3-Methylbutanoate (7.34%), Hexanoic Acid, Methyl Ester(10.93%), cis-3-Hexenyllactate (2.97%), phenol, 2-(1,1-dimethylethyl) (8.50%), 2-(5-methyl-5 vinyltetrah ydro-2-furanyl)-2-propanol (11.63%), 1,3,5-triazine-2,4-diamine,6-chloro-n-ethyl (3.12%), hexadecanoic acid, methyl ester (4.80%), 9,12-octadecadienoic acid (z,z)-, methyl ester (1.85%), 10-octadecenoic acid, methyl ester (2.76%) 2-(5-methyl-5 vinyltetrah ydro-2-furanyl)-2-propanol (11.63%) were the main volatile components.

Table 9
Volatiles composition of methanolic extract of *Solenostemma argel*

No	Compound Name	Retention Time (min)	Molecular Formula	m/z Fragments	Peak Area Percentage *
1	Cis-2,6-Dimethyl-2,6-Octadiene	6.43	C10H18	41,53,69 #, 81, 95, 109, 123, 138	8.95
2	2,6-Octadiene, 2,6 Dimethyl-	6.73	C10H18	27, 41, 53,69 #,81, 95, 109,128, 138	4.19
3 4	Cyclohexene, 1-Methyl-4-(1 Methylethenyl)-, (S)-	7.07	C10H16	27, 41, 53, 68 #, 79, 93, 107, 121, 136	2.70
5	1,3-Dioxolane,4-Methyl-2-Pentadecyl	7.34	C19H38O2	30,43, 46, 57#, 69, 81, 87, 105	9.69
6	Bicyclo[3.1.0]Hexane, 4-Methylene-1-(1-Methylethyl)	7.84	C10H16	41, 69, 77, 93#, 105, 121, 121	1.64
7	N,N'-Bis(3-Aminopropyl) Ethylenediamine	8.12	C8H22N4	30, 44#, 58, 71, 87, 100, 154, 175	3.33
8	2-Furanmethanol, 5-Ethenyltetrahydro-À,À,5-Trimethyl-, Cis	8.44	C10H18O2	43, 55,59#, 68, 81,94, 111, 155	5.01
9	2-Furanmethanol,5-Ethenyltetrahydro-À,À,5-Trimethyl-, Cis	8.85	C10H18O2	43, 59#, 68, 81, 94, 111, 155	4.41
1	Linalool	9.23	C10H18O	41, 55, 71#, 80, 93, 107, 121, 136	2.87
11	Nonanoic Acid, 9-Oxo-, Methyl Ester	10.99	C10H18O3	55, 69, 74#, 87, 100, 127, 143, 186	3.33
12	Methyl 3-Methylbutanoate	11.19	C8H16O5	41, 57, 69, 74#, 85, 101, 116	7.34
13	Hexanoic Acid, Methyl Ester	11.77	C7H14O2	18, 29, 43, 55, 59, 74#, 87, 99,130	10.93
14	cis-3-Hexenyllactate	12.34	C9H16O3	45, 55, 67, 82#, 89, 99, 141, 157, 172	2.97
15	phenol, 2-(1,1-dimethylethyl)-	15.31	C10H14O	65, 77, 91, 107, 115, 135, 135#, 150	8.50

* The most intense ion is indicated by the base peak (tallest peak). # (Peak area of individual volatile compound/total peak areas of all volatiles) x 100.

No	Compound Name	Retention Time (min)	Molecular Formula	m/z Fragments	Peak Area Percentage *
16	2-(5-methyl-5 vinyltetrah ydro-2-furanyl)-2-propanol	17.82	C10H18O2	43, 59#, 68, 94, 111, 137, 155	11.63
17	1,3,5-triazine-2,4-diamine, 6-chloro-n-ethyl	27.44	C5H8ClN5	43#, 55, 71, 85, 97, 111, 125, 125, 145,158,173	3.12
18	hexadecanoic acid, methyl ester	29.10	C17H34O2	43, 74#, 87, 97, 129, 143, 185, 227, 270	4.80
19	9,12-octadecadienoic acid (z,z)-, methyl ester	32.33	C19H34O2	67#, 81, 95, 220, 293, 294	1.85
20	10-octadecenoic acid, methyl ester	32.44	C19H36O2	41, 55#, 69, 111, 180, 222, 264, 296	2.76

* The most intense ion is indicated by the base peak (tallest peak). # (Peak area of individual volatile compound/total peak areas of all volatiles) x 100.

The obtained results were agreement with that obtained by Ertas, et al., (2015) who found that linoleic acid, 17-tetratriacontane, palmitic acid, and hexatriacontane were the major fatty acids isolated from *E. aleppica*, *E. eriophora*, *E. macroclada, E. grisophylla, E. seguieriana subsp. seguieriana, E. craspedia*, *E. denticulata, E. falcata*, and *E. fistulosa*. These results are compatible in agreement with **Marín et al., (2018)** who reported the chemical composition of polar extracts (b and c) from *c. minor* l. leaves by means of GC-MS. also, a screening of the pharmaco-toxicological effects of the four extracts obtained from this species was performed and found the chromatogram of extract b obtained after the gc/ms analysis showed chromatographic peaks from 8 to 23 min approximately and 33 compounds were identified. The molecular ion of compound 18 was m^+ 292 da, while compound 19 had its molecular ion at m/z 278 da, 14 da less than compound 18. Similar recently results were consistent with those of **Soliman et al., (2021)** who found five bioactive substances were identified and categorized based on their chemical structures using GC-MS analysis of methanolic extract of *E. cuneata* Vahl. Hexanal dimethyl acetal (6.59%), hexadecanoic acid, methyl ester (palmitic acid methyl ester) (21.34%), methyl octadeca-9,12-dienoate (13.47%), (9E,12E)-octadeca-9,12-dienoyl chloride (14.21%), and methyl 12-hydroxy-9-octadecenoate (44.39%) were the main volatile components. Our results were agreement with the results that obtained by Abdel-Motaal et al., (2022)

who found that the GC-Ms analysis of *S. arghel* recorded fifty-one volatile organic compounds (VOCs) within EtOAc extraction and thirty VOCs in MeoH extract.

4. Conclusions

In this work several analyses indicated that the aerial parts of *Solenostemma argel* methanolic extract are rich in bioactive compounds. The methanol extract of *Solenostemma argel* methanolic extract had the highest scavenging potency ((79.36%) at a concentration of 10 µg/mL, and the best extract with a powerful MIC was ethyl acetate, acetone, chloroform, and methanol the most powerful activity on most microbial isolates and lastly chloroform. The methanolic extract of *Solenostemma argel* showed sufficient membrane stability to be considered an anti-inflammatory agent. On the other hand, in vitro models revealed that the methanol extract exhibited potent anticancer activity in several cancer cell lines such as lung carcinoma cell line (A549), colon adenocarcinoma cell line (Caco-2), and the human breast cancer cell line (MDA-MB-231). The main phenolic acids in the methanolic extract of *Solenostemma argel* were syringic acid, p-coumaric acid, caffeic acid, gallic acid and ferulic acid) and gallic acid, caffeic acid and syringic acid and the most abundant phenolic compounds was gallic (71.70%) among all the phenolic acids, and GC–MS analysis revealed that the main flavonoids were, rutin, quercetin, kaempferol, luteolin, and 7 hydroxyflavone respectively. Communally, this study indicated that there was potent protective effect of the methanolic extract of *Solenostemma argel* against pathogenic microorganisms and several human cancer cell lines, which may be connected to its antioxidant and anti-inflammatory activities. It can be concluded that methanolic extract of *Solenostemma argel* could be employed as a easily accessible source of natural bioactive compounds with medicinal benefits, which prospective particularly beneficial to the pharmaceutical industry. Futures perspectives of operating an in vivo study and exploration of *Solenostemma argel* as a natural pharmacological agent to replace chemical drugs could be a promising step.

Declarations

Availability of data and materials

The data used and analyzed in this study can be provided from the corresponding

author for scientific, non-profit purpose.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author contribution

R.M.E., Y.A.K. established the study design and provided the research strategy. **M.S.M.S., H.G.O.O.** assessed the experiments and provided the data analysis. **M.A.A.** resources, **A.A**. methodology, tables, and figures, writing review and editing. All authors have read and agreed to the published version of the manuscript

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Figures

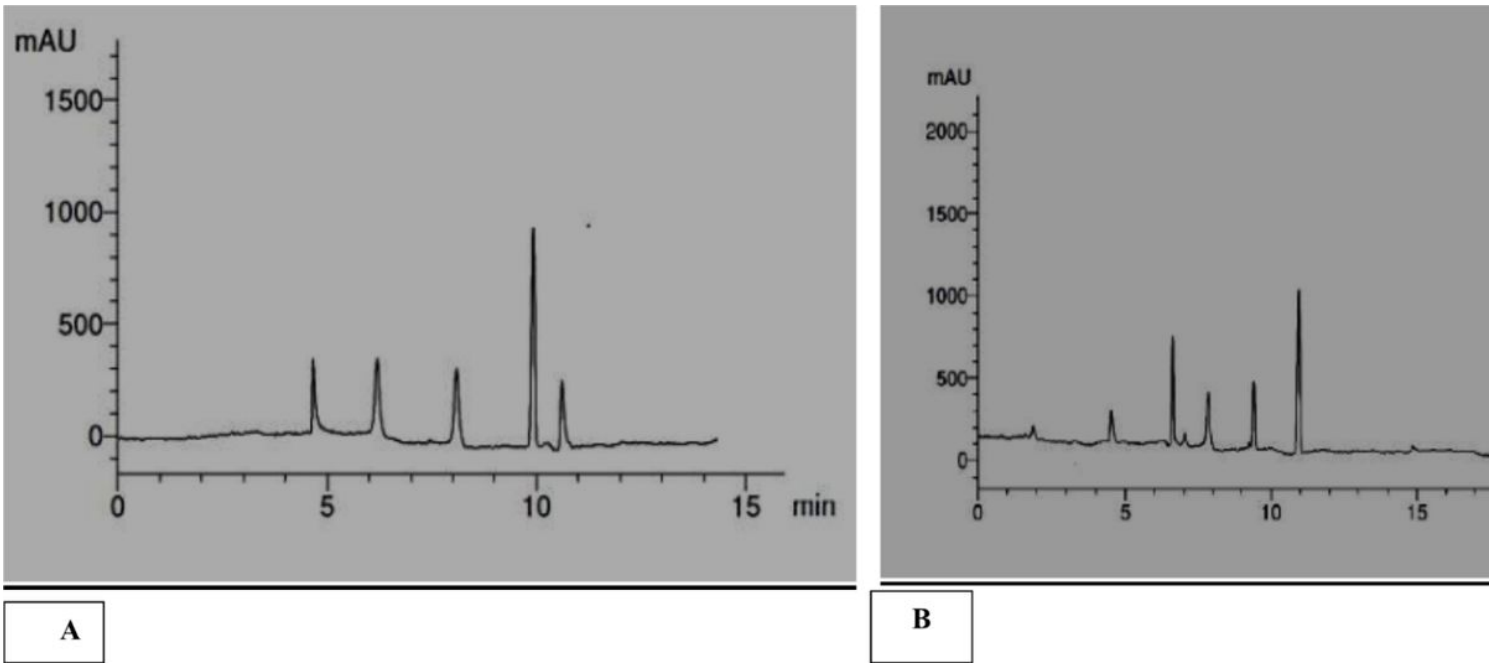


Figure 1

HPLC chromatographic profiles of phenolic acids (A) and flavonoids (B) in methanol extract of *Solenostemma argel*.

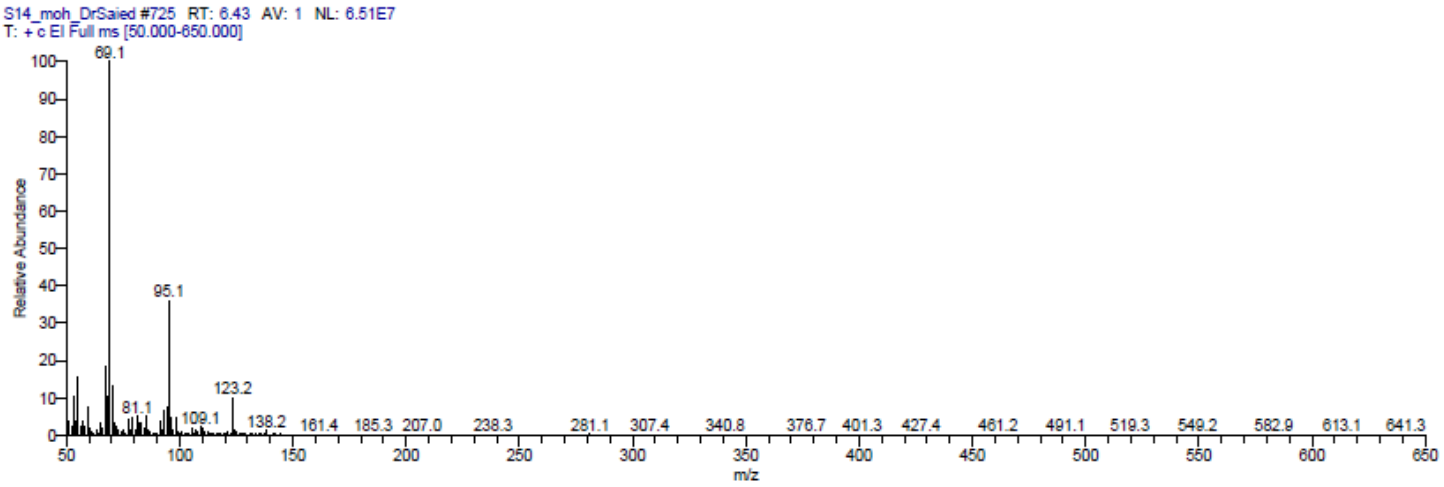


Figure 2

GC-MS chromatographic for identification of compound in methanol extract of *Solenostemma argel*