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Phytochemical Screening, Antioxidant and Cytotoxic Activities Of Some Plants Species Derived From The Northwestern Coast Of Egypt.

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ABSTRACT

Cancer is a grave problem. Searching for treatment from plants is vital. The aim of this study is to estimate the phytochemicals, antioxidants, and the cytotoxicity of nine different plants on human cell lines: liver (HEPG-2), breast (MCF-7), and colorectal (CACO-4). Phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, and phenolics in all extracts. Alkaloids were the maximum in *Marrubium alysson L* (4.5 g % w/w). Flavonoids were the maximum in *Achillea santolina L*. (0.04 g % w/w). Tannins were the highest in *Echinops spinosus Roxb* (2.88 g % w/w). Phenolics were the maximum in *Echinops spinosus Roxb* (0.23 g % w/w). The maximum antioxidants and free radical scavenging activities were in *Haplophyllum tuberculatum* Forsake A. (0.26 ppm), and (0.083 mg/ml) respectively. The ethanolic extracts (µg/mL) of all plants showed cytotoxicity. *Withania somnifera* L. Dunal (*WS*) showed the maximum cytotoxicity against (HEPG-2) IC_{50} (8.6 µg/ml) and (MCF-7) IC_{50} (9.4µg/ml). Thus undergo fractionation using successive polar solvents. The fractions were tested on the same cell lines. Ethyl acetate fraction (EAF) had the maximum cytotoxicity against (MCF-7) IC_{50} (6.63µg/ml). Gas chromatography-mass spectrometry identified 40 compounds as phytol, farnesol, and others that have anticancer action through different mechanisms. It could be concluded that the total plants' extracts showed cytotoxicity against (HEPG-2), (MCF-7) and (CACO-4). *WS* estimated maximum action against (HEPG-2) and (MCF-7). EAF had the highest action against (MCF-7). EAF contained certain compounds which have cytotoxic action especially on (MCF-7). Further investigation can lead to the production of new drug molecules.

Keywords: Cancer, Phytochemicals, Antioxidants, Ethyl acetate fraction, GC-mass.

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INTRODUCTION

Cancer is a brutal metabolic disease and it is one of the principal causes of mortality regardless of expansions in the means of disease diagnosis, curing, and prevention [1]. Moreover, the main causes of cancer are tobacco, smoke, ultraviolet rays, hepatitis B virus, infections from Helicobacter pylori, hepatitis C virus (HCV), in addition to human papillomavirus (HPV), and exposure to ionizing radiation [2]. In 2017, the United States alone had nearly about 688 780 new cancer cases in addition to 600 920 cancer deaths [3]. In fact, there are about 10.9 million new malignancy cases, 24.6 million patients living with cancer and 6.7 million deaths around the world every year [4].

Generally, there are several methods of curing cancer include surgery, immunotherapy, radiotherapy, and chemotherapy. They are associated with fatal side effects as fast clearance, toxicity, no specificity, metastasis, minimizing blood production, immunosuppression, GIT inflammation, heart diseases, hair loss, and nervous disorders. [5].

The South Mediterranean area, especially Egypt, is one of the most important phytogeographical regions because of its moderately high rainfall. It contains 50% of the total flora of Egypt. It is a wealthy source of medicinal plants which have various therapeutic uses. Medicinal plants play an essential role in self-medication alone or in combination with other pharmaceutical preparation especially in the economically weak area of the world, as Africa [6].

Really there are about 80% of the world's inhabitants depend on conventional medicines. Moreover, there are more than 60% of clinically accepted anticancer treatments are derivatives of medicinal flora. Phytochemical compounds present in the stem, leaves, barks, and roots that provide protection against several diseases as alkaloids, flavonoids terpenoids, sugar, saponins, phenols and tannins [7]. Terpenoids are the oldest compounds that are widespread in nature and known for its antibacterial, anti-inflammatory and anti-fungal properties. Alkaloids are mainly anesthetic agents used in several surgical practices in addition to their characters as anti-cancer, anti-asthma, and anti-malaria [8]. Flavonoids are anti-oxidants and improve various health effects [7].

Medicinal plants are important reservoirs of anticancer agents there are various metabolites used in the treatment of cancer as vincristine which is a naturally-occurring alkaloid that was extracted from the dried leaves of *Catharanthus roseus* L. [9]. Paclitaxel which was isolated from *Taxus brevifolia* Nutt. and it is sold generally under the brand name Taxol®. It is one of the most important active anticancer chemotherapy [10]. Homoharringtonine which was isolated from *Cephalotaxus fortunei* Hook. Its bark extract was used in the treatment of cancer in Chinese traditional medicine [11].

Antioxidants are a group of compounds which are used to fight cancer by inhibition of the beginning of cancer during carcinogenesis. Oxidants destroy macromolecules as enzymes, proteins, lipids, and DNA and to fight these radicals, living organisms generate enzymes or depend on non-enzymatic molecules as flavonoids, vitamin K, ascorbic acid, cysteine, and vitamin K for protection [12].

The medicinal value of these plants is derived from the association between the chemical configuration of the effective phytochemicals and the pharmacodynamic effect they produce on the body. Generally, these plants have complex chemical structures vary from 2-3 compounds to tens or maybe hundred these indicate the various pharmacodynamic actions. [13].

As a result of the above mentioned, this study is based on nine medicinal plants of different species to investigate their anti-cancer action that may assist in the production of new anticancer drugs from plant origin and consequently decrease the side effect of chemotherapy [5].

The main objective of this study is the investigation of the phytochemical compounds, antioxidant and cytotoxic activities of some plants that were collected from the Northwestern region of Egypt. The identification of their cytotoxic activities on different cell lines in vitro. The production of several fractions using successive polar solvents to identify the fraction produced the highest cytotoxic action on the same cell lines. The last step is Gas chromatography-mass spectrometry to identify compounds in this fraction that produced the anticancer action and the mechanisms of their action.

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MATERIALS AND METHODS

1-Collection of plant samples: The above- ground parts of the plants were collected from the Northwestern Coast of The Mediterranean Sea region in Egypt during the spring months of the year (2017).

Table (1): Scientific names, locations, latitude and longitude of regions of collected plants at Northwestern coast of the Mediterranean Sea region, Egypt in 2017:

NO	Plants	Location	N	E
1	***Achillea santolina* L**	El-Alamein	30° 43' 36''	31° 32' 13''
2	***Echinops spinosus* Roxb**	El-Alamein	30° 34 ' 35''	31° 32' 13''
3	***Haloxylon scoparium* Pomel**	Marsa Matruh	31° 25 ' 42''	26° 59' 43''
4	***Haplophyllum tuberculatum* Forsake A**	El-Alamein	30° 34' 32''	31° 32' 10''
5	***Marrubium alysson* L**	Alexandria	31° 01' 46''	29° 44' 59''
6	***Ononis vaginalis* Val.**	Marsa Matruh	31° 22' 04''	27° 11' 21''
7	***Phlomis floccosa*. Don**	Marsa Matruh	31° 22' 33''	27° 06' 35''
8	***Thymelaea hirsute*. Ebdle**	Alexandria	31° 06' 52''	29° 44' 59''
9	***Withania somnifera* L. Dunal**	Marsa Matruh	31° 22' 46''	27° 05' 55''

The collected plants were recognized and then authenticated at the herbarium, Desert Research Center (DRC), Egypt. As their voucher specimens were deposited. Floristic identifications were performed according to **(Tackholm, 1974) [14].**

2. Preparation of plant extracts:

Twenty g of every powdered plant were completely soaked in ethanol (200 Ml ×4). Extracts were filtered on Whatman filter paper No. 42 (125 mm), then carefully concentrated under vacuum at 40°C. The dehydrated extracts were kept at 4°C for more study **[15].**

3. Phytochemical screening:

An amount of 0.1 g of the dehydrated extract of every type was re-solubilized in 25 ml of 70% ethanol.

A-Total alkaloids:

Identification of alkaloids was carried out gravimetrically by the method **(Harborne, 1973) [15].**

B-Total tannins:

Total tannins were identified according to method **(Makkar, 2003) [16].**

C-Total flavonoids:

The total flavonoids were identified using the aluminum chloride colorimetric method by (**Arvouet Grand et al., 1994) [17].**

D- Total phenolics:

The Folin–Ciocalteu method was carried out to identify total phenolic contents **[18].**

4. Total antioxidant capacity (by phosphomolebdenum method): The total antioxidant activities of the plants are estimated by the method **(Arvouet Grand et al., 1994) [17].**

5. Method of 2,2-diphenyl- 1 picrylhydrazyl (DPPH):
The scavenging activities of the samples for the radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) were established by **(Brand Williams, 1995) [20].**

Apparatus: Spectrophotometer UV/Vis, SpecorD 250plus, AnalytikJena

6. Preparation of total extracts:
Fifty g of every crushed plant was soaked with 70% ethanol (4 x150ml).The solvent was purified under reduced pressure then the ethanolic extracts were stored at4°Cfor more biological in vitro investigations**.**

7. In-vitro cytotoxic action of the total ethanolic extracts 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT assay):

The cytotoxic activity of every total ethanolic extract was investigated in The National Cancer Institute, Cairo University. Egypt, three tumor cell lines; Breast cell line [MCF-7], Colorectal cell line [CACO4] and, liver cell line (HEPG2) were used. The capacity of cytotoxicity was investigated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide (MTT) method. Cells were located in 96 multi-well plates (104 cells/ well) for nearly 24 hours before healing with the plant extracts under test (0.0, 1.0, 2.5, 5.0 and 10.0 µg/ml.) were added to the cell monolayer, triplicate wells were ready for each individual dose. Monolayer cells were stored in the investigated extracts for about 48 hours at 37°C and in an environment of 5% CO2. Then the cells were incubated carefully with MTT (0.5 mg/ml). Then the blue color MTT formazan was dissolved in detergent (50% of N, N-dimethylformamide and 10% of sodium dodecyl sulfate). Color concentration was investigated in an ELISA reader. The relation between living fraction and extract concentration.Is plotted to obtain the survival curve for every cancer cell line after the particular individual extracts. IC50 for every investigated extract was deliberated from this survival curve. **[21]**.

8. Fractionation of *Withania somnifera L. Dunal:*

The powdered drug was added into Soxhlet apparatus then extraction was occurred with following solvents. 1) Petroleum ether (40-60°C), then 2) Chloroform, followed with 3) Ethyl acetate, finally 4) Methanol. Every time before adding the solvent of elevated polarity marc was dried. Every extract was then carefully concentrated by rotary vacuum evaporator apparatus at 40- 50°C then dried residue was collected in dense glass bottles for more studies.

9. Gas chromatography mass spectrometry analysis: The analysis was occurred using a GC (Agilent Technologies 7890A) interfaced with a mass-selective detector (MSD, Agilent 7000 Triple Quad) prepared with Agilent HP-5ms (5%-phenyl methyl polysiloxane) capillary column (30 m × 0.25 mm i. d. and 0.25 µm film thickness). The transporter gas was helium with the linear velocity of 1 ml/min. Moreover, the injector and detector temperatures were about 200º and 250º C respectively. The volume injected 1µl of the test. The MS working dimensions were as follows: ionization potential 70 eV, associated with interface temperature 250º C, in addition to acquisition mass range 50–600.

10- Statistical analysis:

Statistical analysis was carried out using F-test for significance at $p \leq 0.05$ and computing of Least Significant Difference (LSD) test, values to separate means in different statistical groups according to the described method by **(Little and Hills, 1978) [22]**.

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RESULTS AND DISCUSSION

1-Quantitative investigation of phytochemical compounds of the tested plants:

A-Total alkaloids:

Alkaloids are nitrogenous components produced inside plants for protection against pathogens and herbivores**,** these compounds have anticancer potential by stimulating apoptosis and cell cycle arrest at different stages, in a number of cancer cell lines **[8].**

From the quantitative results outlined in Table (2). It is clear that the values of total alkaloids varied from (4.50 to 0.74 % w/w). The highest values were detected in *Marrubium alysson* L. (4.50 g % w/w) followed by *Achillea santolina* L (3.17 g % w/w)**.** While the lowest values were detected in *Ononis vaginalis* Val. (0.74 g % w/w) followed by *Haloxylon scoparium* Pomel (1.42g % w/w).

Table (2): Phytochemical compounds of 70 % ethanolic extracts of nine tested plants at Northwestern coast of the Mediterranean Sea region. Egypt 2017:

Plants	Alkaloids g % (w/w)	Tannins g % (w/w)	Phenolics g % (w/w)	Flavonoids g % (w/w)
***Achillea santolina* L**	3.17 ± 0.11	1.23 ± 0.04	0.09±2.41	0.04 ± 0.17
***Echinops spinosus* Roxb**	2.64 ±0.08	2.88 ±0.07	0.23±2.43	0.004 0.04
***Haloxylon scoparium* Pomel**	1.42 ±0.09	1.47 ± 0.05	0.07±1.81	0.02 ± 0.04
***Haplophyllum tuberculatum* Forsake A**	3.16 ±0.07	2.18 ±0.07	0.21±2.56	0.02 ± 0.09
***Marrubium alysson* L**	4.5 ± 0.02	0.78 ±0.02	0.03±0.39	0.01 ± 0.23
***Ononis vaginalis* Val.**	0.74 ±0.11	0.45 ±0.02	0.02±0.13	0.003±0.16
***Phlomis floccosa*. Don**	2.29 ± 0.09	1.57 ±0.04	0.11±1.11	0.02± 0.47
***Thymelaea hirsute*. Ebdle**	2.15 ±0.09	0.82 ±0.03	0.04±2.09	0.002±0.19
***Withania somnifera* L.Dunal**	2.85 ± 0.09	0.70 ±0.02	3.03±0.07	0.02 ±1.14
LSD0.05	0.0569	0.0247	0.006	0.005

B-Tannins:

Tannins are polyphenolic compounds. They have not only anticancer but also antioxidant activity **[23]**.

From the Table (2) it is clear that the amount of tannins varied from (2.88 to 0.45 g % w/w). The highest values of tannins were observed in *Echinops spinosus* Roxb (2.88 g % w/w) followed by *Haplophyllum tuberculatum* Forsake (2.18 g % w/w). While the lowest values were detected in *Ononis vaginalis* (0.45 g % w/w) followed by *Withania somnifera* L. Dunal (0.70 g % w/w).

C-Total phenolics:

Phenolics include curcumin, flavonoids, tannins, resveratrol, and gallocatechins which are anticancer compounds their cytotoxicity against a wide range of cancer cells **[24]**.

From the Table (2). It is clear that the amount of phenolics varied from (0.23 to 0.02 g % w/w). The highest values of phenolics were detected in *Echinops spinosus* Roxb (0.23 g % w/w). followed by *Haplophyllum tuberculatum* Forsake A (0.21 g % w/w). While the lowest values were detected in *Ononis vaginalis* Val. (0.02 g % w/w) followed by *Marrubium alysson* L (0.03 g % w/w).

D-Flavonoids:

Flavonoids are polyphenolic compounds that can inhibit injury produced by free Radicals by direct removing of reactive oxygen species, stimulation of antioxidant enzymes and enhancing in antioxidant characters of low molecular antioxidants **[7]**. Flavonoids have anticancer activities against human cancers including; cervical carcinoma (Hela), hepatoma (Hep-G2), and breast cancer (MCF-7) **[25]**.

From the Table (2) it is clear that the amount of flavonoids varied from (0.04 to 0.002 g % w/w). The highest values of flavonoids were detected in *Achillea santolina* L (0.04 g % w/w). Followed by *Withania somnifera* L. Dunal, *Haloxylon scoparium* Pomel, *Haplophyllum tuberculatum* Forsake A and, *Phlomis floccosa* D. Don had the same value (0.02 g % w/w). The lowest values were obtained in *Thymelaea hirsute* L. Ebdle extract (0.002g % w/w) followed by *Ononis vaginalis* Val (0.003 % w/w).

2- **The Total antioxidant capacity of 70 % ethanolic extracts of tested plants:**

All the plants showed antioxidant activities. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers **[13]**.

From the Table (3). It is clear that the highest antioxidant capacity values varied from (0.26 to 0.09 ppm). The highest antioxidant capacity values were detected in *Haplophyllum tuberculatum* Forsake A (0.26 ppm). followed by *Echinops spinosus* Roxb (0.2 ppm). While the lowest value was detected in *Ononis vaginalis* Val. (0.09 ppm) followed by *Haloxylon scoparium* Pomel (0.12 ppm). The tissue destruction caused by ROS involves protein, lipids, DNA destruction and oxidation of vital enzymes **[13]**. Natural antioxidants include phenolics which decrease oxygen-derived free radicals by giving an electron or a hydrogen atom to the free radical **[26]**. The intake of natural antioxidants has been connected with decreased risks of cancer.

Table (3): The Total antioxidant capacity of 70 % ethanolic extracts of nine tested plants at Northwestern coast of the Mediterranean Sea region. Egypt 2017.

Plants	Total anti oxidant capacity (ppm)
***Achillea santolina* L**	0.14 ± 14.58
***Echinops spinosus* Roxb**	0.2 ±24.31
***Haloxylon scoparium* Pomel**	0.12 ± 3.85
***Haplophyllum tuberculatum* Forsake A**	0.26 ±2.88
***Marrubium alysson* L**	0.15 ± 5.03
***Onions vaginalis* Val.**	0.09 ± 13.63
***Phlomis floccosa* D. Don**	0.18 ± 0.13
***Thymelaea hirsute* L. Ebdle**	0.14 ± 8.41
***Withania somnifera* L.Dunal**	0.16 ± 8.48

3- In vitro antioxidant activity of 70 % ethanolic extracts of tested plants on DPPH scavenging activity:

The antioxidant activities of the ethanolic extracts of the tested plants were investigated for their DPPH radical scavenging activity. Antioxidant agents with high scavenging activity should have a low IC_{50} value.

Results in the Table (4) indicated that all extracts possess free radical scavenging potential. The IC_{50} values ranged from (0.083 to 0.103 mg/ml). The highest scavenging activity was observed in *Haplophyllum tuberculatum* Forsake A with IC_{50} value of (0.083 mg/ml) followed by *Echinops spinosus* Roxb with IC_{50}value of (0.087 mg/ml) . While the lowest scavenging activities were observed in *Marrubium alysson* L with IC_{50} value of (0.103 mg/ml). followed by *Withania somnifera* L. Dunal with IC_{50} value of (0.098 mg/ml).

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Table (4): The inhibitory concentration 50 % (IC_{50}) (mg/ml) of 70 % ethanol extracts of nine tested plants at Northwestern coast of the Mediterranean Sea region. Egypt 2017 on DPPH scavenging activity:

Plants	Inhibitory concentration (IC_{50}) (mg/ml)
Achillea santolina **L**	0.088
Echinops spinosus **Roxb**	0.087
Haloxylon scoparium **Pomel**	0.094
Haplophyllum tuberculatum **Forsake A**	0.083
Marrubium alysson	0.103
Ononis vaginalis **Val.**	0.096
Phlomis floccosa**. Don**	0.092
Thymelaea hirsuta**.L. Ebdle**	0.097
Withania somnifera **L.Dunal**	0.098

4- The cytotoxic activity of plants under study:

The in vitro cytotoxic activities of the ethanol extracts of the nine tested plants were investigated against liver (HEPG-2), colorectal (CACO-4) and breast (MCF-7) carcinoma cell lines.

As shown in the Table (5) it is clear that all plant extracts estimated antitumor action with different IC_{50} values and on different cell lines.

Table (5): In vitro cytotoxic activity of 70 % ethanol extracts of tested plants on liver (HEPG-2), colorectal (CACO-4) and Breast (MCF-7) cell lines:

Plant name	IC_{50} µg/ml		
	HEPG2-1	CACO-4	MCF7-1
Achillea santolina **L**	42.5	32	36.5
Echinops spinosus **Roxb**	40	22.7	39
Haloxylon scoparium **Pomel**	23.5	24	36.5
Haplophyllum tuberculatum **Forsake A**	18	10	22.3
Marrubium alysson **L**	25	20	23
Onions vaginalis **Val.**	25	21	31.5
Phlomis floccosa**. Don**	41.5	24	37.5
Thymelaea hirsute **L. Ebdle**	50	23	36
Withania somnifera **L.Dunal:**	8.6	10.5	9.4

Liver cell line (HEPG-2):

Withania somnifera L. Dunal produced the highest cytotoxic action with IC_{50} value of (8.6 µg/ml). these results are in accordance with **(Ahmed et al., 2018) [27].** That study indicated that *Withania somnifera* L. Dunal extract had a strong anticancer action against HepG2 cells. It produced a marked effect on the cells as shrinkage and gathering of dead HepG2 cells when compared with control untreated cells. followed by *Haplophyllum tuberculatum* Forsake A with IC_{50} value of (18 µg/ml). While *Thymelaea hirsuta*.L. Ebdle produced the lowest cytotoxic action against HEPG-2 with IC_{50} value of (50 µg/ml). followed by *Echinops spinosus* Roxb with IC_{50} Of (40 µg/ml).

B-Colorectal cell line (CACO-4):

Data in the Table (5) indicated that the all plants ethanolic extracts produced anticancer action against CACO-4 with different IC_{50} values. The highest cytotoxic action against CACO-4 was found in *Haplophyllum tuberculatum* Forsake A with IC_{50} value (10 µg/ml) similar results were obtained by **(Al-Muniri et al., 2017) [28]**. Explained that all the extracts of *H. tuberculatum* species from the leaves showed antioxidant and cytotoxic activities as it contains several bioactive compounds which are responsible for its biological activities. *Withania somnifera* L. Dunal with IC_{50} value (10.5 µg/ml) followed *H. tuberculatum* . The lowest value was obtained in *Achillea santolina* L with IC_{50} value of (32 µg/ml).

C-Breast cell line (MCF-7):

Data in the Table (5) indicated that the all plants ethanolic extracts produced anticancer action against MCF-7 with different IC_{50} values. The highest cytotoxic action against MCF-7 was found in *Withania somnifera* L. Dunal with IC_{50} value of (9.4µg/ml). Several studies indicated that treatment with *WS* ethanol extract produced a significant increase in G2/M phase (investigating cell cycle arrest and inhibition of mitosis). And also it produced an increase in the sub-G0 region **[27]**. *Haplophyllum tuberculatum* Forsake A followed to *W. somnifera* with IC_{50}value of (22.3 µg/ml).

From the Table (5) it is clear that *Withania somnifera* L. Dunal had the highest cytotoxic action. The ethanol extract of *Withania somnifera* L. Dunal had the highest cytotoxic action among the nine tested plant extracts on both livers (HEPG-2) cell line followed by breast cell line (MCF-7). The second cytotoxic action on the colorectal cell line (CACO-4). Several investigations have identified the roots and leaves of *W. somnifera* as a wealthier source of withanolides that are cytotoxic to breast cancer, neuroblastomas, myeloid cells, prostate, neuroprotective, and had an immunomodulatory action **[27]**.

5- Fractionation of *Withania somnifera* L. Dunal:

On the Table (5) it was concluded that the ethanolic extract of *W. somnifer*a was the most active thus subjected to fraction using different polar solvents these solvents are (petroleum ether, chloroform, ethyl acetate, and methanol) to investigate the fraction of *W. somnifer*a that produces the highest cytotoxic activity as the polarity of solvent used for extraction has a great effect on the yield of different phytochemical classes present in the plant.

On the Table (6) it is clear that all *Withania somnifera* L. Dunal fractions produced lower cytotoxic action than total extracts on the three cell lines except ethyl acetate fraction on breast cell line (MCF-7). This fraction showed the highest cytotoxic effect with IC_{50} value of (6.63 µg/ml). To discuss the active components of ethyl acetate fraction, it was subjected to analysis on GC-mass.

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Table (6): In vitro cytotoxic activity of different Withania somnifera L. Dunal fractions on liver (HEPG-2), colorectal (CACO-4), and Breast (MCF-7) cell lines:

Extracts	IC_{50} µg/ml			
	HEPG-2	CACO-4	MCF-7	HCT-15
Petroleum ether	22.9	12.8	27	23
Chloroform	14.8	17	No result	11.7
Ethyl acetate	12.5	13	6.63	14.5
Methanol	13.9	12.5	12.7	16

7-Gas chromatography mass spectrometry (GC-MASS) of the ethyl acetate fraction of *W. somnifera:*

Forty peaks of phytochemicals were obtained which were investigated and identified in Table (7). The investigation of these components was rooted on a relationship of their mass spectra and retention time the authentic compounds and by computer corresponding with NIST and WILEY library in addition to the comparison of the disintegration pattern of the mass spectral records in the literature. All 40 compounds were identified from the (GC-MS) analysis of ethyl acetate fraction of *W. somnifera* producing different phytochemical activities that responsible for the main biological activities.

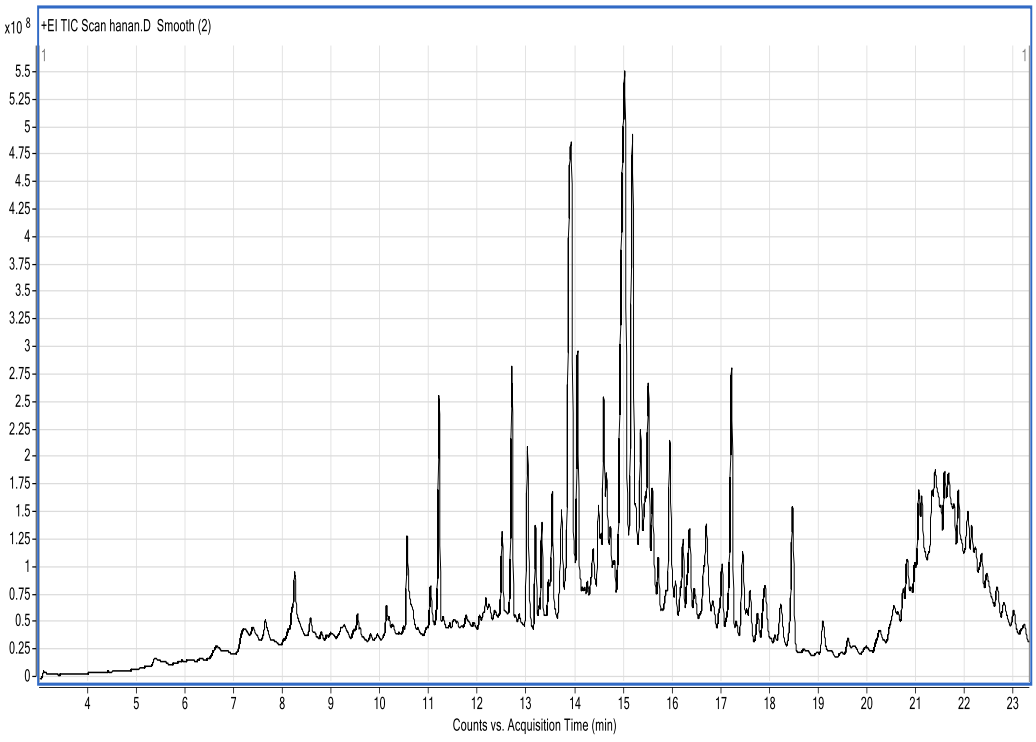

Figure (1): Gas chromatography mass spectrometry (GC-mass) of the ethyl acetate fraction of *Withania somnifera* L. Dunal.

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Table (7): Quantitative investigation of active constituents in the ethyl acetate fraction of *Withania somnifera* L. Dunal:

No.	R.t.	Name	Percentage
1	3.107	Piperidin-4-carboxylic acid	0.35
2	5.401	Solanidine	0.44
3	6.639	Tropinone	0.35
4	7.397	5,7,3',4',5'-Pentahydroxyflavone	0.56
5	7.658	Gardenin	0.56
6	8.236	6-Propyl-2-thiouracil	2.3
7	8.578	β-Sitosterol	0.28
8	8.786	3-Hydroxy-7,8,2',3'-tetramethoxyflavone	1.23
9	8.986	5,7-Dimethoxy-4-methylcoumarin	0.25
10	9.251	Isovitexin	0.63
11	9.809	Quercetin 3',4',7-trimethyl ether	0.22
12	9.943	Kampferol-3,4'-dimethyl ether	0.18
13	10.143	Coumarin-6-ol, 3,4-dihydro-4,4,7,8-tetramethyl-	0.37
14	10.562	Scoparone	2.26
15	11.027	Datiscetin	0.68
16	11.198	Phytol	3.29
17	12.164	Dihydrokaempferol	0.67
18	12.477	Estradiol, 3-deoxy-	1
19	12.681	Pyrimido[1,2-a]azepine, 2,3,4,6,7,8,9,10-octahydro-	3.26
20	13.015	Ouabagenin	3.02
21	13.186	Citronellol	1.46
22	13.312	Astilbin	1.09
23	13.512	5,7,3',4',5'-Pentahydroxyflavone	2.13
24	13.708	2'-Hydroxy-2,4,4'-trimethoxychalcone	1.75
25	13.883	4',6-Dimethoxyisoflavone-7-O-β-D-glucopyranoside	12.67
26	14.05	Amobarbital	3.16
27	14.469	Strophanthidin	1.86
28	14.579	Hexobarbital	1.99
29	15.003	Farnesol	17.44
30	15.17	5,7,3',4'-Tetramethoxyisoflavone	7.76
31	15.337	1-(2-Pyrimidyl)piperazine	1.32
32	15.488	6-Methoxyluteolin	2.75
33	15.944	3',5'-Dimethoxy-3,5,7,4'-tetrahydroxyflavone	2.78
34	16.344	Isolongifolene	1.8
35	16.686	Nalorphine	3.08
36	17.183	1-(2,3-Dimethylphenyl)-2-pyrrolidinimine	5.58
37	17.423	3,5,3',5'-Tetra-tert-butyldiphenoquinone	2.48
38	18.442	Norphytan	3.06
39	19.077	2-Valerylimidazole	1.02
40	21.676	Hexadecane	2.92

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9- Classification of compounds in GC-mass:

These 40 compounds are classified into 13 categories according to their classes and pharmacological action these categories are summarized in Table (8). It is clear that there are 3 main categories having the major constituents of this fraction. They are about 68% ethyl acetate fraction and they have the highest cytotoxic action against MCF-7.

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Table (8) Classes and percentages of compounds in GC mass:

No	Class	Percentage
1	**Flavonoids**	35.66
2	**Terpenoids**	27.05
3	**Pyrrolidinimine**	5.58
4	**Barbiturates derivatives**	5.50
5	**Phytosterols**	4.24
6	**Alkaloids**	3.87
7	**Coumarin derivatives**	3.57
8	**Pyrimidol derivative**	3.26
9	**Aliphatic hydrocarbon**	2.92
10	**Diphenoquinone**	2.48
11	**Uracil derivative**	2.30
12	**Piperazine derivative**	1.32
13	**Others**	3.30

A-Flavonoids:
The first category which contributes nearly 35.66%. It has both antioxidant and cytotoxic action against different types of cell lines as stomach, prostate, lung, breast, Colon, and Leukemia **[29]**. The structures, number of hydroxyl groups and substitution greatly influence mechanisms of antioxidant activity as radical removing and metal ion chelating capacity. Flavonoids also prevent free radical production**[8].** Glucopyranoside had an anticancer action against several types of cell lines particularly estrogen-dependent human breast cancer (MCF7). In addition to human cervix epitheloid carcinoma (HeLa) **[29].**

B-Terpenoids:
Contribute about 27.05%. It had an anticancer action against different types of cell lines such as oral squamous carcinoma, lung adenocarcinoma, hepatoma, lymphoblastic leukemia, melanoma, pancreatic adenocarcinoma, and colorectal carcinoma **[30]**.

B. 1-Farnesol:

Figure (2): Chemical Structure of farnesol

Sesquiterpenes alcohol formed from three isoprenoids units with15-carbon which contributes about 17.44%. As shown in Fig (2). It has both antioxidant and anticancer action. FAR is an effective inducer of cell cycle arrest and apoptosis in a variety of carcinoma cell lines. FAR has antiproliferative activity against (HeLa)

cervical cancer by apoptosis induction and loss of mitochondrial membrane potential. It has a cytotoxic action against breast cancer as it produces endoplasmic reticulum stress in addition to initiation of MAP-kinases stimulation of the apoptosome **[31]**.

B. 2- Phytol:

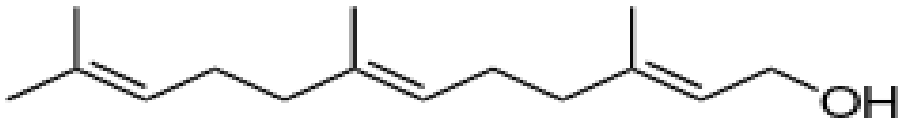

Figure (3): Chemical Structure of Phytol.

Contributes about 3.29%. As shown in Fig (3) It has an antioxidant action which protects lipid biomolecules. In addition to its cytotoxic action through different mechanisms. It stimulates peroxisome proliferators-activated receptor α (PPARα) and so controls the expression of genes correlated with lipid metabolism. Phytol produces concentration-dependent anticancer action against 6 cell lines (breast (MDA-MB-231), kidney (A-549), breast (MCF-7), colon (HT-29), breast (Hs294T), and cervical cancer **(**HeLa) cell) **[32]**.

C-Phytosterols: Contributes about (4.24%). It produces anticancer activity against several types of cell lines, breast, lung, prostate, human leukemic U937 cells, ovary and colon cancer **[33]**.

CONCLUSION

All the nine studied plants showed cytotoxic action on different cell lines due to the presence of various phytochemicals and antioxidants, *Haplophyllum tuberculatum* Forsake A produced the highest cytotoxic action against CACO-4. *Withania somnifera L*. Dunal showed the highest cytotoxic action against HEPG-2 and MCF-7 especially ethyl acetate fraction on breast cancer due to the presence of active compounds such as farnesol, phytol, phytosterols, and others.

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