



Research Article

ANTIBACTERIAL, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF THE HYDROALCOHOLIC EXTRACT OF THE LEBANESE PLANT: *EPHEDRA CAMPYLOPODA*

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ABSTRACT

In order to determine the chemical composition of the hydroalcoholic extract obtained from the stems of the Lebanese plant *Ephedra campylopoda*, a phytochemical screening was carried out. The latter aids in identifying the content of this extract in primary and secondary metabolites in order to study and for the first time, some of the biological activities (as the antioxidant, antiproliferative and antibacterial) of this plant. The obtained results showed that the stems of *Ephedra campylopoda* are rich in several bioactive molecules such as polyphenols, flavonoids, tannins, etc. This wealth presented a remarkable antioxidant activity at a concentration of 0.5 mg/mL. It also presented an antiproliferative effect on two types of cancer cells: epithelial HT-29 and human colon HCT-116. Finally, its antibacterial effect was noticed against five bacterial strains. The obtained results encourage further research to be conducted in order to confirm the antibacterial, antioxidant and antiproliferative effects of this Lebanese plant.

Keywords: *Ephedra campylopoda*, phytochemical screening, antioxidant activity, antiproliferative activity, antibacterial activity.

INTRODUCTION

Lebanon is characterized by a relatively large flora due to its geographic location, varied topography, distinct soil types and climatic variations. In fact, about 2,600 wild species (92 of which are endemics), can be found merely in Lebanon. Among these species, only few hundreds of plants are used in treating various diseases as gastrointestinal disorders, kidney and urinary diseases, cardiovascular diseases, diabetes, asthma, sexual disorders, hair problems and various tumors^{1,2}. For this reason, it is definitely interesting and necessary to conduct new scientific studies on such plants, especially those used locally as a traditional medicine.

Ephedra campylopoda belongs to the Ephedraceae family. It is distributed along the Mediterranean towards Italy and Greece, Albania, Bulgaria, Cyprus, Egypt, Ethiopia, Jordan, Lebanon, Somalia, the Syrian Arab Republic and extends further south into Sinai, Saudi Arabia and Yemen. Recently, it was reported to be discovered in North Africa Eritrea and Djibouti³. This plant has been traditionally used to treat kidney problems such as kidney stones. The stems of most members of this genus are known to contain ephedrine which is an alkaloid useful in the treatment of asthma and many other respiratory system diseases.

Till now the literature lacks studies that have examined the antioxidant, antibacterial and antiproliferative activities present in the stems of *E. campylopoda*. Due to this reason, our study

aims primarily to evaluate for the first time, the effects of the stem's hydroalcoholic extract on five bacterial strains: Gram-positive and Gram-negative bacteria. Secondly, the study intends to investigate the plant's antiproliferative capacity on two types of cancer cell lines: epithelial HT-29 and human colon HCT-116.

MATERIALS AND METHODS

Plant Collection and Powder Preparation

E. campylopoda was collected from the South of Lebanon in a field located at an altitude of 100m during March – April 2016. The biological authentication was carried out by the Professor George Tohme, the president of the Lebanese C.N.R.S.

The plant's fresh stems were washed well then were cut into small pieces and dried in the shade at room temperature, away from the sunlight. During the drying process, the stems were turned over repeatedly for homogeneous drying. After that, the dried stems were grinded by a grinder. The obtained powder was then preserved in a container away from light, heat, and moisture for later use.

The powdered stems (weighing 228.1g) were then placed in water-methanol (v/v) of pH 3.5 for 48 hours along with stirring at room temperature. The macerate obtained was filtered to remove insoluble residues. Subsequently, the filtrate was

condensed by evaporation, using a rotary evaporator, in order to eliminate the remaining methanol solvent. Finally, the filtrate was frozen before being a lyophilized powder to be processed later⁴.

All of the chemicals used were of an analytical grade. Methanol was purchased from BDH, England.

Phytochemical screening

To study the chemical composition of water-methanol extracts in the stems of *E. campylopoda*, qualitative detection of primary and secondary metabolites was performed (Table 1).

Total phenolic content (TPC)

The Folin–Ciocalteu reagent method was used to estimate the total phenolic quantities according to Farhan et al.¹³ with minor modifications. Briefly, 100 µL of the stems' extracts were taken and mixed with 1 mL of Folin–Ciocalteu reagent (1/10 dilution in water). After 5 min, 1.5 mL of Na₂CO₃ 2% (w/v) was added. The blend was incubated in the dark, at room temperature for 30 min. The absorbance of blue-colored solution for all samples was measured at 765 nm using a Gene Quant 1300 UV-Vis spectrophotometers. The results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of plant powders.

$$\text{Total phenol content} = \text{GAE} \times V \times D / m,$$

Where GAE is the gallic acid equivalence (mg/mL);

V is the volume extract (mL), D is dilution factor and m is the weight (g) of the pure plant extract.

The blank was formed by 0.5 mL water-MeOH and 1.5 mL of Na₂CO₃ (2%).

Total flavonoids content (TFC)

The aluminum chloride method was used according to Quettier-deleu *et al.*¹⁴ to determine the TFC of the studied plant with minor modifications. Briefly, 1 mL of water-methanol extract was mixed with 1 mL of methanolic aluminum chloride solution (2 %). After an incubation period at room temperature in the dark for 1 h, the absorbance of all samples was determined at 415 nm using a Gene Quant 1300 UV-Vis spectrophotometers. The results were expressed in mg per g of rutin equivalent (RE).

$$\text{Flavonoids content} = \text{RE} \times V \times D / W$$

Where RE is Rutin equivalent (µg/mL), V is the total volume of sample (mL), D is dilution factor, W is the sample weight (g). The blank was formed by 1 mL water-MeOH and 1 mL of 2 % methanolic aluminum chloride solution.

Total alkaloids content (TAC)

The quantification method for alkaloids determination was used according to Harborne¹⁵. 100 mL of 10 % acetic acid in ethanol was added to 1 g of dry powdered plant and then covered and allowed to stand for 4 h. After that, the extracts were filtrated and concentrated in a water bath to 25 mL of its original volume. Droplets of concentrated ammonium hydroxide were added to the extract until the whole solution precipitated. The precipitates were then washed with dilute ammonium hydroxide and filtered using filter paper Whatman. The residue was dried in the oven at 40 °C and weighed. The alkaloid content was determined using the following formula:

$$\% \text{ Alkaloid} = [\text{final weight of the sample} / \text{initial weight of the extract}] \times 100$$

Total tannins determination

Tannins were determined by the Folin–Ciocalteu method¹⁶. 0.4 mL (10 mg/mL) of the water-methanol extract of the stems were added to 2 mL of Folin–Ciocalteu reagent and to 4 mL of Na₂CO₃ (35%). The mixture was stirred well and kept at room temperature for 30 min. Standard solutions of gallic acid (20, 40, 60, 80 and 100 µg/mL) were prepared in the same method as described previously. The absorbance of the test and standard solutions were measured at 765 nm with a UV-Visible spectrophotometer. Blank was formed by 0.5 mL water-MeOH and 1 mL Na₂CO₃ (35%). The tannin content was expressed in mg GAE / g of extract.

Total saponin determination

Stems' powder (20 g) was put into a conical flask and 100 cm³ of ethanol (20%) were added. The sample was heated over a hot water bath (about 55°C) for 4 h with continuous stirring. The mixture was then filtered and the residue was re-extracted with another 200 mL ethanol (20%). The combined extracts were reduced to 40 mL over water bath at about 90°C. The concentrate was transferred into a 250 mL separatory funnel in which 20 mL of diethyl ether were added and shaken vigorously. The aqueous layer was recovered and the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of aqueous sodium chloride (5%). The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight and the saponin's content was calculated¹⁷.

Saponin's content was calculated using the following formula:

$$\% \text{ Saponin} = [\text{final weight of sample} / \text{initial weight of extracts}] \times 100$$

Moisture content¹⁸

Dried stems (1.5 g) were placed in an oven at 105 °C for 1 h. Then, it was placed in a desiccator for half an hour. The mass of the content was recorded and was returned again to the oven for another 1 h. After heating, it was placed again in the desiccators for half an hour. These steps yielded a dry powder in which its mass was recorded again in order to calculate the percentage of humidity in the sample. All samples were done in triplicate.

$$\% \text{ Humidity} = [(\text{Initial weight} - \text{final weight}) / \text{powder weight}] \times 100$$

With:

Initial weight = Sample weight + crucible weight (before heating)

Final weight = Sample weight + crucible weight (after heating)

Proportion of ash

Stems' powder (2 g) were placed and burned in a furnace burning (muffle furnace) at 550 °C for 5 h till obtaining a powder having an ovary gray color. The residues were then weighed and the percentage of ash was estimated according to the essential dry weight of plant powder¹⁹.

$$\% \text{ Ash} = (\text{final weight} / \text{initial weight}) \times 100$$

Initial weight = Sample weight + crucible weight (before heating).

Final weight = Sample weight + crucible weight (after heating).

Mineral content

Acid digestion was performed to determine the minerals content. 1g of stems' powder was placed in the oven at 80 °C for 24 h. 10 mL of concentrated HCl was then added at 80 °C with stirring followed by covering the beaker. From time to time, drops of H₂O₂ (35 %) were added. The beaker was put to warm for 15 h. After the evaporation of HCl, 10 mL of HNO₃ were added. Vacuum filtration was performed for the obtained mixture followed by syringe filtration.

The minerals: iron, calcium, magnesium, lead, copper, cadmium, chromium, manganese and zinc were determined by the atomic absorption spectrometry.

Total proteins

Proteins were determined using the method of AOAC²⁰. 1 g of powdered stems was placed into specific tubes (500 mL) with a catalyst (containing 5 g of K₂SO₄ and 0.25 g of CuSO₄). 12-15 mL of H₂SO₄ (96-98%) and 10 mL of H₂O₂ (30-35%) were added to the sample. The sample digestion was kept for 20 min at 100 °C. After cooling the tubes, distillation was carried out by automatically adding 50 mL of water and 50 mL of NaOH (35%) for 5 min. The released NH₃ was captured in an erlenmeyer flask containing 25 mL of boric acid (4%). Titration of ammonium ion was made using a solution of H₂SO₄ (0.1M) in the presence of 3-5 drops of Tashiro indicator. The protein content was calculated by multiplying the mineral nitrogen content by 6.25.

$$\text{Protein content} = 6.25 * \text{volume H}_2\text{SO}_4$$

Total lipids

Total lipids were evaluated according to the method described by Aberoumand²¹. 2g of powder were extracted by Soxhlet apparatus containing petroleum ether (bp: 40-60 °C) till the extraction of total lipids. After that, the extract was put in a beaker and placed in the oven at 100 °C in order to evaporate the entire solvent. Finally, it was cooled in a desiccator and weighed.

$$\% \text{ Lipids} = [\text{lipid weight} / \text{powder weight}] \times 100$$

Evaluation of the antioxidant activity by DPPH assay

The method of Rammal et al.^{22,23} was used for the scavenging ability of DPPH antioxidant test. 1 mL of different concentrations (0.1, 0.2, 0.3, 0.4 et 0.5 mg/mL) of diluted extracts of the plant stems was added to 1mL of DPPH (0.15 mM in methanol) and at the same time, a control consisting on 1mL DPPH with 1mL methanol was prepared. The reacting mixtures were mixed manually very well and then were incubated in the dark at room temperature for 30 min. The absorbance was measured at 517 nm by a Gene Quant 1300 UV-Vis spectrophotometer. The ascorbic acid was used as a positive control and the water-methanol was used as blank. The DPPH scavenging ability of plant extracts was calculated using the following equation:

$$\% \text{ Scavenging activity} = \frac{[(\text{Abs control} - \text{Abs sample}) / (\text{Abs control})] \times 100}$$

The Abs control is the absorbance of DPPH + water-methanol; Abs sample is the absorbance of DPPH radical + sample. Also, three controls have been prepared.

Evaluation of the anti-proliferative activity

To study the antiproliferative activity of water-methanol stems' extracts from the studied plant, cell culture was performed using the epithelial cells HT-29 and HCT-116 cells of the human colon. Then the measure of inhibition of cell proliferation was applied using the yellow tetrazolium MTT technique.

Cell culture was performed in 96-well plates, each containing 100 µL DMEM at 10.000 cells for HT-29 and 15.000 cells for HCT-116. The water-methanol stems' extracts were diluted with the DMEM culture medium in decreasing concentrations (200, 100, 50, 25 and 5 µg/mL) and were then added to the wells after pre-incubation for 24 h. The plates were then incubated under 5% CO₂ and at a temperature of 37 °C during 24, 48 and 72 hours respectively.

After incubation, 10 µL of MTT solution were added per well and incubated for 3 h at 37 °C. Then a 100 µL solubilization solution was added to each well. Finally, the absorbance was measured with a spectrophotometer at 570 nm. This quantity is directly proportional to the number of cells with an intact membrane.

Antibacterial activity assay

Bacterial strains: The strains used in this study were three Gram positive bacteria (*Staphylococcus epidermidis* CIP 444, *S. aureus* ATCC 25923, and *Enterococcus faecalis* ATCC 29212) and two Gram-negative strains (*Escherichia coli* ATCC 35218 and *Pseudomonas aeruginosa* ATCC 27853). The Gram-positive CIP 444 strain is a clinical strain that was isolated from an infected implanted device in a patient hospitalized in the Mignot Hospital of Versailles, France²³.

Ali Chokr has identified and characterized the properties of these strains and deposited it to be enclosed within the collection of microorganisms of Pasteur Institute in 2007²⁴⁻²⁶. The other strains were ATCC that were stored in glycerol stocks at -80°C and used as required. Brain heart infusion (BHI), Brain heart agar (BHA), and Mueller–Hinton broth (MHB) were purchased from HIMEDIA (Mumbai, India), that were prepared and then autoclaved as indicated by the manufacturer before their use.

MIC and MBC assays: plant's extracts were tested for its corresponding Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal Concentrations (MBC) by broth micro-dilution assay, as recommended by the Clinical Laboratory and Standard Institute (CLSI)²⁷. A concentration of 200 mg/mL of stems extract was prepared. In a 96-well plate (200 µL/well) (Greiner Bio-One, Essen, Germany), serial two-fold dilutions in MHB of the different extracts were done. The wells were inoculated with 5 × 10⁵ bacteria/mL. After incubating the plates at 37°C for 24 hours, the MIC (which is defined as the lowest concentration that yielded no growth) was determined. In addition, the wells with no visible growth were plated on BHA in order to determine the MBC (which is defined as the lowest concentration which killed ≥99.9% of the initial inoculum). The Petri plates were incubated overnight at 37°C, and the MBC was determined.

Table 1 : Detection of primary and secondary metabolites by phytochemical screening

Metabolites	Added reagent	Expected result
Alkaloids ⁵	Dragendorff reagent	Red-orange Precipitate
Tannins ⁵	FeCl ₃ (1%)	Blue color
Resines ⁶	Acetone + water	Turbidity
Saponines ⁷	Agitation	Formation of Foam
Phenols ⁵	FeCl ₃ (1%) + K ₃ (Fe(CN) ₆) (1%)	Blue-green
Terpenoids ⁷	Chloroform + H ₂ SO ₄ conc	Reddish brown
Flavonoids ⁸	KOH (50%)	Yellow color
Carbohydrates ⁷	α -naphthol + H ₂ SO ₄	Purple ring
Reducing sugar ⁷	Fehlings (A+B)	Brick red precipitate
Quinones ⁹	HCl conc	Yellow precipitate
Sterols et Steroids ^{7,8}	Chloroform + H ₂ SO ₄ conc	Red (surface) + greenish yellow fluorescence
Cardiac glycosides ^{7,8}	Glacial acetic acid + FeCl ₃ (5%) + H ₂ SO ₄ conc	Rings
Diterpenes ⁵	Copper acetate (or sulfate)	Emerald green
Anthraquinones ⁹	HCl (10%) + chloroform + Ammonia (10 %)	Pink color
Proteins & aminoacids ¹⁰	Ninhydrin 0.25%	Blue color
Lignines ¹⁰	Safranine	Pink color
Phlobatannins ¹¹	HCl (1%)	Blue color
Anthocyanines ¹²	NaOH (10%)	Blue color
Flavanones ¹²	H ₂ SO ₄ conc	Purple red color
Fixed oils and fats ⁷	Spot Test	Oil stain

Table 2 : Chemical composition of the water-methanol extracts of the stems of *E. campylopoda*

	Water/methanol
Tannins	+
Phenols	+
Terpenoids	+
Flavonoids	+
Carbohydrates	+
Reducing sugar	+
Sterols and steroids	+
Cardiac glycosides	+
Diterpenes	+
Proteins & amino acids	+
Lignin	+
Saponin	+
Flavanones	+

Table 3 : Mineral values determined by Atomic Absorption Spectrometry

	Ca	Pb	Zn	Fe	Cu	Mn	Mg
mg/L	4188	66	8	11,52	0,72	1,44	880
mg/g	41.88	0.66	0.08	0.1152	0.0072	0.0144	8.8

Table 4 : Results of MIC and MBC for the water/methanol stems extracts

	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. epidermidis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>
MIC (mg/mL)	12.5	12.5	12.5	50	25
MBC (mg/mL)	25	50	50	-	100

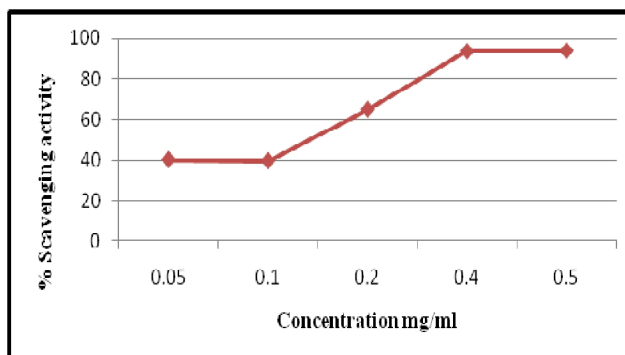


Figure 1: Antioxidant activity of the methanol/water extract from the stems of *E. campylopoda*

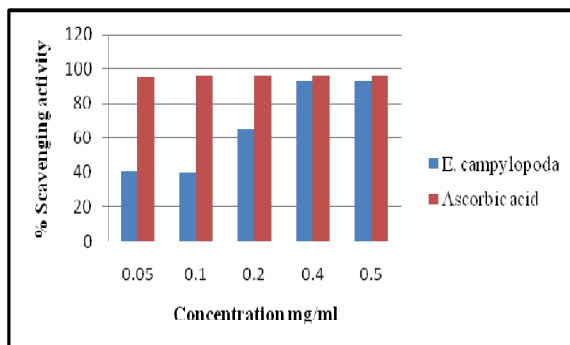


Figure 2: Antioxidant activity of the ascorbic acid and methanol/water extract from the stems of *E. campylopoda*

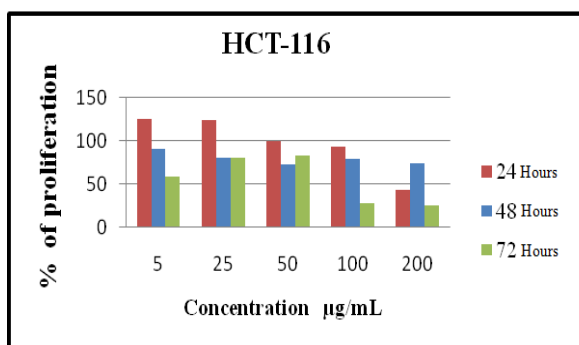


Figure 3: Percentage of proliferation of the HCT116 human colon cell according to the incubation time (h) and the concentration of the extract (µg/mL)

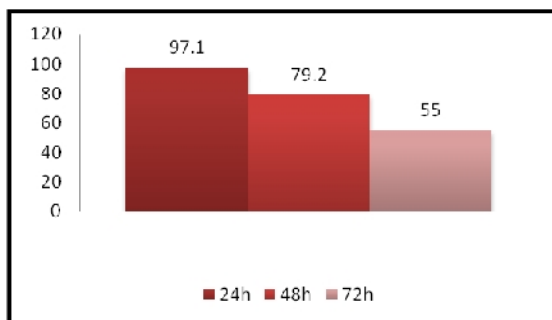


Figure 4: Means of the percentage of proliferation of HCT-116 human colon cell for each incubation time

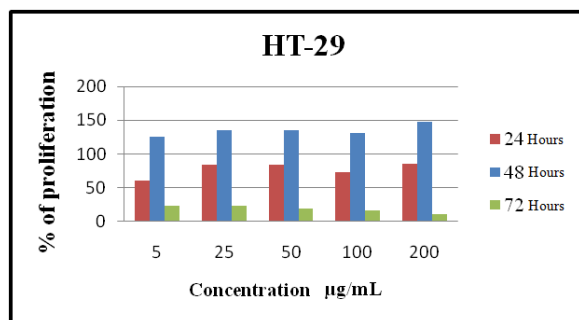


Figure 5: Percentages of proliferation of epithelial HT29 cells according to the incubation time (h) and the concentration of the extract (µg/ml)

RESULTS AND DISCUSSION

Phytochemical screening (qualitative tests)

Due to the strong relationship between the chemical compounds and the medicinal uses of plants, a phytochemical screening was performed to find out the primary and secondary metabolites present in the stems of *E. campylopoda* (Table 2).

The results obtained by this screening (Table 2) show that there are several primary and secondary metabolites in the stems of *E. campylopoda* with a difference in the amounts depending on the solvent used. Despite the absence of alkaloids, quinones, anthraquinones, phlobatannins and anthocyanins, the hydroalcoholic extracts were found to be rich in phenols, carbohydrates, tannins, terpenoids, flavonoids, reducing sugars, sterols and steroids, cardiac glycosides, diterpenoids, proteins and amino acids, lignin and flavanones in the water-methanol extract.

TPC and TFC

TPC and TFC are expressed respectively by equivalent gallic acid and rutin. The obtained results indicate 4.835 mg TPC and 19.241 mg TFC per g of dry powder. It is important to note that Ibragic and Sofic²⁸ found that in *Ephedra alata* TPC and TFC were higher (53.3 ± 0.1 mg GAE/g and 2.8 mg QE (quercetin equivalent)/g dry weight). This difference might be due to different factors such as species, soil, climate, etc.

Total alkaloids content

No precipitate formation was observed during the addition of concentrated ammonium hydroxide. This indicates that the stems of the *E. campylopoda* don't contain alkaloids unlike *Ephedra Sinica* which is rich in alkaloids especially ephedrine²⁹.

Total tannins content

The absorbance readings using a UV-visible spectrophotometer show that the tannin content expressed in gallic acid equivalent is of 0.0527 mg/g of dry matter.

Total saponin content

The obtained results indicate that 1g of powdered stems of the plant contains 0.0151g of saponin which corresponds to a percentage of 1.51%.

Moisture and Ash content

The obtained results indicate that 1g of powdered stems of the plant contains 7.85% moisture. Therefore, ash represents 93% of dry matter.

Lipids content

The method of Soxhlet indicated that the total content of lipids existing in 2g of powdered stems of *E. campylopoda* was 0.8 g which is equivalent to 40 % dry matter of the plant.

Protein content

The results obtained by the Kjeldahl technique showed that in each gram of powdered stems of *E. campylopoda*, there is 87.5mg of protein which is equivalent to 8.75% dry matter of the plant.

Mineral content

The presence of minerals (Table 3) in the stems of *E. campylopoda* has been detected using the AAS technique. The results show that the stems of this plant are much richer in calcium than in any other metal. It has a high content of calcium (41.88 mg/g) unlike the copper's content which is almost negligible.

Biological properties

Antioxidant activity

Even if free radicals can possess some beneficial physiological roles, an excess of those radicals along with unbalanced reactive biomolecules generates oxidative stress which is harmful for human being. The reason is that it plays a major role in the initiation and development of chronic and degenerative pathologies such as cardiovascular and neurodegenerative diseases, autoimmune disorders, aging and cancer³⁰. Thus, identifying plants with potent antioxidant capacity is of a great interest. In the present study, we assessed the antioxidant power of the hydroalcoholic extracts from stems of Lebanese *E. campylopoda* by evaluating their ability to scavenge free DPPH radicals. The obtained data showed that the concentration of methanol/water extract, and that of ascorbic acid, presented a positive correlation with the DPPH test. And an increase in the antioxidant activity of the stems of *E. campylopoda* was noticed from 40% at the concentration 0.05 mg/mL till 94% at the concentration 0.5 mg/mL (Figure 1).

Therefore, there was a slight increase from 93% to 96% in ascorbic acid because it has a very important antioxidant effect at low concentrations.

It can also be noted that at a concentration of 0.5 mg/mL, the stems of *E. campylopoda* and ascorbic acid have similar antioxidant activity (Figure 2).

Antiproliferative activity

The richness in phenolic and flavonoids compounds was demonstrated by the phytochemical screening. The results obtained in the DPPH test validated those obtained by the phenols' screening. To determine the antiproliferative activity of the stems of *E. campylopoda*, the technique of yellow tetrazolium MTT was performed and the results obtained are presented in Figures 3, 4 and 5.

For HCT-116 human colon cells at the concentration of 100 µg/mL of the methanolic/water extract, the percentage of proliferation after incubation for 24 h was 93.57% which then decreased with increasing incubation time to reach 79% after 48 h and 28.18 % after 72 h (Figure 3).

In addition, a correlation was noticed between the percentage of proliferation and the concentration used. It is noted that the proliferation decreased with an increasing concentration for each incubation period. For example, at a concentration 5 µg/mL the percentage of proliferation was 125.36%, 90.43% and 59.06%, respectively after 24, 48 and 72 h then it decreased to 93.57%, 79% and 28.18% for the same time.

In general, the percentages of proliferation obtained after 24 h were very high with an average of 97.1% for the five concentrations. After 48 h, it decreased to 79.2% and reached 55% after 72 h of incubation (Figure 4).

Regarding the epithelial cells HT-29, a similar correlation was obtained. The proliferation percentage was 84.66% after 24 h of incubation at a concentration 25 µg/mL which decreased to 23.37% after 72 h.

When the concentration increases to 100µg/mL, the values become 73.12% and 15.59% respectively at 24 and 72 h. Furthermore, the proliferation percentage for a concentration 5 µg/mL was 22.8% after 72 h while the increase in the concentration to 200 µg/mL decreases it to 11.35% (Figure 5).

In general, the average percentages of proliferation after 24 h were 77.4% and fall to 18.48% after 72 h.

Antibacterial activity

The method of micro-dilution in wells, used for the determination of MIC, led to the results presented in Table 4. The MIC values for gram positive bacteria (12.5 mg/mL) were lower than those for gram negative bacteria (25 mg/mL for *P. aeruginosa* and 50 mg/mL for *E. coli*). Also Gram positive bacteria are more sensitive to the extract than gram negative bacteria which may be due to the presence of a capsule that makes Gram negative bacteria more resistant.

In addition, the results of this study also showed that the values of MBC were higher than those of MIC. The values were two times greater for *S. aureus* and four times greater for *E. faecalis*, *S. epidermidis* and *P. aeruginosa*. Moreover, the values were also higher in Gram negative bacteria (100 mg/mL for *P. aeruginosa*) than in Gram positive bacteria (eg. 25 mg/mL for *S. aureus*).

Based on these results, we can say that this plant extract can act as a bacteriostatic agent which can also exert a lethal effect in the most dangerous and sometimes deadly microorganisms.

CONCLUSION

The obtained results demonstrated for the first time, that water/methanol extract of the stems of *E. campylopoda* has a promising antioxidant effect, an anti-proliferative effect on two types of cancer cells, as well as an antibacterial effect on different types of bacteria (Gram positive and Gram negative bacteria).

Further investigations are required i) to determine the specific compounds that are responsible for these activities and ii) to discover other interesting activities that might be present in this plant, such as the antibiofilm property.

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REFERENCES

- Marc EB, Nelly A, Annick D-D, Frederic D. Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. J Ethnopharmacol 2008; 120: 315–34.
- Deeb T, Knio K, Shinwari ZK, Kreydiyyeh S, Baydoun E. Survey of medicinal plants currently used by herbalists in Lebanon. Pak J Bot 2013; 45: 543–55.

3. Freitag H, Maier-Stolte M. The genus *Ephedra* in NE Tropical Africa. Kew Bulletin 2003; 58: 415-26.
4. Hijazi A, Bandar H, Rammal H, Hachem A, Saad Z, Badran B. Techniques for the Extraction of Bioactive Compounds from Lebanese *Urtica dioica*. Am J Phytomed Clin Ther 2013; 6: 507-13.
5. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complement Altern Med 2010; 10: 1-8.
6. Al-Balany MR. Effect of crude plant Extracts and Vasicine alkaloid of *Adhatoa vasica* in some pathogenic Microorganisms. Msc. Thesis, faculty of Science. Baghdad University, Iraq; 2004.
7. Fred JA, Adisa A. Phytochemical and Pharmacognostic Studies of *Telosma africanum* (N.E.Br) Colville Leaf and Stem. Int J Pharm Sci and Res 2012; 3(6):1860-62.
8. Khandelwal KR. Practical Pharmacognosy–Techniques and Experiments. Nirali Prakashan, Pune, India; 2005.
9. Siddiqui AA, Ali M. Practical Pharmaceutical Chemistry. Ist. ed: CBS Publishers and distributors, New Delhi; p. 126-31; 1997.
10. Rani N Paval, Moorthi C, Sentharama RI, Kathiresan K. A study to explore the pharmacognostic and phytochemical screening of *Artemisia nilagirica* leaves found in nilgiris district of Tamil Nadu. Int J Pharm Pharm Sci 2012; 4(4): 441-47.
11. Krishnaiah D, Devi T, Bono A, Sarbatly R. Studies on phytochemical constituents of six Malaysian medicinal plants. J Med Plants Res 2009; 3(2): 067-072.
12. Mir MA, Sawhney SS, Jassal MMS. Qualitative and quantitative analysis of phytochemicals of *Taraxacum officinale*. W J Pharm Pharmacol 2013; 2(1): 001-005.
13. Farhan H, Malli F, Rammal H, Hijazi A, Bassal A, Ajouz N et al.. Phytochemical screening and antioxidant activity of Lebanese *Eryngium creticum* L. Asian Pac J Trop Biomed 2012; 2(3): S1217-S20.
14. Quettier-Deleu C, Gressier B, Vasseur J, Dine T, Brunet C, Luyckx M et al.. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. J Ethnopharmacol 2000; 72: 35-42.
15. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. 3rd ed: New Delhi: Springer Pvt. Ltd; 2005.
16. Singh R, Pawan Kumar V, Singh G. Total phenolic, flavonoids and tannin contents in different extract of *Artemisia absinthium*. J Intercult Ethnopharmacol 2012; 1(2): 101-4.
17. Obdoni B, Ochuko P. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci 2001; 8: 203–8.
18. Prasanna K, Yuvaranni S. Preliminary Phytochemical Screening and Antibacterial activity of *Datura metel* And *Vitex negundo* Against Bacterial Cold Water Disease Causing Organism. Int J of Pharm and Pharm Sci 2014; 6(5): 230-3.
19. Manjulika Y, Sanjukta C, Sharad KG, Geeta W. Preliminary Phytochemical Screening of Six Medicinal Plants Used In Traditional Medicine. Int J of Pharm and Pharm Sci 2014; 6(5): 539-42.
20. Helrich K. Official methods of analysis of the Association of Official Analytical Chemist “AOAC”, 15th ed. Arlington, Virginia; 1990.
21. Aberoumand A. A comparative study on composition analysis of plants consumed in India and Iran. Am Eurasian J Agric Environ Sci 2010; 8: 3329-333.
22. Farhan H, Rammal H, Hijazi A, Hamad H, Badran B. Phytochemical screening and extraction of polyphenol from stems and leaves of a Lebanese *Euphorbia macrolada* schyzoceras Boiss. Ann Biol Resh 2012; 3(1):149-56.
23. Rammal H, Farhan H, Mohsen H, Hijazi A, Kobeissy A, Daher A, et al.. Antioxidant, cytotoxic properties and phytochemical screening of two Lebanese medicinal plants. Int Res J Pharm 2013; 4(5):132-6.
24. Chokr A, Watier D, Eleaume H, Pangon B, Ghnassia JC, et al. Correlation between biofilm formation and production of polysaccharide intercellular adhesin in clinical isolates of coagulase-negative staphylococci. Int J Med Microbiol 2006; 296: 381-8.
25. Chokr A, Leterme D, Watier D, Jabbouri S. Neither the presence of ica locus, nor *in vitro*-biofilm formation ability is a crucial parameter for some *Staphylococcus epidermidis* strains to maintain an infection in a guinea pig tissue cage model. Microb Pathog 2007; 42: 94-7.
26. Sadovskaya I, Kogan G, Chaignon P, Chokr A, Vinogradov E, et al. Carbohydrate-containing components of biofilms produced *in vitro* by some staphylococcal strains related to orthopaedic prosthesis infections. FEMS Immun Molecular Microbiol 2006; 47: 75-82.
27. Wikler MA, Low DE, Cockerill FR, Sheehan DJ, Craig WA, Tenover FC, et al. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard (7thedn.) Document M7-A7. Wayne, PA: CLSI; 2006.
28. Ibragic S, Sofić E. Chemical composition of various *Ephedra* species. Bosn J Basic Med Sci 2015; 15(3): 21-7.
29. Gurley BJ, Gardner SF, White LM, Wang PL. Ephedrine pharmacokinetics after the ingestion of nutritional supplements containing *Ephedra sinica* (ma huang). Ther Drug Monit 1998; 20(4): 439-45.
30. Rahman T, Hosen I, Islam MMT, Shekhar HU. Oxidative stress and human health. Adv Biosci Biotechnol 2012; 3: 997–1019.

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