



Research Article

ANTIBACTERIAL, ANTIOXIDANT AND ANTIPROLIFERATIVE ACTIVITIES OF THE HYDROALCOHOLIC EXTRACT OF THE LEBANESE *ANNONA SQUAMOSA* L. SEEDS

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ABSTRACT

To determine the chemical composition of the seeds of the Lebanese *Annona squamosa* L., a phytochemical screening was performed on a water/methanol (v/v) extract. Following that, several quantitative tests were carried out to determine the content of the seeds in the secondary metabolites. Also, the nutritive and medical importance was evaluated in order to realize some biological activities such as antibacterial, antioxidant and anti-proliferative activities. The obtained results showed that the hydroalcoholic extract from the seeds of this fruit contains a variety of primary and secondary metabolites like alkaloids, resins, saponin, phenols, terpenoids, flavonoids, carbohydrates and quinones. In addition, it has a low antioxidant activity and a bacteriostatic activity that is greater than a bactericidal agent. Finally, the seeds' extract presented an anti-proliferative activity that is more effective on HCT-116 colon cells than on HT-29 epithelial cells. Further research studies need to be conducted to confirm the antibacterial, antioxidant and anti-proliferative effects of the seeds found in this fruit.

Keywords: *Annona squamosa* seeds, phytochemical screening, secondary metabolites, biological properties.

INTRODUCTION

Medicinal plants are important for both: the pharmacological research and the development of medicinal products; its constituents are used either directly as therapeutic agents or indirectly as raw materials for drug synthesis or models for pharmacologically active compounds. Such medicines are increasingly accepted for the maintenance of personal health even among people living in urban areas. This might be probably due to the increasing inefficiency of many modern medicines used to control different infections such as typhoid fever, gonorrhoea and tuberculosis. In addition, it might be due to the increased resistance of several bacteria to various antibiotics and the rising cost of the drugs ¹.

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 ^{2,3}. For this reason, it is interesting and necessary to conduct new scientific studies on such plants, especially those used locally as a traditional medicine.

Annona squamosa is also known as apple sugar, sweetsop or custard-apple. It belongs to the Annonaceae family, which in turn belongs to the order of Magnoliales, and to the class of

Magnoliopsida, under the phylum Magnoliophyta ⁴. It is traditionally used for the treatment of epilepsy, dysentery, cardiac problems, worm infections, constipation, hemorrhage, antibacterial infections, fever, and ulcers. It also has antifertility, antitumor and abortifacient properties ⁴. The leaves, bark, seeds and roots of this plant have shown many therapeutic activities including anticancer effects, CNS depressants, analgesics, antihyperglycemic effects, anti-inflammatory effects, anti-proliferative effects, wound healing results and antiulcer activities ⁵.

Till now the literature lacks studies that have examined the antioxidant, antibacterial and anti-proliferative activities present in the seeds of the Lebanese *A. squamosa*. Due to this reason, our study aims primarily to evaluate for the first time, the effects of the seeds' hydroalcoholic extract on five bacterial strains: Gram-positive and Gram-negative bacteria. Secondly, the study intends to investigate the antioxidant and anti-proliferative capacity of the seeds on two types of cancer cell lines: epithelial HT-29 and human colon HCT-116.

MATERIAL AND METHODS

Plant Collection and Powder Preparation

A. squamosa was collected from the South Lebanon during the period "November-January" 2016. The biological authentication was carried out by the Professor George Tohme, the president of the Lebanese C.N.R.S.

Fresh seeds obtained from the fruit 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 seeds were turned over repeatedly for homogeneous drying. After that, the dried seeds were grinded by a grinder. The obtained powder was then preserved in a container away from light, heat, and moisture for later use. Next, the powdered seeds (500 g) were placed in water-methanol (v/v) of pH 3.5 for 48 h with stirring at room temperature. The macerate was filtered to remove insoluble residues. Subsequently, the filtrate was condensed by evaporation, using a rotary evaporator, to eliminate the remaining methanol solvent. Finally, the filtrate was frozen before being a lyophilized powder to be processed ⁶.

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

Phytochemical screening

To study the chemical composition of water-methanol extract of the seeds of *A. squamosa*, qualitative detection of primary and secondary metabolites was performed according to Nasser et al. ⁷ (Table 1).

Total phenolic content (TPC)

The Folin–Ciocalteu reagent method was used to estimate of total phenolic quantities according to Farhan et al. ⁸ with minor modification. Briefly, 100 µL of seeds' 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 spectrophotometer. The results were expressed in mg of gallic acid equivalent (GAE) per g of dry weight of seeds' 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 the dilution factor and m is the weight (g) of the pure seeds 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. ⁹ to determine the TFC of the studied seeds with slight modification. 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 hour, 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 the 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 determinations was used according to Harborne ¹⁰. 100 mL of 10 % acetic acid in ethanol was added to 1 g of dry powdered seeds 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:

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

Total tannins determination

Tannins were determined by the Folin-Ciocalteu method ¹¹. 0.4 mL (10 mg/mL) of the water-methanol extract of the seeds was added to 2 mL of Folin-Ciocalteu reagent and 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

Seeds' powder (2 g) was put into a conical flask and 100 cm³ of ethanol (20%) were added. The samples were heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was then filtered and the residue re-extracted with another 200mL 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 samples were dried in the oven to a constant weight and the saponins' content was calculated ¹².

Saponin's content was calculated using the following formula:

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

Moisture content ¹³

Seeds' powders (1.5 g) were placed in an oven at 105 °C for 1 h. Then, it was placed in a dessiccator for half an hour. The mass of the content was recorded was returned 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 noticed again to calculate the percentage of humidity in these samples. All samples were done in triplicate.

$$\% \text{ Humidity} = \left[\frac{\text{Initial weight} - \text{final weight}}{\text{powder weight}} \right] \times 100$$

With:

$$\text{Initial weight} = \text{Sample weight} + \text{crucible weight (before heating)}$$

$$\text{Final weight} = \text{Sample weight} + \text{crucible weight (after heating)}$$

Proportion of ash

2 g of seeds' powder 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 weighted and the percentage of ash was estimated according to the essential dry weight of seeds' powder¹⁴.

$$\% \text{ Ash} = [\text{final weight} / \text{initial weight}] \times 100$$

$$\text{Initial weight} = \text{Sample weight} + \text{crucible weight}$$

(before heating).

$$\text{Final weight} = \text{Sample weight} + \text{crucible weight (after heating)}.$$

Mineral content

Acid digestion was performed to determine the minerals' content. 1 g of seeds' powder was put 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 left 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 "AOAC"¹⁵. 1 g of powdered seeds was placed into specific tubes of 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 done for 20 min at 100 °C. After cooling of 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 Aberoumand¹⁶. 2 g of powdered seeds 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 to evaporate the entire solvent. Finally, it was cooled in a dessiccator 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.^{17,18} 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 seeds 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 a blank. The DPPH scavenging ability of seeds' extracts was calculated using the following equation:

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

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 anti-proliferative activity of water-methanol seeds' extracts from the studied plant, cell culture was performed using 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 seeds' 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 membrane⁷.

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 of a patient who is hospitalized in the Mignot Hospital of Versailles, France¹⁹.

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 2007¹⁹⁻²¹. 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 its use.

MIC and MBC assays: seeds' extracts were tested for their 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)²². A concentration of 200 mg/mL of seeds' 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	Dragendorff reagent	Red-orange Precipitate
Tannins	FeCl ₃ (1%)	Blue color
Resines	Acetone + water	Turbidity
Saponines	Agitation	Formation of Foam
Phenols	FeCl ₃ (1%) + K ₃ (Fe(CN) ₆) (1%)	Blue-green
Terpenoids	Chloroform + H ₂ SO ₄ conc	Reddish brown
Flavonoids	KOH (50%)	Yellow color
Carbohydrates	α-naphtol + H ₂ SO ₄	Purple ring
Reducing sugar	Fehlings (A+B)	Brick red precipitate
Quinones	HCl conc	Yellow precipitate
Sterols et Steroids	Chloroform + H ₂ SO ₄ conc	Red (surface) + greenish yellow fluorescence
Cardiac glycosides	Glacial acetic acid + FeCl ₃ (5%) + H ₂ SO ₄ conc	Rings
Diterpenes	Copper acetate (or sulfate)	Emerald green
Anthraquinones	HCl (10%) + chloroform + Ammonia (10 %)	Pink color
Proteins & aminoacids	Ninhydrin 0.25%	Blue color
Lignines	Safranine	Pink color
Phlabetannins	HCl (1%)	Blue color
Anthocyanines	NaOH (10%)	Blue color
Flavanones	H ₂ SO ₄ conc	Purple red color
Fixed oils and fats	Spot Test	Oil stain

Table 2 : Chemical composition of the water-methanol extracts of the seeds of *A. squamosal*

	Water/methanol
Phenol	+
Terpenoids	+
Flavonoids	+
Carbohydrates	+
Quinones	+
Diterpenes	+
Proteins & aminoacids	+
Lignins	+
Flavonones	+
Fixed oil & Fat	+

Table 3: Quantification of some active compounds in the seeds

TPC	TFC	Tannin	Saponin	Lipid	Protein	Ash	Moisture
3.83 mg/g	12.17 mg/g	1.138 mg/g	1.21 %	35 %	0.0625 %	94 %	4.85 %

Table 4: Mineral values determined by Atomic Absorption Spectrometry

	Ca	Pb	Zn	Fe	Cu	Mn	Mg
mg/L	716.01	13.08	276.6	10.83	4.47	1.23	735.84
mg/g	5.012	0.092	1.936	0.0758	0.0313	0.0086	5.151

Table 5: Results of MIC and MBC for the water/methanol seeds extracts

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

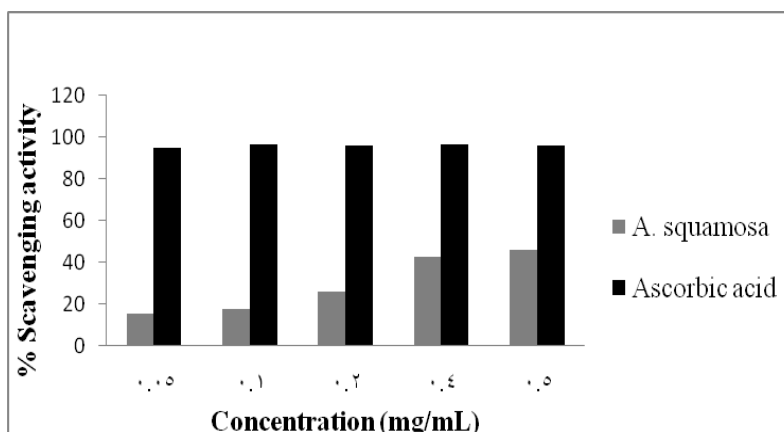


Figure 1: Antioxidant activity of the hydroalcoholic extract from seeds of *A. squamosa*

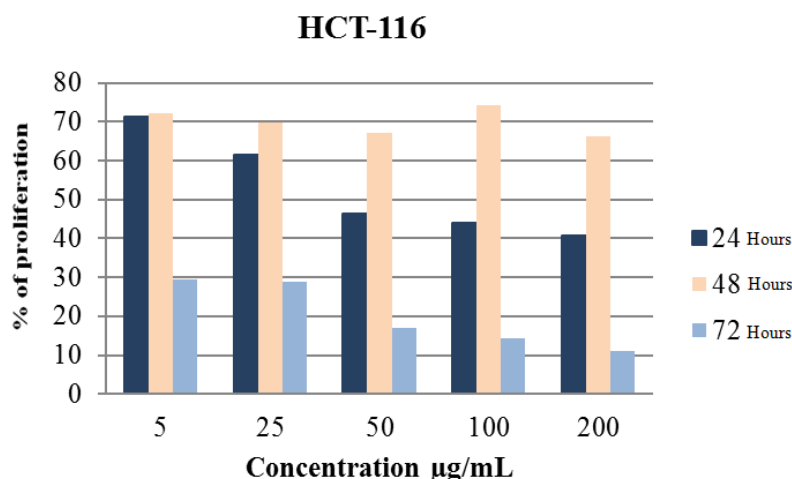


Figure 2: Percentage of proliferation of the HCT116 human colon cell according to the incubation time (h) and the concentration of the extract (µg/mL)

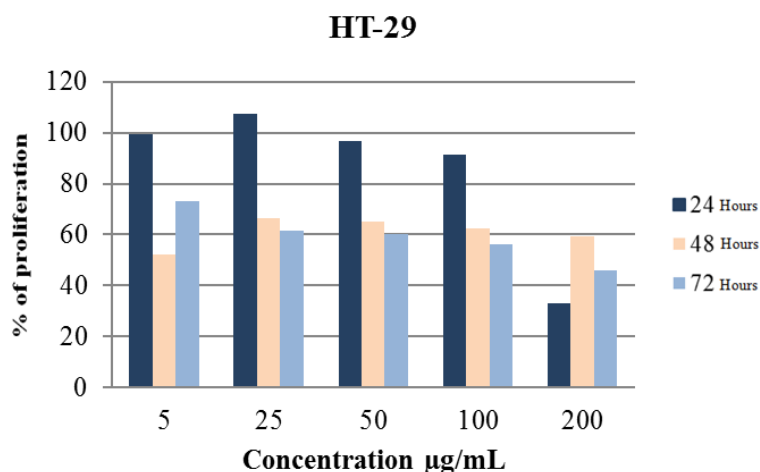


Figure 3: Percentages of proliferation of epithelial HT-29 cells according to the incubation time (h) and the concentration of the extract (µg/mL)

RESULTS AND DISCUSSION

Phytochemical screening (qualitative tests)

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

The results obtained by the phytochemical screening (Table 2) show that there are several primary and secondary metabolites in the hydroalcoholic extract from the seeds of *A. squamosa*. It can be noticed from these results that the seeds of this fruit are rich in secondary metabolites such as phenols, terpenoids, flavonoids, carbohydrates, quinones, diterpenes, proteins, amino acids, lignins, flavonones and oils.

Quantification of active compounds

Mineral content

The presence of minerals (Table 4) in the seeds of *A. squamosa* has been detected using the AAS technique. The calcium and magnesium contents show that the seeds of *A. squamosa* have a good nutritional value.

Biological properties

Antioxidant activity

Even if free radicals can possess some beneficial physiologic roles, an excess of those radicals along with unbalanced reactive biomolecules generates oxidative stress which is harmful for human beings. 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 cancer²³. 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 the seeds of Lebanese *A. squamosa* by evaluating its ability to scavenge free DPPH radicals. The obtained data showed that the concentrations of methanol/water extract and those of ascorbic acid presented a positive correlation with the DPPH test. And an increase in the antioxidant activity of the seeds was noticed from 15.3 % at the concentration 0.05 mg/mL till 46.11 % 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.

Anti-proliferative activity

The richness in phenolic and flavonoids compounds has been demonstrated by the phytochemical screening. The results obtained in the DPPH test validated those obtained by the screening of phenols. To determine the antiproliferative activity of the seeds of *A. squamosa*, the technique of yellow tetrazolium MTT was performed and the results obtained are presented in Figures 2 and 3.

For HCT-116 human colon cells at the concentration of 25 µg/mL of the water/methanol extract, the percentage of proliferation was much greater after an incubation period for 24 h (61.37%) than for 72 h (28.91%). In addition, the percentage of proliferation for 24, 48 and 72 h decreased with an increased concentration of the extract (Figure 2).

In addition, a correlation was noticed between the percentage of proliferation and the concentration used. It was noted that the proliferation decreased with increasing concentration for each incubation period.

On the other hand, for the epithelial cells HT-29, the same correlations were found. According to the Figure 3, the percentage of proliferation decreases with an increasing concentration of the seeds' extract and with the incubation period. For example, the cell proliferation percentages after 24, 48 and 72 h for an extract concentration 50 µg/mL were 96.77%, 65.21% and 60.02%, respectively, which are greater than those at a concentration 100 µg/mL which were 91.41%, 62.57% and 56.03%, respectively.

Previous studies have shown that the organic and aqueous extracts of the degreased seeds of *A. squamosa* on a rat histolytic tumor cell line AK-5, caused the death of apoptotic tumor cells with better caspase-3 activity and a negative regulation of the anti-apoptotic Bcl-2 and Bcl-x1 genes²⁴. These results showed that the seeds of this fruit exert an anti-proliferative effect depending on the concentration of the extract, and therefore the solvent used and the duration of the treatment.

Antibacterial activity

The method of micro-dilution in wells that is used for the determination of MIC led to the results presented in Table 5. These results showed that at a concentration 50 mg/mL of the hydroalcoholic extract from the seeds of *A. squamosa*, the growth of all the studied bacterial strains was totally inhibited. Thus, the MIC on *in vitro* growth of *S. aureus*, *E. faecalis*, *S. epidermidis*, *E. coli* and *P. aeruginosa* is 50 mg/mL. This growth inhibiting effect is therefore independent of the bacterial strain.

In addition, the results of this study also showed that the values of MBC were higher than those of MIC. They were two times greater for all bacterial strains. Based on these results, we can conclude that the hydroalcoholic extract from the seeds of this studied fruit can act as a bacteriostatic agent which can also exert a lethal effect in the most dangerous and sometimes deadly microorganisms.

CONCLUSION

The objectives of the present study were to determine the chemical composition of the hydroalcoholic extract from the seeds of Lebanese *Annona squamosa*, and to evaluate some of its pharmacological properties. Our results demonstrated for the

first time, that water/methanol extract of the seeds of *A. squamosa* 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). The presence of those biological activities may be referred to the presence of phenol and flavonoids in the studied seeds.

Further investigations are required to determine the specific compounds responsible for these biological activities and to describe the mechanism in which these seeds exert an anti-proliferative activity. On the other hand, other interesting activities such as the antibiofilm property of this fruit can be evaluated.

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REFERENCES

1. Doughari JH. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents; 2012; DOI: 10.5772/26052.
2. 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.
3. Deeb T, Knio K, Shinwari ZK, Kreydiyeh S, Baydoun E. Survey of medicinal plants currently used by herbalists in Lebanon. Pak J Bot 2013; 45: 543–55.
4. Freitag H, Maier-Stolte M. The genus Ephedra in NE Tropical Africa. Kew Bulletin 2003; 58: 415-26.
5. Gajalakshmi S, Divya R, Divya V, Deepika Mythili S, Sathivelu A. Pharmacological activities of *Annona squamosa*: a review. Int J Pharm Sci Rev Res 2011; 10(3): 24-9.
6. 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.
7. Nasser M, As-sadi F, Jaafar F, Kanaan H, Hijazi A, Chokr A, et al.. The antibacterial, antioxidant and antiproliferative activities of the hydroalcoholic extract of the Lebanese plant: "*Ephedra campylopoda*". Int Res J Pharm 2016; 7(12).
8. 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.
9. 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.
10. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis. 3rd ed: New Delhi: Springer Pvt. Ltd; 2005.
11. 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.
12. 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.
13. Prasanna K, Yuwvaranni 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.
14. 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.
 15. Helrich K. Official methods of analysis of the Association of Official Analytical Chemist "AOAC", 15th ed. Arlington, Virginia; 1990.
 16. 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.
 17. 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.
 18. 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.
 19. Chokr A, Watier D, Eleaume H, Pangon B, Ghnassia JC, Mack D, 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(6): 381-8.
 20. 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.
 21. Sadovskaya I, Chaignon P, Kogan G, Chokr A, Vinogradov E, Jabbouri S. Carbohydrate-containing components of biofilms produced *in vitro* by some staphylococcal strains related to orthopaedic prosthesis infections. FEMS Immunol Molecular Microbiol 2006; 47(1): 75-82.
 22. 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 (7th edn.) Document M7-A7. Wayne, PA: CLSI; 2006.
 23. Rahman T, Hosen I, Islam MMT, Shekhar HU. Oxidative stress and human health. Adv Biosci Biotechnol 2012; 3: 997-1019.
 24. Pardhasaradhi BV, Reddy M, Ali AM, Kumari AL, Khar A. Antitumour Activity of Annona Squamosa Seed Extracts Is through the Generation of Free Radicals and Induction of Apoptosis. Indian J Biochem Biophys 2004; 41(4):167-72.

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