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Antibacterial and cytotoxic activities of some Libyan medicinal plants

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ABSTRACT

The total alcoholic extracts prepared from the aerial parts of Capparis spinosa L., Juniperus phoenicea, Ruta graveolanse and Artimisia herba alba growing in Al-jabal Al-akhdar in Libya showed a significant cytotoxic activity against both breast and colon cell lines MCF-7 and HCT-116 as well as a significant antibacterial activity against some tested Gram positive and Gram negative bacteria. At the same time, preliminary photochemical screening of their aerial parts was carried out. This study was undertaken to justify the beneficial medicinal uses of these studied plants and their alcoholic extracts.

Key words: *Capparis spinosa L*, Cytotoxic activity, Antibacterial activity

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INTRODUCTION

According to the World Health Organization, over 80% of the world's population, or 4.3 billion people, rely upon such traditional plant-based systems of medicine to provide them with primary health care. The use of such alternative medicines has become increasingly popular in the developed world. Libya has tremendous wealth of medicinal plants scattered in all over a vast area especially in Al-jabal Al-akhdar region. These plants are used in Libyan folkore medicine for their medicinal values. Al-jabal Al-akhdar has a high diversity of plant species that show both economic and medicinal importance (**1**).

Capparis spinosa (Capparaceae) is present in almost all the Circum-Mediterranean countries, and is included in the floristic composition of most of them. The salted and pickled Caper bud (also called simply Capers) is often used as a seasoning or garnish. Capers is a common ingredient in Mediterranean cuisine, especially Italian one (**2**). The flower buds are pickled and used as a flavouring in sauces. The young fruits and tender branch tips can also be pickled and used as a condiment. The flower buds are harvested in the early morning and wilted before pickling them in white vinegar. Young shoots - cooked and used like Asparagus. Petroleum ether, methanol, hexane, butanol and aqueous crude extracts of the whole aerial parts of *Capparis spinosa* exhibited variable degrees of antimicrobial activity (**3**). The methanolic extract showed strong antioxidant activities which may be attributed to its phenolic content (**4**). Trombetta *et al* (5) investigated the anti-allergic properties of 2 lyophilized extracts obtained from *Capparis spinosa* L. Aghel *et al* (**6**) evaluated the protective action of *Capparis spinosa* ethanolic root bark extract in an animal model of hepatotoxicity, which is induced by carbon tetrachloride. The root-bark is analgesic, anthelmintic, antihaemorrhoidal, diuretic, emmenagogue, expectorant, tonic and vasoconstrictive. It is used internally in the treatment of gastrointestinal infections, diarrhoea, gout and rheumatism. Externally, it is used to treat skin conditions, capillary weakness and easy bruising (7). Phytochemical studies have shown the presence of many beneficial compounds such as spermidine, rutin, quercetin, kaempferol, stigmasterol, campesterol, tocopherols, and carotenoids.

Biological studies reveal important antimicrobial, anti-oxidative, anti-inflammatory, immune modulatory, antitumor and antiviral properties (**8,9,10** and **11**).

Juniperus Phoenicea L. (Cupressaceae) is an evergreen plant usually growing as a bush or a tree. The tree's essential oil is especially rich in the tricyclic sesquiterpene thujopsene; the heartwood contains an estimated 2.2% of this hydrocarbon (**12, 13, 14, 15**,and **16**). Even though some studies have been done on members of the genus; little information is available about the medical use of *Juniperus Phoenicea.* The plant *Juniperus phoenicea* L. is widely growing on the rocky soils of the mediterranean regions. It is used as a folk medicine to treat rheumatism, oedema and urinary tract diseases. The aqueous extract of *Juniperus Phoenicea* leaves are used for the treatment of various diseases such as diarrhea, gout and poor appetite (**17**). It also eliminates gastrointestinal bacteria and parasites (**18**). Currently, the juniper berry (the fruits) is being researched as a possible treatment for diet controlled diabetes (**19**). It is also said to have been used by some tribes as a female contraceptive (**20**).

Ruta graveolens (Rutaceae) is a perennial herb or small shrub that grows erect up to 1 meter high with alternate leaves. It is commonly known as Rue and its oil is used as flavoring in foods and beverages and as a fragrant ingredient in manufacturing soaps and cosmetics. The leaves, stems and flowers are the parts used. Common medicinal uses include: fertility regulation, menstrual cramps, earache, headache, nasal bleeding, and insect repellent. Decoction of Rue is used in Venezuela and Haiti as an emmenagogue and to expedite delivery (**21**, **22**, **23**, and **24**). Other uses: diaphoretic, emetic, digestive stimulant, treatment of measles, scarlet fever, and remedy for epilepsy, vermifuge, treatment of ulcers and gum problems. Bruised leaves are placed on a tooth or in the ear to relief pain. It has been reported to possess antifungal, antibacterial (**25**), anti-inflammatory (**26** and **27**), antitumor (**28**), antioxidant and cytotoxic activity (**29** and **30**). In addition, Ruta 6 (which is a diluted potency of the mother tincture (Ruta Q), in combination with calcium phosphate has shown potent antitumor in patients with brain cancer (**31**).

Artemisia herba-alba (Asteraceae) is a short shrub usually found in Northern Africa and the Middle East. The parts that grow above the ground are used as medicine. *Artemisia herba-alba* is a good fodder for grazing animals, mainly sheep and in the Algerian steppes cattle. People take *Artemisia herba-alba* for cough, stomach and intestinal upset, the common cold, measles, diabetes, yellowed skin (jaundice), anxiety, irregular heartbeat, and muscle weakness. It is also used for parasitic infections such as roundworms, pinworms, tapeworms, hookworms, and flukes (**32**). Its essential oil showed antibacterial activity (**33**). Iriadam *et al* (**34**) investigated the effects of *Artemisia herba-alba* on blood glucose levels and some other biochemical parameters to demonstrate their possible therapeutic effects on diabetes. Mighri *et al* (**35**) examined the antimicrobial and antioxidant activities of its essential oil extracted by hydrodistillation from the aerial parts of *Artemisia herba-alba* cultivated in southern Tunisia.

Recently, biologically active compounds and extracts isolated from plant species used in herbal medicine in Libya have been the center of interest . In this report, we present data on the antibacterial activity of total alcohol extracts prepared from the four Libyan plants mentioned above against some tested Gram positive and Gram negative bacteria; As well as their *in-vitro* cytotoxic activity against both breast and colon cell lines MCF-7 and HCT-116**.** The aim of this study is to investigate these four plant species and to justify the beneficial medicinal uses of their extracts.

MATERIALS AND METHODS

Plant materials:
The aerial parts of the four studied plants *Capparis spinosa* L., *Juniperus phoenicea*, *Ruta graveolanse* and *Artimisia herba alba* were collected from Al –Jabal Al Akhdar, Al-bayda city, Libya during 2013. The plant materials were kindly identified by Prof. Dr. Mahmoud Ali Hassanain, Professor of medicinal and aromatic plants, Faculty of Agriculture, Omar AL-Mukhtar University, Al-bayda. Leaves and stems of the plants under investigation were separately air-dried, powdered and kept in tightly closed amber colored containers.

1-Preliminary phytochemical screening:
The air-dried powdered fruits and aerial parts [stems and leaves] of each studied plant were screened for their contents of volatile oils, carbohydrates and/or glycosides, tannins, free and combined flavonoids, unsaturated sterols and/or triterpenes, alkaloids, anthraquinones and cardiac glycosides . The results are recorded in table (1).

2-Preparation of Total alcohol extracts:
30 gm of the air dried aerial parts of each studied plant were separately extracted with alcohol 90% using soxhlet apparatus till exhaustion. Each of the resulted extract was concentrated under vacuum by rotary evaporator. The residues left after distillation of solvent were weighed and kept in a desiccators.

The percentages of each extract were calculated [16.38% (cappar), 45.5% (juniper), 34.18% (Rue) and 43.7% (artimisia)].

Biological investigation:

1. Anti-bacterial activity for each prepared total alcohol extract:

The antimicrobial screening was performed applying the disc agar diffusion method using nutrient agar media for bacteria, the following microorganisms were tested; Gram positive bacteria [*Bacillus subtilis*, *Staphylococcus aureus*], Gram negative bacteria [*Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonilla typhi*]. Susceptibility test discs of Ceftriaxone [30µg/disc] HIMEDIA laboratories Pvt. Limited. The total alcohol extract was given in a concentration of 0.25 and 0.5 mg/disc. Each total alcohol extract was tested against representative strains of bacteria applying the disc agar diffusion method (**36** and **37**)**.** The discs were then placed on to the surface of the inoculated plates previously prepared. The plates were incubated inverted at 37°C for 24 hours. After incubation, zones were recorded in mm. in diameter, Diameter less than 5mm. indicates no effect. A disc impregnated with alcohol is used as a negative control as well as discs of Ceftriaxone was used a positive control for each micro-organism. Results are recorded in table (**2**).

2. In-vitro Cytotoxic activity of the total crude ethanol extracts:

Potential cytotoxic activity of each crude ethanol extract was carried out in National Cancer Institute, Cairo University Egypt, using two tumor cell lines; Breast cell line [MCF-7] and Colon cell line [HCT-116]. Measurement of cytotoxicity was carried out using Sulfo-rhodamine-B assay method (**38** and **39**). Were Cells plated in 96 multi-well plate (104 cells/ well) for 24 hours before treatment with the extracts under test (0, 1, 2.5, 5 and 10 µg/ml.) were added to the cell monolayer, triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the tested extracts for 48 hours at 37°C and in an atmosphere of 5% CO2. After 48 hours, cells were fixed, washed and stained with Sulfo Rhodamine-B- stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and extract conc. is plotted to get the survival curve for each tumor cell line after the specified extracts. IC50 for each tested extract were calculated from this survival curve. The results are recorded in tables (3&4) and illustrated in figures (**1-8**)**.**

RESULTS AND DISCUSSION

Table (1) recorded the results of preliminary phytochemical screening for the four plant species under investigation. This screening revealed the presence of carbohydrates and/or glycosides, unsaturated sterols and/or triterpenes, tannins and flavonoides [both aglycones and glycosides] in the studied plants and presence of volatile oil was shown the four plants. Presence of alkaloids and or/ nitrogenous bases was only seen in the plant *Capparis spinosea* with the absence of cardiac glycosides, saponins and anthraquinones . Although the results revealed the absence of alkaloids, cardiac glycosides, saponins and anthraquinones in *Juniperus pheonicea*, *Ruta graveolens and Artimisia herba alba.*

Table1. Preliminary phytochemical screening of the aerial parts of each studied plants

Name of the test	*Capparis spinosa*	*Juniperus pheonicea*	*Ruta graveolens*	*Artimisia herb- alba*
Carbohydrates and/or glycosides	+	+	+	+
Saponins	-	-	-	-
Unsaturated sterols and/or triterpenes:				
a-Leibermann's and Burchard's test	+	+	+	+
b-Salkwask's test	+	+	+	+
Tannins	+	+	+	+
Flavonoid aglycones	+	+	+	+
Flavonoid glycosides	+	+	+	+
Anthraquinone aglcones	-	-	-	-
Anthraquinone glycosides	-	-	-	-
Cardiac glycosides	-	-	-	-
Alkaloids and/or nitrogenous bases	+	-	-	-
Volatile oil	+	+	+	+

Table (**2**) recorded the results of antimicrobial activity of alcoholic extracts. It revealed that ethanol extract prepared from the four studied plants possessed a broad spectrum effect against both the tested Gram positive and Gram negative bacteria. Ethanolic extract obtained from the the four investigated plant species showed lower antibacterial activity against all the tested bacteria at low concentration [0.25mg/disc] with the higher activity at higher *spinosa* showed the least antimicrobial concentration [0.5mg/disc]. Meanwhile the extract obtained from *Capparis* activity against *Bacillus subtilis* with the high activity against *Staphylococcus aureus* at both examined

concentrations. At the same time this extract showed a moderate activity against the other tested organisms [*E. Coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Salmonilla typhi*. Ethanolic extract obtained from the aerial parts of *Juniperus pheanicea* showed moderate antimicrobial activity against both gram positive and gram negative tested bacteria especially at high concentration [0.5mg/disc]. Ethanolic extract obtained from *Ruta graveolens* showed higher antibacterial activity against both *Klebsiella pneumonia* and *Salmonilla typhi* at the two tested concentrations. Ethanolic extract obtained from *Artimisia herba alba* showed the least antibacterial activity against most of the tested gram negative bacteria *[Klebsiella pneumonia*, *E.coli* and *Pseudomonas aeruginos*a] with the high activity against *Staphylococcus aureus* at the lower concentration [0.25mg/ml]. Meanwhile, the same extract possessed the highest activity against both *Klebsiella pneumonia* and *Salmonilla typhi* , with the lowest activity against *Bacillus subtilus*. All these results agreed with what is mentioned in the previous studies dealing with both the antibacterial activities of the entitled plant species. Also the total ethanolic extracts obtained from the four plant species exhibited a moderate antibacterial effect against both the examined Gram positive and Gram negative bacteria under investigation in comparison with the standard antibiotic Ceftriaxone.

Table 2. Antimicrobial activity of alcoholic extracts prepared from plants under investigation

Micro-organism	*Capparis spinosa*		*Juniperus phoenicea*		*Ruta graveolanse*		*Artimisia herba alba*		Ceftria-xone
Gram- positive	**0.25mg/ disc**	**0.5mg/ disc**	**0.25mg/ disc**	**0.5mg/ disc**	**0.25mg/ disc**	**0.5mg/ disc**	**0.25mg/ disc**	**0.5mg/ disc**	
Staphylococcus aureus	13 +	15 +	11 +	12 +	8 +	14 +	11 +	14 +	20
Bacillus subtilis	8 +	10 +	10 +	11 +	9 +	13 +	6 +	9 +	13
Gram negative									
E. Coli	9 +	11 +	9 +	12 +	9 +	12 +	7 +	13 +	30
Pseudomonas aeruginosa	9 +	10 +	9 +	11 +	8 +	14 +	8 +	14 +	23
Klebsiella pneumoniae	10 +	12 +	8 +	12 +	12 +	17 ++	5 -	18 ++	26
Salmonilla typhi	10 +	10 +	9 +	12 +	11 +	16 ++	10 +	18 ++	27

Inhibition zones in mm = <6mm [-] , 6-15 mm[+] , 16-25mm [++] , >25 mm[+++]

Table 3. Cytotoxic activity of the ethanol extracts obtained from each plant under investigation against breast cell lines MCF-7

Conc. in ug/ml	*Juniperus phoenicea* Surviving Fraction	*Capparis spinosa* Surviving Fraction	*Ruta graveolens* Surviving fraction	*Artimisia herba alba* Surviving fraction
0.000	1.000	1.000	1.000	1.000
5.000	0.534	0.850	0.827	0.570
12.500	0.405	0.591	0.551	0.463
25.000	0.274	0.334	0.314	0.238
50.000	0.316	0.289	0.266	0.221
IC50*	6.98	17.0	15.2	10.1

** IC50: The conc. of alcoholic extract that inhibit 50% of the tested cells*

Table 4. Cytotoxic activity of the ethanol extracts obtained from each plant under investigation against colon cell lines HCT-116

Conc. in ug/ml	*Juniperus pheonicea* Surviving Fraction	*Capparis spinosa* Surviving Fraction	*Ruta graveolens* Surviving fraction	*Artimisia herba alba* Surviving fraction
0.000	1.00	1.000	1.00	1.000
5.000	0.566	0.866	0.77	0.780
12.500	0.451	0.572	0.567	0.556
25.000	0.346	0.428	0.348	0.311
50.000	0.331	0.328	0.289	0.236
IC50*	9.38	18.8	16.3	15.2

** IC50: The conc. of alcoholic extract that inhibit 50% of the tested cells*

From tables (**3&4**) and figures (**1-8**) it was observed that; The total alcohol extract obtained from the four plants under investigation showed cytotoxic activity against both examined cell lines breast MCF-7 and colon cell line HCT-116. Measurement of the potential cytotoxic activity was concluded according to the calculated IC50 values [µg/ml].

The total alcohol extract obtained from *Juniperus phoenicea* showed the most potent effect against both breast cell lines IC_{50}=6.98 µg/ml. and colon cell lines IC_{50}=9.38 µg/ml. followed by that of *Artimisia herba-alba* IC_{50}=10.1 and

15.2 µg/ml., then that of *Ruta graveolens* IC_{50} =15.2 and 16.3 µg/ml. and finally the alcohol extract of *Capparis spinosea* IC_{50}=17.0 and 18.8 µg/ml. respectively.

Figure 1. Survival curve for breast cell line MCF-7 for *Capper spinosa*

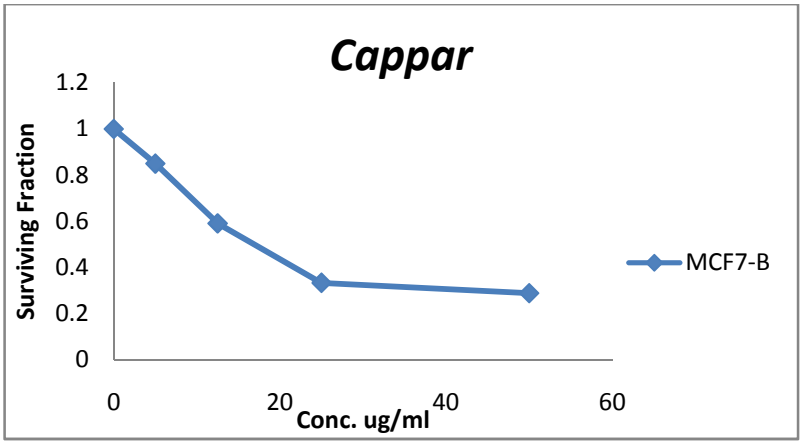


Figure 2. Survival curve for breast cell line MCF-7 for *Juniper phoenicea*

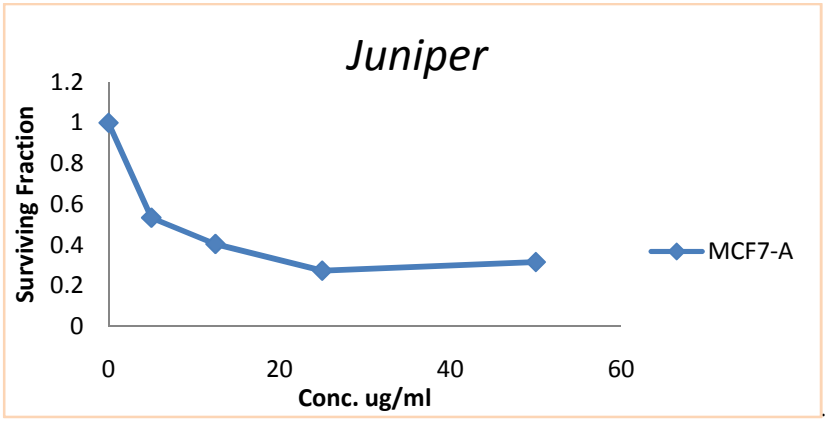


Figure 3. Survival curve for breast cell line MCF-7 for *Ruta graveolens*

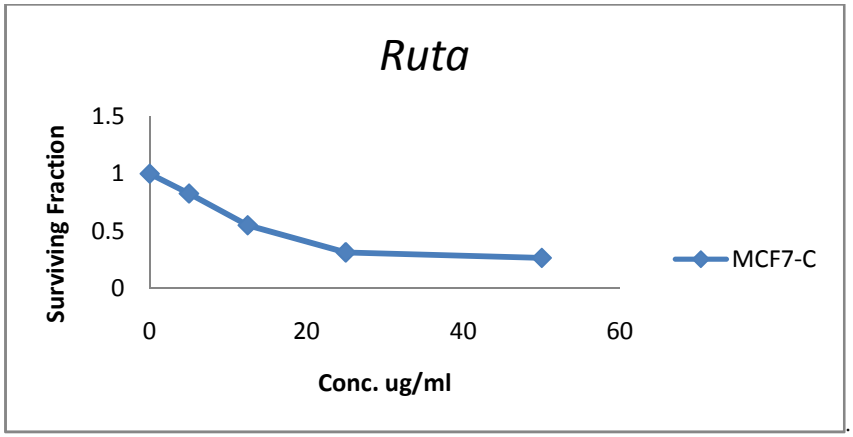


Figure 4. Survival curve for breast cell line MCF-7 for *Artimisia herba alba*

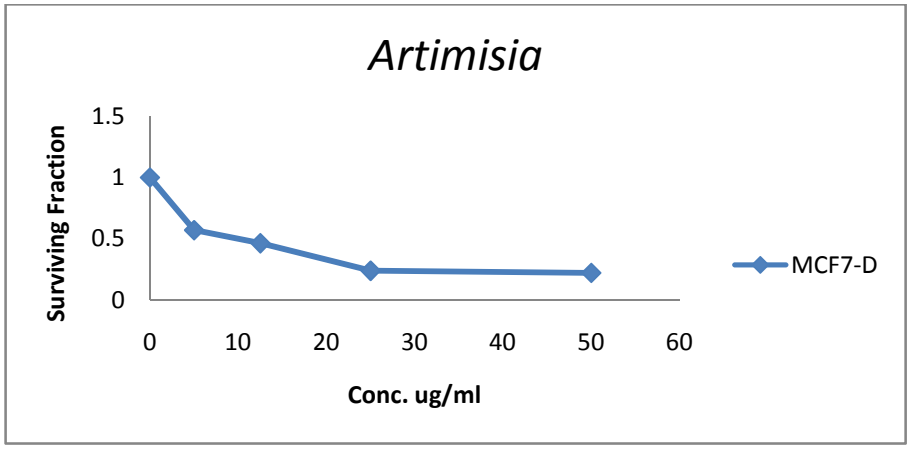


Figure 5. survival curve for colon cell line HCT-116 for *Capper spinosa*

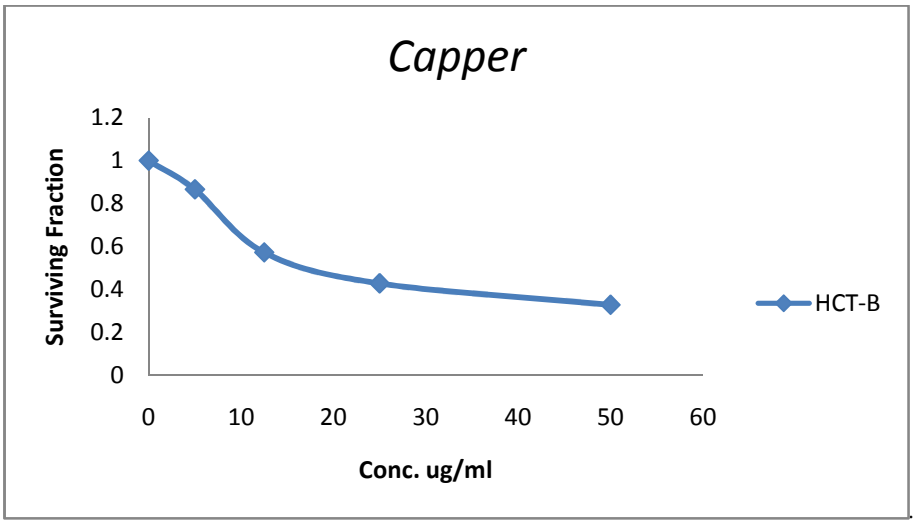


Figure 6. Survival curve for colon cell line HCT-116 for *Juniper phoenicea*

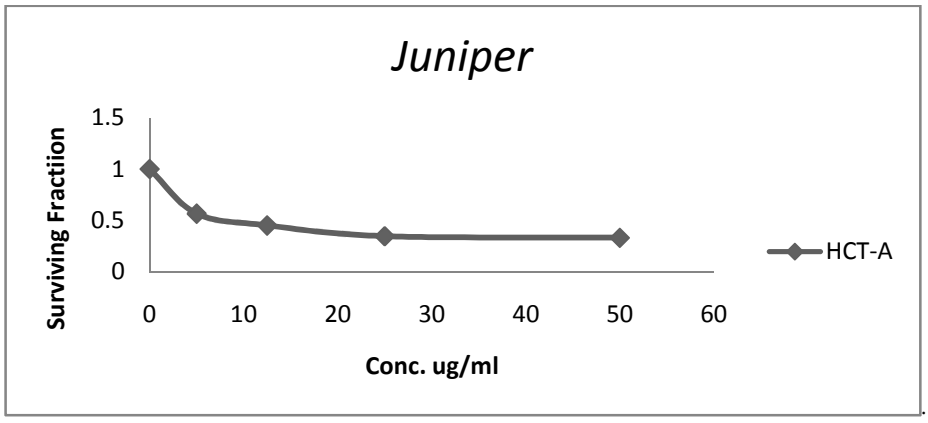


Figure 7. Survival curve for colon cell line HCT-116 for *Ruta graveolens*

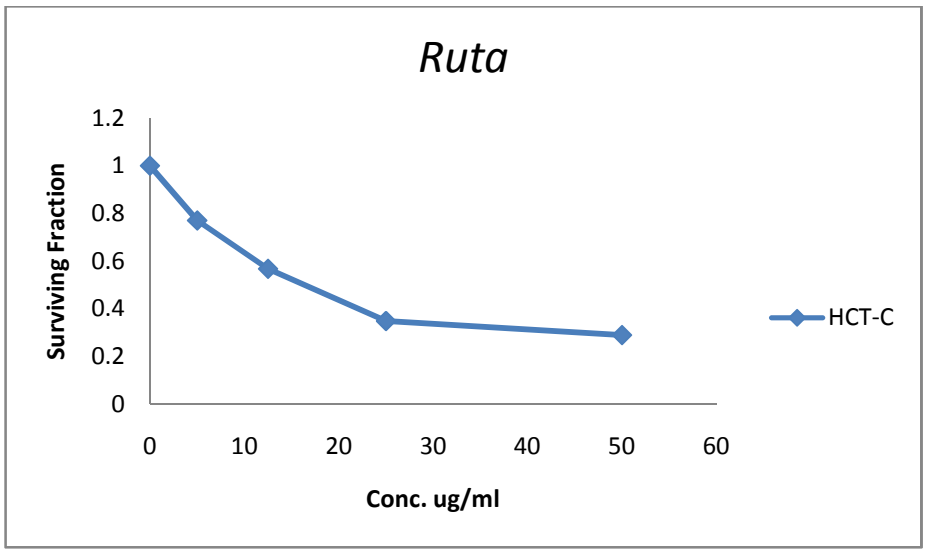
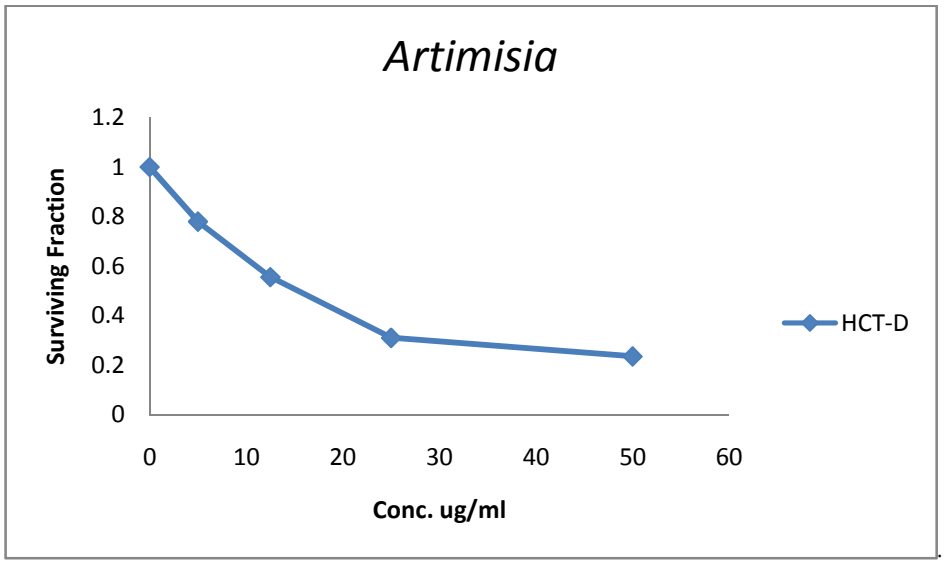


Figure 8. Survival curve for colon cell line HCT-116 for *Artimisia herba alba*



It is important to mention that these results complies with what was carried out by (**8**) as they evaluated the cytotoxic effects of methanolic extract of mature fruit of *Capparis spinosa* on Human larynx carcinoma (Hep-2) and Human cervix adenocarcinoma (HeLa) tumor cell lines *in vitro.* Kulisic-Bilusic *et al* (2011) studied the influence of essential oil and aqueous infusion from wild-grown caper (*Capparis spinosa*) on cell growth, NF-kB activation, apoptosis and cell cycle in the human colon carcinoma cell line, HT-29 and they suggested that capper contains volatile and non-volatile compounds which potentially can play an important role in colon cancer prevention.

It is important to mention that (**31)**, investigated the brain cancer cell-killing activity of *Ruta graveolen*. They treated human brain cancer and HL-60 leukemia cells *in vitro* with different concentration. They proved that *Ruta graveolens* could be used for effective treatment of brain cancers. Furthermore, Preethi *et al* (**28**)**,** they found that an extract of *Ruta graveolens* to be cytotoxic to Dalton's lymphoma Ascites **(DLA)** and Ehrlich Ascites Carcinoma **(EAC**).

At the same time, there are no available reports dealing with cytotoxicity of *Juniperus pheonicea* and *Artimisia herba-alba* Libyan plants.

This study is considered as the first report dealing with the *in vitro* cyototoxic activities for the alcoholic extracts of aerial parts of the four plants under investigation against breast [MCF-7] and colon cell lines [HCT-116] in Libya.

CONCLUSION

Alcoholic extracts obtained from aerial parts of *Capparis spinosea, Juniperus phoenicea, Ruta graveolens* and *Artimisia herba-alba* exhibited cytotoxic and antibacterial activities .

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