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Research Article

Chemical Composition And Pharmacological Potential Of Green And Orange Lebanese *Citrus Aurantium* peels: A Comparative Study

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ABSTRACT

To discover the importance of the Lebanese *Citrus aurantium*, we first determined the chemical composition of the dried peels of the green and orange fruits. Second, we evaluated some of their *in vitro* pharmacological potentials. The antioxidant property of the hydroalcoholic extract was studied using the DPPH test. Then, the antiproliferative power was determined on different types of cancer cells and finally, the antibacterial ability was studied on five bacterial strains (three Gram positive and two Gram negative). The obtained results showed that the hydroalcoholic extract of both green and orange dried peels of *Citrus aurantium* contains different secondary metabolites such as alkaloids, phenols, flavonoids and coumarin. In addition, it showed an antioxidant activity due to their high content of polyphenols. It also presented an antiproliferative effect on two types of cancer cells: epithelial cell and HT-29 cell HCT-116 human colon and exerted an antibacterial effect against five bacterial strains. All these results show that peels of this fruit might be important for use in different business sectors such as the pharmaceutical, food and chemical industries.

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INTRODUCTION

Plant-derived antioxidants, especially polyphenolic compounds, have proven success in minimizing the levels of toxic free radicals and relieving different oxidative-stress mediated dieases. In addition, daily intake of natural antioxidants has been correlated with reduced occurrence of different diseases including cancer, diabetes and cardiovascular disease. Hence, the phenolics and flavonoids of various medicinal plants exhibit potent anti-inflammatory and anti-proliferative capacities (Sayed-ahmad ET AL., 2014; Farhan et al., 2013).

Lebanon is distinguished by a great wealth of plant species especially with medicinal properties. In fact, 2607 wild species of which 92 are endemics can be found in only 10452 km^2. For that, it will be very necessary to conduct scientific studies on these Lebanese plants, especially those used locally as medicine.

Citrus plants belonging to the family *Rutaceae* which includes fruits such as orange, mandarin, lime, lemon, sour orange and grapefruit are of multiple beneficial nutrients for human beings. It is native to tropical Asia but it is also found in all tropical and subtropical country. Global production of citrus fruit has significantly increased during the past few years and has reached 82 million tons in the years 2009–2010. Where oranges commercially the most important citrus fruit accounts for about 50 million tons and 34% of which was used for juice production, yielding about 44% peel as by-product (Li et al., 2006).

To valorize this by-product, and for the first time, we conducted a scientific research on the dried green and orange peels of the Lebanese *Citrus aurantium* L. in order to:

- Evaluate their chemical composition (alkaloids, polyphenols, flavonoids, saponins, tannins, total protein, total lipid, and minerals).
- Determine their total phenolic and total flavonoid contents.
- Investigate their antioxidant activity using an *in vitro* antioxidant test (DPPH).
- Estimate their antibacterial capacity on five bacteria stain.
- Evaluate their antiproliferative potential on different cancer cell lines.

Material and Methods

Plant collection and preparation of powders

C. aurantium was collected from Beirut. Fresh green and orange peels of the fruit were well washed, cut into small pieces and dried in the shade at room temperature, away from sun light. During the drying process, they were turned over to allow homogeneous drying. After this period, the dried peels were grinded by a grinder to obtain a powder form and then preserved in a container away from light, heat, and moisture for later use.

Powdered peels (300 g) were placed in methanol (50%) with a pH 3.5 and the mixuture was exposed to sonication by ultrasonic for 1 h at 60 °C (Hijazi *et al*., 2013). Then, the mixture was filtered to remove insoluble residues. Subsequently, the filtrate was condensed by evaporating to eliminate the methanol using a rotary evaporator. Finally, the filtrate was frozen before being lyophilized into powder to be processed.

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

Phytochemical Screening (Qualitative Tests)

To study the chemical composition of water-methanol extracts of *C. aurantium* green and orange peels, qualitative detection of primary and secondary metabolites was performed according to Nasser et al., (2017) (Table 1).

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

Metabolites	**Added reagent**	**Expected result**
Alkaloïds	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
Terpenoïds	Chloroform + H_2SO_4 conc	Reddish brown
Flavonoïds	KOH (50%)	Yellow color
Carbohydrates	α-naphtol + H_2SO_4	Purple ring
Reducing sugar	Fehlings (A+B)	Brick red precipitate
Quinones	HCl conc	Yellow precipitate
Sterols et Steroïds	Chloroform + H_2SO_4 conc	Red (surface) + greenish yellow fluorescence
Cardiac glycosides	Glacial acetic acid + $FeCl_3$ (5%) + H_2SO_4 conc	Rings
Diterpenes	Copper acetate (or sulfate)	Emerald green color
Anthraquinones	HCl (10%) + Chloroform + Ammonia (10 %)	Pink color
Proteins & aminoacids	Ninhydrin 0.25%	Blue color
Lignines	Safranine	Pink color
Phlabotannins	HCl (1%)	Blue color
Anthocyanines	NaOH (10%)	Blue color
Flavanones	H_2SO_4 conc	Purple red color
Fixed oils and fats	Spot Test	Oil stain

Quantitative Tests

Total Penolic Content (TPC)

The Folin–Ciocalteau reagent method has been used for the estimation of total phenol according to (Farhan *et al*., 2012). Briefly, 100 µL of green and orange dried peels hydroalcoholic extract have been taken and mixed with 1 mL of Folin–Ciocalteau reagent (1/10 dilution in water). After 5 min, 1.5 mL of Na_2CO_3 2% (w/v) has been added. The blend was incubated in the dark at room temperature for 30 min. The absorbance of blue-colored solution of 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.

Total phenol content = GAE x V x D /W,

Where **GAE** is the gallic acid equivalence (mg/mL), **V** is the volume of the extract (mL), **D** is dilution factor and **W** is the weight (g) of the plant extract.

The blank was formed by 0.5 mL water-MeOH and 1.5 mL of Na_2CO_3 (2%).

Total Flavonoids Content (TFC)

The aluminum chloride method was used according to (Quettier-deleu *et al*., 2000) for the determination of TFC. 1 mL of water-methanol extract was mixed with 1 mL of methanolic aluminum chloride solution (2 %). After an incubation period for 1 h at room temperature, in the dark, 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).

Flavonoids content = RE×V×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 has been used according to (Harborne, 2000). 100 mL of 10 % acetic acid in ethanol was added to 1 g of dry powdered peels and then covered to be stand for 4 h. After that, the extract has been filtrated and concentrated on a water bath to 25 mL of its original volume. Droplets of concentrated ammonium hydroxide were added to the extract until the precipitation of the whole solution, and then the precipitates were 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:

% Alkaloid = [final weight of the sample / initial weight of the extract] × 100.

Total Tannins Determination

Tannins were determined by the Folin-Ciocalteau method (Singh et al., 2012). 0.4 mL (10 mg/mL) of the water-methanol extract of peels was added to 2 mL of Folin-Ciocalteau reagent and 4 mL of Na_2CO_3 (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 manner as described previously. The absorbance of the test and standard solutions was measured at 765 nm with a UV-Visible spectrophotometer. Blank was formed by 0.5 mL water-MeOH and 1 mL Na_2CO_3 (35%). The tannin content was expressed in mg GAE / g of extract.

Total Saponin Determination

Peels powder (20 g) was put into a conical flask and 100 cm^3 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 filtered and the residue 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 and 20 mL of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while 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; the saponin content was calculated (Obdoni & Ochuko, 2001).

Saponin content was calculated using the following formula:

% Saponin = [final weight of sample / initial weight of extracts] × 100

Moisture Content

Peels (1.5 g) have been taken and placed in an oven at 105 °C for 1 h. Then, they were putted in a desicator for half hour. After that, the mass of the content has been noticed. Then, they were returned again to the oven for another 1 h. After heating, they were putted again in the desiccators for half hour. These steps have led to dry powders and their mass has been noticed again in order to calculate the percentage of humidity in these samples. All samples have been done in triplicate (Prasanna & Yuwvaranni, 2014).

% Humidity = [(Initial weight – final weight)/ powder weight] ×100

With:

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

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

Proportion of Ash

Peels powder (2 g) were putted in and burned in a furnace burning (muffle furnace) at 550 °C for 5 h till the obtaining of an ovary gray color of the powders. Then, the residues have been weighted and the percentage of ash has been estimated according the essential dry weight of plant powder (Manjulika *et al.,* 2014).

% Ash = [final weight / initial weight] ×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. 1 g of peels powder was put in the oven at 80 ˚C for 24 h. Then, 10 mL of concentrated HCl was added at 80 ˚C with stirring. The beaker was covered. From time to time, drops of H_2O_2 (35 %) were added. The beaker was left to warm for 15 h. After evaporation of HCl, 10 mL of HNO_3 were added. Vacuum filtration was performed to the obtained mixture followed by filtration by syringe.

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

Total Protein

Proteins were determined using the method of "AOAC" (Helrich, 1990). 1 g of powdered peels was placed in specific tubes of 500 ml with catalyst (containing 5 g of K_2SO_4 and 0.25 g of $CuSO_4$). 12-15 mL of H_2SO_4 (96-98%) and 10 mL of H_2O_2 (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 NH_3 was captured in a Erlenmeyer flask containing 25 ml of boric acid (4%). Titration of ammonium ion was made using a solution of H_2SO_4 (0.1M) in the presence of 3-5 drops of Tashiro indicator. The protein content is calculated by multiplying the mineral nitrogen content by 6.25.

Protein content = 6.25 x Volume H_2SO_4

Total Lipids

Total lipids are evaluated according to the method described by (Aberoumand, 2010). 2 g of peels powder were extracted by Soxhlet apparatus containing petroleum ether (bp: 40-60 ºC) till the extraction of total lipids. After that, the extract was poured into a beaker then sitted in the oven at 100 °C to evaporate the whole solvent then cooled in a dessiccator and weighed.

% Lipids = [lipid weight / powder weight] ×100

Evaluation of the Antioxidant Activity by DPPH assay

The method of (Rammal et *al*., 2012) has been used for the scavenging ability of DPPH antioxidant test. 1 mL of different concentrations (0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL) of diluted extracts of the peels was added to 1 mL of DPPH (0.15 mM in methanol) and at the same time, a control consisting on 1 mL DPPH with 1 mL methanol was prepared. The reaction mixtures were mixed very well by hand, and then incubated in the dark at room temperature for 30 min then 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:

***% scavenging* activity = [(Abs control − Abs sample)]/ (Abs control)] ×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 Antiproliferative Activity

To study the antiproliferative activity of water-methanol peels extract from the studied plant, cell culture was performed using epithelial cells HT-29 and HCT-46 cells of the human colon. Then a measure of inhibition of cell proliferation by the yellow tetrazolium MTT technique was applied.

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 peels extracts were diluted with the DMEM culture medium at decreasing concentrations (200, 100, 50, 25 and 5 µg/mL) and were then added to the wells after pre-incubation of the cells for 24 h. The plates were then incubated under 5% CO_2 and at a temperature of 37 ° C during 24, 48 and 72 hours.

After incubation, 10 µL of MTT solution have been added per well and incubated for 3 h at 37 °C. Then a 100 µL solubilization solution was added to each well. Finally reading 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 is isolated from an infected implanted device of a patient who is hospitalized in the Mignot Hospital of Versailles, France (Chokr *et al.,* 2006). The other strains are ATCC. The latter 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), in which they were prepared and then autoclaved as indicated by the manufacturer before their use.

MIC and MBC assays: extracts of plant peels 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) (Wikler *et al*., 2006). A concentration of 3 mg/mL of peels 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 5×10^5 bacteria/mL. After incubating the plates at 37°C for 24 h, 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.

RESULTS AND DISCUSSION

In order to determine the composition of the hydroalcoholic extract prepared from the green and orange peels of *C. aurantium*, a classical phytochemical screening was performed, as several studies highlighted the positive correlation between the chemical composition of medicinal plants and their medical uses.

Phytochemical Screening

The results of the phytochemical screening shown in Table 2 showed that peels of *C. aurantium* are rich in various secondary metabolites.

Table 2: Composition of the hydroalcoholic extract of the dried green and orange peels of Citrus aurantium

	Green peels	Orange peels
Alkaloids	+	+
Tannin	+	-
Resines	+	-
Coumarines	+	
Saponins	_	+
Phenols	+	+
Terpenoids	+	+
Volatile Oil	+	+
Flavonoids	+	+
Carbohydrates	+	+
Quinons	+	+
Phytosterols	+	+
Steroids	+	+
Cardiac glycosides	+	-
Diterpenes	+	+
Anthraquinones	+	-
Protein and amino acid	+	-

Phlabotannins	+	-
Anthocyanins	_	-
Flavanones	+	+
Fixed oils and fats	+	+
Carotenoids	+	+

From the above observation, we notice the presence of alkaloids, resins, coumarins, phenols, terpenoids, volatile oil, flavonoids, quinons, steroids, cardiac glycosides, diterpene, anthraquinons, phlabotannins, anthocyanins, flavanones, fixed oils and fats and carotenoids in water/methanolic extract. Consequently, peels of *C. aurantium* by its richness in various secondary metabolites may have several medical approaches such as:

- Analgesic thanks to the presence of alkaloids (Khader *et al*., 2010).
- Anti tumor especially due to the presence of flavonoids (Kanadaswami *et al*., 2005).
- The presence of coumarin gives important pharmacological activities such as anti-inflammatory, cytotoxic, antitumoral, anti-osteoporotic, antiallergic, regulator of cardiac oxidative stress and lipid metabolism (Witaicenis et al., 2014).
- Antioxidant due to its richness in phenolic compounds that are considered free radical scavengers helping in the prevention of several diseases of aging.
- Antimicrobial and antiinflammatory effects due to the presence of diterpene.
- Control of cholesterol ester accumulation that lead for heart disease risk can be thanks to the presence of phenolic compounds (Meyer *et al*., 1997).
- Heart beats regulation and heart failure treating by lowering blood pressure thanks to the presence of cardiac glycosides.

Quantitative Tests

The TPC, TFC, alkaloids, lipids, protein, ash and humidity of the green and organge dried peels of *Citrus aurantium* were quantified and the results are presented in Table 3. These results showed that green peels are richer in phenols, alkaloids, lipids and protein than orange peels that have a higher content in flavonoids.

Table 3: Quantitaive tests of dried green and orange peels of Citrus aurantium

	TPC mg/g	TFC mg/g	Alcaloid	Humidity	Ash	Lipids	Protein
Orange peels	16.08	83.7	5.70 %	0 %	0 %	5 %	8.5 %
Green peels	26.4	28.1	7.4 %	0.43 %	5.6 %	40 %	8.8 %

Determination of Mineral Content

The presence of minerals (Table 4) in the dried peels of *C. aurantium* has been detected using the atomic absorption spectrophotometer technique.

Table 4: Mineral values in the extract obtained by Atomic Absorption Spectrometry

	Ca	Mg	Pb	Cr	Zn	Fe	Mn
mg/L	33.5	40.05	0.83	1.04	9.9	92.06	0.65
mg/g	0.67	0.801	0.0166	0.0208	0.198	1.8412	0.013

Antioxidant Activity

Even if free radicals can possess beneficial physiologic roles, excess of them and reactive biomolecules that cannot be balanced or destroyed generates oxidative stress which is harmful for humans since 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 cancer (Rahman *et al*., 2012). Therefore, identifying plants with potent antioxidant capacity is therefore of great interest. In our present study, we evaluated the antioxidant activity of the water/metahnol extracts from dried green and orange peels of *C. aurantium* grown in Lebanon by evaluating their ability to scavenge free DPPH radicals. Our obtained results showed that the concentrations of methanol/water extract presented a positive correlation with the DPPH test. There was an increase of the antioxidant activity of peels of *C. aurantium* with the increased concentration. It was increased from 8 % at the concentration 0.05 mg/mL till 84 % at the concentration 0.5 mg/mL (Figure 1). Our results indicated that orange peels exerted higher antioxidant capacity at different concentrations reaching the 88 % at the concentration 0.5 mg/mL.

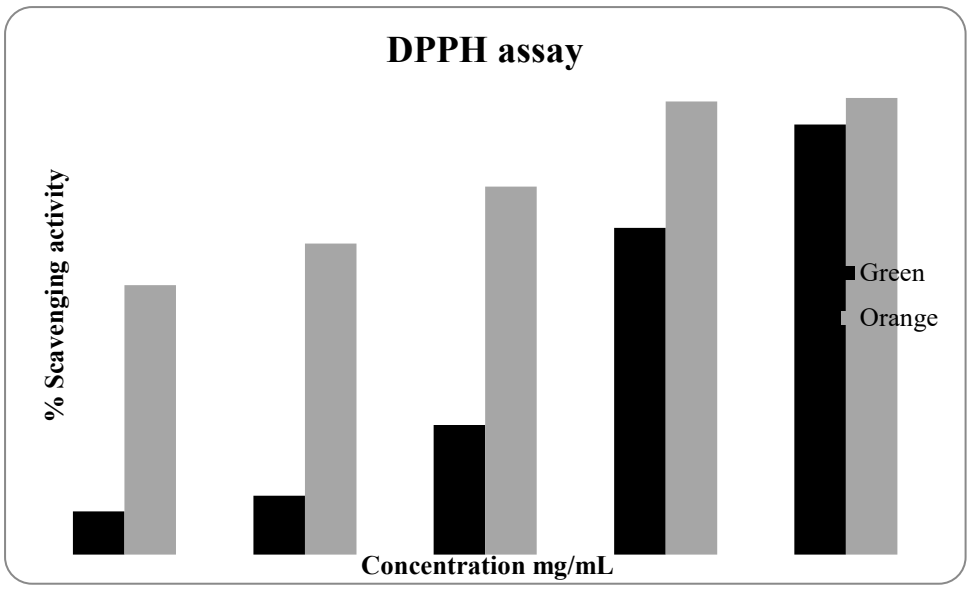

Figure 1: Antioxidant activity of the hydroalcoholic extract from the peels of C. aurantium

Antiproliferative Activity

The richness of *C. aurantium* peels in phenols 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 dried peels of *C. aurantium*, the technique of yellow tetrazolium MTT was performed and the results obtained are presented in Figures 2 and 3.

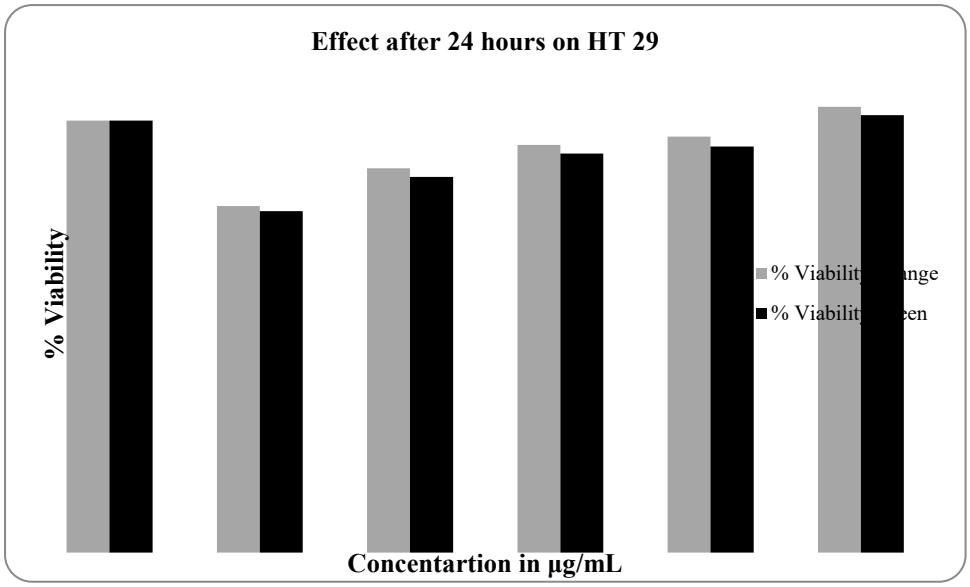

Figure 2: Effect of Citrus peel extract on HT29 cell

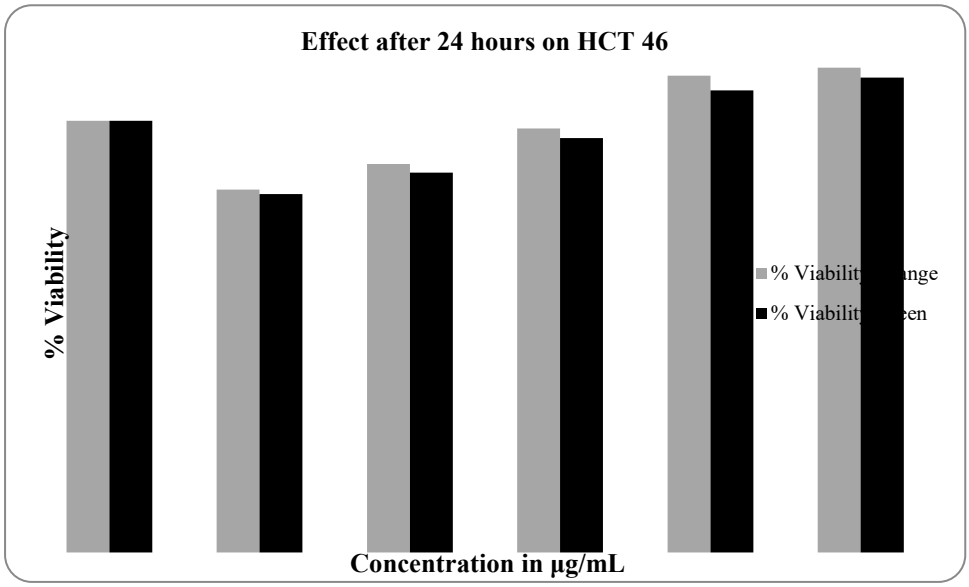

Figure 3: Effect of Citrus peel extract on HCT46 cell

The obtained results indicated that at low doses (5 and 25 µg/mL) the % of viability of cancer cells decreased. However at high doses this percetange was increased. Based on these observations, we can conclude that there is no significant impact of orange and green peels of C. aurantium of these types of cancer cells.

Antibacterial Activity

The antibacterial activity could be classified as bactericidal (MBC / MIC < 2) or bacteriostatic (MBC very far from the MIC). In order to evaluate our results, the MBC / MIC ratio calculation was done. Our results showed that this ratio was around 2, indicating that their effect is bactericidal.

The activity of a plant extract depends on several factors including the concentration of active ingredients. Our results obtained throughout the phytochemical screening done on the dried orange and green peels of this plant followed by the TPC and TFC determination indicated the prensence of good amount of phenols and flavonoids. And it was

demonstrated that the polyphenolic compounds as flavonoids have a remarkable antibacterial effect as indicated in a previous research (Thangaraj et al., 2000).

Our obtained results showed for the first time, that orange peels exerted more antibacterial activity because of inhibition of bacterial growth. This result reveals the higher sensitivity of *S. epidermidis*, which is Gram-positive, againt the peels extract (Table 5).

Table 5: Antibacterial activity of the peels pf C. aurantium.

Plant	MIC (mg/mL)					MBC (mg/mL)				
	S. aureus	*E. faecalis*	*S. epidermidis*	*E. coli*	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*S. epidermidis*	*E. coli*	*P. aeruginosa*
Green	1.5	0.75	0.38	0.75	0.75	>1.5	1.5	0.75	1.5	1.5
Orange	1.3	0.68	0.32	0.65	0.65	>1.5	1.1	0.7	1.2	1.2

CONCLUSION AND PERSPECTIVES

Our study indicated that Lebanese *Citrus aurantium* peels are a potential natural source of phenols, falvonoids, tannins among others. The antibacterial results showed that the extract of the citrus peels has MIC and MBC effects on five bacterial strains (*S. aureus, E. faecalis, S. epidermidis, E. coli, P. aeruginosa*). In addition to that, high antioxidant activity 88% showed that *C. aurantium* contributes in the prevention of oxidation as antioxidants and free radical scavengers.

For anticancer activity, we obtained slight effect. In order to obtain a significant anticancer result we can perform isolation of molecules that might have anti-cancer potential effect after being incubated for 48 and 72 hours.

Thus, this species can be used as a potential source of drugs against various diseases and can be introduced in the food sector as natural preservatives and antioxidants. This research may be of interest from a functional point of view and for the valorization of *Citrus aurantium* in Lebanon and the wider Mediterranean region.

REFERENCES

1. Aberoumand A . A comparative study on composition analysis of plants consumed in India and Iran. American-Eurasian Journal of Agriculture & Environnmental Sciences, 2010, 8: 3329-333.
2. Bandar H., Hijazi A., Rammal H., Hachem A., Saad Z., Badran B. Techniques for the Extraction of Bioactive Compounds from Lebanese *Urtica dioica.* American Journal of Phytomedicine and Clinical Therapeutics 2013, 1(6): 507-513
3. Chokr A., Watier D., Eleaume H., Pangon B., Ghnassia JC, Mack D., Jabbouri S. Correlation between biofilm formation and production of polysaccharide intercellular adhesin in clinical isolates of coagulase-negative staphylococci. International Journal of Medical Microbiology, 2006, 296: 381-388.
4. Farhan H., Rammal H., Hijazi A., Daher A., Reda M., Annan H., Chokr A., Badran B. Chemical composition and antioxidant activity of a Lebanese plant *Euphorbia macroclada schyzoceras.* Asian Pacific Journal of Tropical Biomedicine 2013, 3(7): 542-548.
5. 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*. Annals of Biological Research, 2012, 3 (1):149-156

6. Harborne J.B. and Williams C.A. Advances in flavonoid research since 1992. Phytochemistry 2000 55: 481-504.
7. Helrich K. Official methods of analysis of the Association of Official Analytical Chemist "AOAC", 15th edition. Arlington, Virginia,1990.
8. Kanadaswami C., Lee L.T., Lee P.P.H., Hwang J.J., Ke F.C., Huang Y.T., Lee M.T. The antitumor activities of flavonoids. In vivo, 2005, 19(5), 895-909
9. Khader M., Bresgen N., Eckl P.M. Antimutagenic effects of ethanolic extracts from selected Palestinian medicinal plants. Journal of ethnopharmacology, 2010, 127(2), 319-324.
10. Li J., Melenhorst J., Hensel N., Rezvani K., Sconocchia G., Kilical Y., Hou J., Curfman B., Major E., Barrett AJ. T-cell responses to peptide fragments of the BK virus T antigen: implications for cross-reactivity of immune response to JC virus. Journal of General Virology, 2006, 87(10):2951-2960
11. Manjulika Y., Sanjukta C., Sharad K.G., Geeta W. Preliminary Phytochemical Screening of Six Medicinal Plants Used In Traditional Medicine. International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(5): 539-42.
12. Meyer A., Yi O., Pearson D., Waterhouse A.L., Frankel E. Inhibition of human low-density lipoproteins oxidation in relation to phenolic antioxidants in grapes. Journal of Agricultural and Food Chemistry, 1997, 43: 1638– 1643.
13. Nasser M., As-sadi F., Jaafar F., Kanaan H., Hijazi A., Chokr A., Rammal H. Antibacterial, Antioxidant and Antiproliferative activities of the hydroalcoholic extract of the Lebanese plant: "*Ephedra campylopoda"* International Research Journal of Pharmacy, 2017, 7(12): 23-29.
14. Obdoni B. and Ochuko P. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta States of Nigeria. Global Journal of Pure and Applied Sciences, 2001, 8: 203–208.
15. Prasanna K. and Yuwvaranni S. Preliminary Phytochemical Screening and Antibacterial activity of *Datura metel* And *Vitex negundo* Against Bacterial Cold Water Disease Causing Organism. International Journal of Pharmacy and Pharmaceutical Sciences, 2014, 6(5): 230-3.
16. Quettier-Deleu C., Gressier B., Vasseur J., Dine T., Brunet C., Luyckx M., Cazin M., Cazin J. C., Baileul F., Trotin F. Phenolic compounds and antioxidant activities of buckwheat (*Fagopyrum esculentum* Moench) hulls and flour. Journal of Ethnopharmacology, 2000, 72: 35-42.
17. Rahman T., Hosen I., Islam M.M.T., Shekhar H.U. Oxidative stress and human health. Advances in Bioscience and Biotechnology, 2012, 3: 997–1019
18. Rammal H., Farhan H., Malli F., Hijazi A., Bassal A., Ajouz N., Badran B. Phytochemical screening and antioxidant activity of Lebanese *Eryngium creticum* L. Asian Pacific Journal of Tropical Biomedicine, 2012, 2(3): S1217-S1220
19. Sayed-ahmad B., Hijazi A., Kazan H.F., Rammal H., Kobeissy A., Badran B., Saad Z. Extraction, phytochemical screening, chemical quantification and identification of bioactive compounds from Lebanese *Urtica dioica*. American Journal of PharmTech Research 2014; 4(2): 592-604.
20. Singh R., Pawan Kumar V., Singh G. Total phenolic, flavonoids and tannin contents in different extract of *Artemisia absinthium*. Journal of Intercultural Ethnopharmacology, 2012, 1(2): 101-104.
21. Thangaraj H.S., Adjei O., Allen B.W., Portaels F., Evans M.R.W., Banerjee D.K., Wansbrough-Jones M. H. In-vitro activity of ciprofloxacine, sparfloxacine, ofloxacin, amicacin and rifamoicin against Ghania isolates of *Mycobacterium ulcerans.* Journal Antimicrobial Agent Chemoterapy, 2000, 45(12): 231-233.
22. Wikler M.A., Low D.E., Cockerill F.R., Sheehan D.J., Craig W.A., Tenover FC, et al.

(2006) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard (7thedn.) Document M7-A7. Wayne, PA: CLSI 2006.

23. Witaicenis A., Seito L.N., da Silveira Chagas A., de Almeida Junior L.D., Luchini A.C., Rodrigues-Orsi P., Cestari S.H., Di Stasi L.C. Antioxidant and intestinal anti-inflammatory effects of plant-derived coumarin derivatives. Phytomedicine, 2014, 21: 240–246

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