Research Article

Biological Activities of Total Oligomeric Flavonoids Enriched Extracts of *Nicotiana tabacum* from Eight Lebanese Regions

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Received: 10-01-2020; **Revised:** 18-02-2020; **Accepted:** 25-02-2020.

ABSTRACT

Secondary metabolites from tobacco exhibit good biological activities. Therefore, in the present study, the total phenolic (TPC) and flavonoids (TFC) content, the antioxidant and antitumor properties of the total oligomeric flavonoids (TOF) enriched extracts from Nicotiana tabacum L. were evaluated. Eight samples of tobacco leaves grown in different regions of Lebanon were the subject in this work. The total phenolic content was determined by the Folin–Ciocalteu assay, while total flavonoid content was determined by aluminium chloride colorimetric assay. The antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH•). To investigate the antitumor activity, TOF extracts were evaluated on breast adenocarcinoma MDA-MB-231 cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cytotoxicity assay. The TOF samples showed significant variations in the chemical composition and in the biological activities. Thereby, N. tabacum TOF extracts varies significantly and rely on the geographic and plant origin of leaves collection. These findings require further investigations on this plant; therefore, TOF extracts must be tested on other cancer cell-lines. As well as much works remains to determine the active principles and their mode of action.

Keywords: *Nicotiana tabacum*, Total Oligomeric Flavonoids, Antioxidant, Antitumor, Total phenolic content.

INTRODUCTION

Cancer, which remains a fatal disease, is still an alarming condition with high mortality and poor prognosis. Numerous studies have provided evidence that elevated levels of reactive oxygen species (ROS) and alteration in the redox balance are one of the common hallmarks of cancer progression and resistance to treatment[1]. In normal cells, ROS, by-products of normal cellular metabolism, are generated in a highly regulated manner at low concentrations and serve as signaling molecules to regulate numerous physiological and biological responses, including proliferation, cell division and immune regulation[2]. However, the uncontrolled generation of these oxidants can lead to oxidative stress, a harmful process that can cause damage to lipids, proteins, and DNA, promoting genomic instability and tumorigenesis[3].

To neutralize the toxic effect of these bioactive molecules, the human body will defend itself by synthesizing antioxidants or intake of food or supplements that contain antioxidants[4]. Thus, identifying exogenous sources of antioxidants molecules is therefore of great importance. Historically, plant-derived antioxidants, especially flavonoids and polyphenolic compounds, have proven success in minimizing the levels of toxic free radicals and treating oxidative stress-related disorders[5,6].

As part of the evaluation of Mediterranean endemic species, we were interested in known species, among these species the Lebanese *Nicotiana tabacum*. Commonly known as tobacco, *Nicotiana tabacum* is a perennial herbaceous plant native of tropical and subtropical America but it is now commercially cultivated worldwide[7]. In Lebanon, Tobacco is a very important economic crop; Tobacco leaf production in Lebanon was about eight thousand tons in 2015 and approximately 25,000 families benefit from the production of tobacco and tunbac[8]. Hence its importance in the Lebanese economy and society.

It was found that over 20 % of tobacco resources are discarded as processing waste[9] since only leaves are used. The discarded tobacco leaves are economically valuable because of their content in bioactive compounds, such as polyphenols, proteins and aromatic compounds[10,11]. Pharmacological studies have shown that the constituents of *N. tabacum* have potential biological activities. For instance, Wang *et al.*[12] identified the polyphenols in tobacco leaves and their antioxidant and antimicrobial activities were also investigated. Furthermore, tobacco was also used in plant biotechnology as a host system for pharmaceutical recombinant protein production such as recombinant subunit vaccines, monoclonal antibodies and therapeutic cytokines; in response to the Ebola outbreaks in 2014 and 2015, efforts using tobacco have resulted in an experimental drug named ZMapp consisting of three monoclonal antibodies[13].

Secondary metabolites in general are extremely dependent on weather conditions[14], since they are a

plastic adaptive response of plants to their environment. Such chemical interaction often includes variations in the production of plant metabolites[15]. In particular, the environmental parameters were found to influence the growth and the chemical composition in *Nicotiana tabacum*[16–20]. Lebanese climate is classified as typically Mediterranean. It presents, however, some specific aspects resulting from the country's location and physiography. This results in the individualization of six Bioclimatic levels: arid, semi-arid, subhumid, humid, prehumid and oromediterranean, all are remarkable by their variability over short distances[21].

However, to date, antioxidant and antitumor activity and chemical analysis of *N. tabacum* in Lebanon are not yet studied. The purpose of the present study was to quantify the total polyphenols and flavonoids contents by the colorimetric methods Folin–Ciocalteu and aluminium chloride respectively, of TOF extracts of *N. tabacum* from eight different regions in Lebanon. Then the antioxidant activity of TOF extracts was assayed by radical scavenging assay and their cytotoxic effect against MDA-MB-231 cells, an epithelial human breast cancer *cell* line using the MTT assay. The class of tobacco studied was the original Saada Six. That was named after the Regie's Saadiyat Laboratory. Developed with a blend of Bulgarian and Azmirly tobacco, planting commenced in 1973[8].

MATERIALS AND METHODS

Materials and reagents

All of the chemicals used were of analytical grade. Methanol, chloroform, Folin-ciocalteu, ascorbic acid and aluminum chloride hexahydrate were purchased from BDH (England). 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Rutin and sodium carbonate anhydrous were purchased from Sigma Aldrich (USA). Samples were weighed using an analytical and numerical balance (Melter Toledo). The dried leaves were grinded using a POLYMIX (PX-MFC 90 D) grind mill. The absorbance values of the solutions were measured using a VWR UV-6300PC double beam spectrophotometer and the extracts were concentrated using HEIDOLPH rotavapor apparatus.

Plant material

The *N. tabacum* leaves, grown at the same periods (3 months) were the target sample materials. They were harvested in September 2018 from 8 villages that cover the main crop areas, different heights and the 3 regions of Lebanon (North, South and Bekaa). The tobacco leaves were grounded into powder using a mill.

Extraction of T*otal Oligomeric flavonoids* (*TOF*)

Solid-liquid extraction followed by a series of liquid-liquid extractions to share the constituents of the crude extract in fractions of different polarities between two immiscible liquid phases. The fresh leaves of *N. tabacum*, were dried at room temperature and reduced to powder using a mill. In order to obtain the Total Oligomers Flavonoids (TOF), 20 g of Leaves powder were macerated in water/acetone mixture (1v/2v), for 24 h with magnetic stirring. The crude acetonic extracts obtained were filtered using Whatman paper, then the supernatant was collected and acetone was evaporated under reduced pressure at 45°C to obtain the aqueous phase. Condensed tannins were partially removed by precipitation with an excess of sodium chloride (NaCl) during 30 min at room temperature, and the supernatant was recovered after filtration. The latter was extracted with 3 x 10 mL ethyl acetate, concentrated, and precipitated with chloroform (volume equal to 5 volumes of the extract residues). After magnetic stirring for 5 minutes, the samples were left under the hood for 24 hours to remove chloroform.

The resulting precipitate was separated and yielded the TOF extract, which was dissolved in suitable volumes of methanol to a concentration of 2 $g.L^{-1}$ and stored in the refrigerator at 4 °C until use.

Determination of Percentage Yield (%)

The extraction yield was calculated according to the following equation (1):

$$\textbf{Yield}\ \% = \frac{W_2}{W_1} . 100 \quad \text{(equation 1)}$$

Where W_1 is the dry weight of the used material and W_2 is the weight of collected extract after evaporation of solvent.

Phytochemical investigations

Determination of total phenolic content (TPC) in tobacco leaves

Total phenolic content (TPC): The phenolic compounds present in the TOF extracts were determined according to the of Folin–Ciocalteu method[22]. The protocol used was based on Singleton and Rossi method[23], with a slight modification. Briefly, an aliquot (20 µL) of tobacco extract was mixed with 0.1 µL of Folin–Ciocalteu reagent in a test tube, followed by 1.5 mL of distilled water. After 5 min, 300 µL of Na_2CO_3 (7.5% w/v) were added. Subsequently the mixture was stirred and incubated at room temperature for 45 min in the dark. The absorbance was measured at 760 nm using a dual-beam spectrophotometer. The TPC in the extract was extrapolated from the calibration curve derived by repeating the same operating conditions for methanolic solutions of Gallic acid (3-300 $\mu g.L^{-1}$). The results were expressed in mg of Gallic acid equivalent (GAE) per gram of dry weight of plant powder, according to the following equation (2). All data were recorded as mean ± SD for three replicates.

Total Phenolic Content= GAE x V x DF/m (equation 2)

Where GAE is the Gallic acid equivalent ($g.mL^{-1}$); V is the volume extract (mL); DF is the dilution factor; and m is the weight (g) of the pure plant extract.

Determination of total flavonoids content (TFC)

The Total flavonoid contents of TOF extract was determined using aluminum chloride spectrophotometric method[24]. Briefly, 1 mL of extract solution and the same volume of 10 % aluminium chloride ($AlCl_3$) methanolic solution were mixed. The mixture was allowed to stand for one hour at room temperature. The absorbance was measured at 415 nm. The blank sample consisted of 1 mL of extract solution in 1 mL methanol without $AlCl_3$. Rutin solutions of various concentrations in methanol (10-160 $\mu g.mL^{-1}$) were used to derive the standard calibration curve. The data of the TFC were expressed as milligrams of Rutin equivalent per gram of plant's dry weight (mg RE/g d w). All data were recorded as mean ± SD for three replicates.

Biological investigations

Antioxidant capacity

The antioxidant activity of TOF extracts of *N. tabacum* leaves was determined using the traditional method of Blois[25], Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, with slight adjustment. First, the extracts were prepared by dissolving 10 mg of each sample in 5 mL of methanol in order to obtain a concentration of 2 $g.L^{-1}$. A stock solution of DPPH (32 $mg.L^{-1}$) was prepared in methanol. The reaction mixture consisted of 1 mL of diluted methanolic extract and 1 mL of DPPH solution. The mixture was incubated in the dark at room temperature for 30 min, then the absorbance was read at 520 nm, using a corresponding blank prepared by adding 1 mL of methanol instead of the extract solution in the reaction mixture. Ascorbic acid (2.7 - 54 $\mu g.mL^{-1}$) was used as a reference standard. All the reaction mixtures were carried out in triplicates. Absorbance was measured using UV-Vis spectrophotometer. The percentage inhibition and IC_{50} were calculated using equation (3). All data were recorded as mean ± SD for three replicates.

$$DPPH\ scavenged\ (\%) = (A_{DPPH} - A_{sample})/A_{DPPH} \times 100 \quad \text{(equation 3)}$$

A_{DPPH} is the absorbance of the blank control; A_{sample} is the absorbance of the samples (extracts or ascorbic acid).

Determination of antitumor activity

a-. **Cell viability**

The viability of MDA-MB-231 cells obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2-5 diphenyl tetrazoliumbromide) assay. MTT is reduced intracellularly in a mitochondrion-dependent reaction to yield insoluble formazan crystals. The ability of cells to reduce MTT indicates mitochondrial activity and serves as a measure of cell viability. Briefly, MDA-MB-231 cells, an epithelial human breast cancer *cell* line, were seeded in 96-well plates (5×103 cells/well). The following day, cells were treated with the different extracts at concentrations ranging from 25 to 250 $\mu g.mL^{-1}$ for 24 and 48h. Every 24 h, 10 µL of MTT solution (Sigma) was added to each well of each plate. After 3 h of incubation at 37 °C, formazan crystals were solubilized with 100 µL of acidified isobutanol. The absorbance was measured spectrophotometrically with an ELISA microplate reader (ELISA reader/Biotech) at 595 nm wavelengths. The number of viable cells was directly correlated to the amount of purple formazan crystals formed.

b- Western blot analysis

MDA-MB-231 (2.5×10^5) were seeded in 6 well plate and incubated with 100 $\mu g.mL^{-1}$ of extracts for 24 h. Then, the cells were washed, collected and lysed in RIPA lysis buffer [1 % Nonidet P-40, 1% sodium deoxycholate, 0.1 % SDS, 0.15 M NaCl, 0.01 M sodium phosphate (pH 7.2), 2 mM EDTA, 50 mM sodium fluoride, 0.2 mM sodium vanadate and 100 units/mL Aprotinin]. After incubation of the lysate for 30 min on ice, the cell lysates were centrifuged at 1200 rpm for 5 min and the supernatant was collected and used or immediately stored at −20 °C. Protein concentration of total cell lysate was measured by the Bradford assay and a total of 50 µg were subjected to 12 % SDS-polyacrylamide gels electrophoresis and the proteins were then transferred onto nitrocellulose membrane. After blocking the transfer with a blocking buffer (1.5 mM Tris–HCl (pH 8), 5 mM NaCl, 0.1 % Tween 20, 5% non-fat dry milk) for 1 h at room temperature, the blots were incubated overnight at 4° C with primary antibodies then with HRP conjugated secondary antibodies. The antibodies used: anti-PARP, anti-Bcl2, anti-procaspase-3 (cell signaling) and anti-GAPDH (abcam). Protein bands on the membrane were visualized by an enhanced chemiluminescence detection system (ECL) according to the manufacturer's instructions.

Statistical analysis

All the experiments for determination of total phenolics, total flavonoids, antioxidant and antitumor assays were conducted in triplicates. The values were expressed as the mean ± standard deviation (SD). The statistical analysis of the results was done using GraphPad Prism software. The values of $p < 0.05$ are considered statistically significant. Correlation coefficients (r) and coefficients of determination (r^2) were calculated using Microsoft Excel 2013 (Microsoft Corporation, Redmond, WA, USA).

RESULTS AND DISCUSSION

Extraction yield

Figure 1 shows the comparison in the percentage of extraction yield from the eight Lebanese regions. Values ranged from 0.4 % for Al-Hissa region to 3.6 % for Baalbeck. It can be seen that the TOF extract yield in the Baalbek district is higher than those of other districts. Studies had shown that during in vivo growth, the plant physiology and ecological function are influenced by the fluctuation in the abiotic factors[26]. The aforementioned fluctuation also known as stressors, such as the adverse environmental stress and climatic factors that includes drought, extremes temperature (freezing and heat) and

light irradiance, have a stimulatory effect on secondary metabolites in crops and medicinal plants. For instance, synthesis and accumulation of many cinnamic acids and flavonoids compounds have been shown to be light depend[27]. As a result, the biosynthesis of the metabolites, in other terms the richness in chemical compounds may evolve in the adaptation of plants to their growth environment. Baalbek District is between the peaks of the western chain and the eastern chain of Lebanon, where an arid semi-desert climate prevails. And as mentioned before bioclimatic variations are well remarkable over short distances in Lebanon. Therefore, this difference in TOF yield ($p = 0.001 < 0.05$) between the Lebanese regions may be due to the impact of climate on the variation in secondary metabolite profiles.

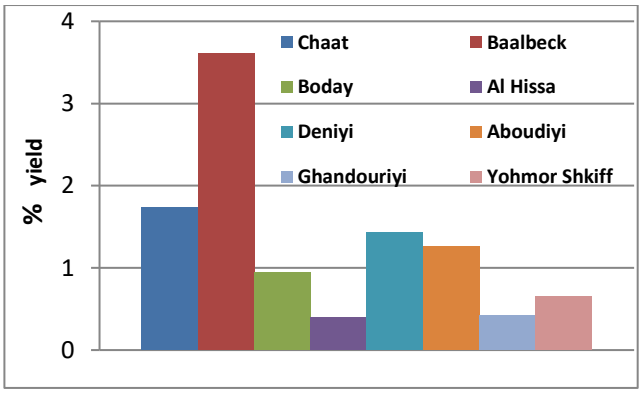

Figure 1. Extraction yield of extracts obtained from *N. tabacum* leaves.

Determination of polyphenols and total flavonoids in Lebanese tobacco leaves

Phenolic content in the TOF extract of tobacco leaves was carried out based on Folin-Ciocalteau method. The TPC are determined from the equation of the standard curve of Galic acid ($y = 0.751 x - 0.007$, $R_2 = 0.994$). Total flavonoid contents were determined by aluminium chloride colorimetric method and results are determined from the equation of the calibration curve of Rutin ($y = 14.47 x + 0.026$, $R_2 = 0.999$). The TPC and TFC in the different samples of TOF extracts of *N. tabacum* are presented in Figure 2.

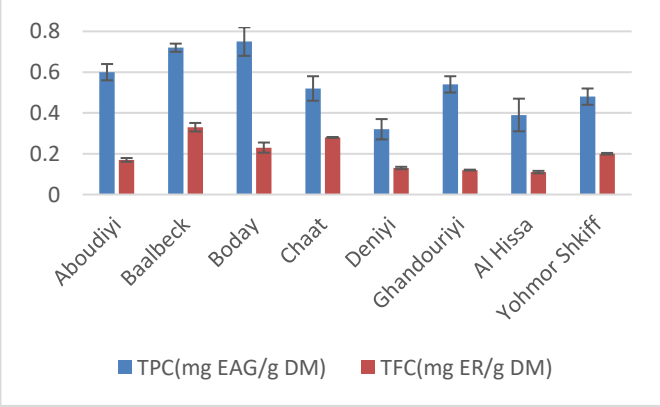

Figure 2 : The results of the total polyphenol and flavonoid contents are expressed in mg of GAE/g (dry weight) and mg of RuE/g (dry weight) of dry extract in the average form ± standard deviation.

The total phenolic contents in the different TOF extracts ranged from 0.32 ± 0.04 to 0.75 ± 0.07 mg GaE/g (dry weight). Furthermore, the TPC values were significantly different ($P < 0.05$). Thus, results revealed that the TOF leafy extract of *N. tabacum leaves from Boday* and Baalbeck are slightly higher in total phenolic content than the other extracts Deniyi had the lowest content. On the other hand, the flavonoid content ranged between 0.11 ± 0.006 to 0.33 ± 0.021 mg RuE/g (dry weight). Among the eight TOF extracts, Baalbeck extracts had the higher content in flavonoids, while Al Hissa had the lowest content.

The extraction yields and quantitative study of secondary metabolite lead to a synergistic result. Regions where stressors occur presented a powerful stimulus to produce secondary metabolites. For instance, drought was reported to show an increase in the amounts of flavonoids and phenolic acids in willow leaves[28], since these metabolites have protective functions during drought stress. Furthermore, temperature strongly influences metabolic activity and plant ontology, and high temperatures can induce premature leaf senescence[29]. A study in which tobacco cultivation was carried out under two different conditions, either in soil, exposed to the full range of climatic variations, or in vase, protected from extreme climatic variation showed that when tobacco grew in soil, 3-*O*-methylquercetin (a flavonoid aglycone) concentration increased in most of the studied varieties[20]. Recently, Bo Fu et al. (2016) have found an effect of different wavelengths of light on the accumulation of flavonoid in tobacco[30]. Despite that the effects were different between flavonoid methyl derivatives and flavonoid glycoside. The TPC and TFC found in TOF extracts of leaves in the present work were significantly lower than those reported in the literature[9,31,32]. Moreover, there are no figures regarding the quantitative study as to the best of our knowledge this is the first study addressing the TOF extract of *N. tabacum.* However, these results aren't surprising since researchers have dealt with non-selective extraction of *N. tabacum*[31–33], contrary to what we have done.

Antioxidant activity

The DPPH method is commonly used as a preliminary test to investigate the reactivity of tested compounds with a stable free radical. In this spectrophotometric assay the stable radical i.e., α,α-diphenyl-β-picrylhydrazyl (deep violet color) reacts with reducing agents, then electrons become paired off. Therefore DPPH· is converted to α,α-diphenyl-β-picrylhydrazine, and the solution loses color, stoichiometrically, depending on the number of electrons consumed[25]. However, the researchers who had studied the antioxidant activity of *N. tabacum* used different methods for this purpose[9,31,34,35]. The antiradical activity or the IC_{50} (extract concentration providing 50% inhibition)

was calculated from the graph plotted from inhibition percentage against different extract concentrations.

All TOF extracts showed dose and region dependent antioxidant activity. It should be noted that there are three samples with a much higher antioxidant capacity (lower IC_{50}) than others, as clearly shown in table 1.

Table 1: The values of IC50 of the TOF extracts of N. tabacum form the Lebanese regions

Sample	Average (mg.mL^{-1})
Ascorbic Acid	0.01 ± 0.002^{a}
Aboudiyi	0.16 ± 0.020^{b}
Al-Hissa	0.06 ± 0.005^{a}
Baalbeck	0.23 ± 0.008^{c}
Boday	0.12 ± 0.004^{b}
Chaat	0.16 ± 0.007^{b}
Deniyi	0.18 ± 0.010^{b}
Ghandouriyi	0.03 ± 0.002^{a}
Yohmor Shkiff	0.03 ± 0.006^{a}
The numbers followed by the same letter in the same column were not significantly different ($p < 0.05$)	

Compared with the IC_{50} of ascorbic acid (0.01 ± 0.002 mg.mL^{-1}), the highest antioxidant activity was obtained with Ghandouriyi and Yohmor Shkiff extracts (0.03 ± 0.002 mg.mL^{-1} and 0.03 ± 0.006 mg.mL^{-1} respectively) they showed no significant difference compared to ascorbic acid, followed by that of Al-Hissa extract (0.06 ± 0.005 mg.mL^{-1}) while the higher IC_{50} is that of Baalbeck (0.23 ± 0.008 mg.mL^{-1}).

Although they possess TPC and TFC relatively low among their peers, our results revealed that those three extracts (Ghandouriyi, Yohmor Shkiff and Al-Hissa) had the highest antioxidant potential. *So those extracts contain higher amount of compounds* having hydrogen donating capabilities to act as an antioxidant than others samples. Despite high correlation has been observed between the TPC and TFC of plants and their antioxidant activity; our results were contradictory and showed no relationship. Many authors have found no such correlation or only a very weak one[36,37].

Cytotoxic effect of Lebanese *N. tabacum* on MDA-MB-231 cancer cells

The effects of various extracts of Lebanese *N. tabacum* were evaluated on breast adenocarcinoma MDA-MB-231 cells using MTT cytotoxicity assay. This is a colorimetric assay during which the yellow water-soluble substrate MTT is reduced to a highly colored formazan product by succinate dehydrogenase enzymes in metabolically active cells. This conversion takes place only in viable cells; therefore, the amount of the formed formazan is proportional to the concentration of viable cells in the sample. MDA-MB-231 cancer cells were treated with different concentrations (25–250 µg.mL^{-1}) of various extracts for different periods of time (24 and 48 h). The extracts from Al-Hissa, Yohmor Shkiff and Ghandouriyi exerted no significant effect on cell viability in all tested conditions (Figure 1B, C and E), while extracts from Chaat, Deniyi, Baalbeck and Boday exerted dose- and time-dependent inhibitory effects (Figure 1 A, D, G and H). In the case of extracts from Chaat and Deniyi, the IC_{50} values (dose required to inhibit cell growth by 50%) corresponded to 200 µg.mL^{-1} and 225 µg.mL^{-1} after 24 h respectively (Figure 3 A and D), and prolonged treatment for 48 h caused a more striking inhibition of cell growth, as the IC_{50} values were 100 µg.mL^{-1} and 200 µg.mL^{-1}, respectively. Baalbeck and Boday extracts showed a more potent inhibitory effect than Chaat and Deniyi extracts; after 24 h of treatment with Baalbeck and Boday extracts, the IC_{50} values corresponded to only 125 µg.mL^{-1} and 100 µg/ml respectively and prolonged treatment reduced these values to 100 µg.mL^{-1} and 75 µg.mL^{-1} after 48 h respectively. (Figure 1G and H).

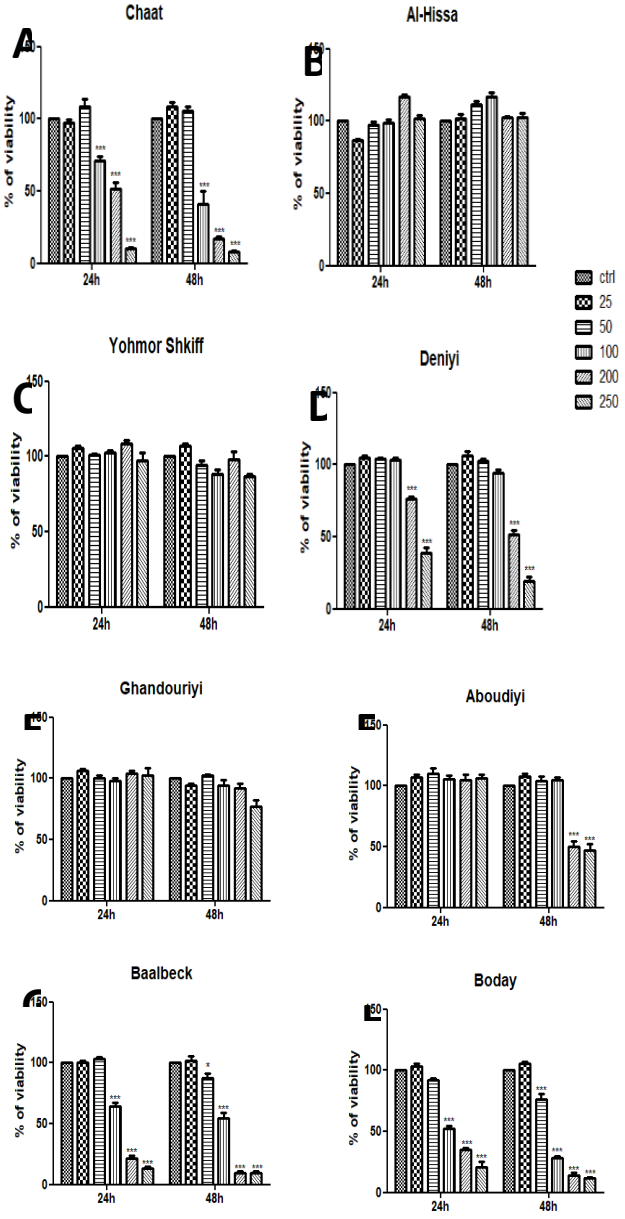

Figure 3: Effect of N. Tabacum extracts on MDA-MB-231 cell proliferation. Cells were treated with various

concentrations (25, 50, 100, 200 and 250µg/ml) of extracts for 24 and 48h and ant proliferative activities were measured by MTT assay. Each value represents a mean ± SEM for 3 independent experiments (n=3) each done in triplicate. ** p<0.01, *** p<0.001 vs. control untreated cells (one way annova test).

PARP cleavage and apoptosis upon exposure to Lebanese *N. tabacum*

Apoptosis is a widely accepted mechanism for anti-proliferative activity of many naturally occurring as well as synthetic agents. Apoptotic cell death is characterized by a number of morphological and cellular changes, including chromatin condensation, membrane blebbing, DNA fragmentation, and cleavage of key cellular proteins such as PARP. We conducted experiments to address the question of whether anti-proliferative activity of Lebanese *N. tabacum* against MDA-MB-231 cells was due to apoptosis induction. Apoptosis inducing effect of *N. tabacum* was assessed by western blot analysis.

An immunoreactive band corresponding to cleaved PARP (89 kDa) was observed with a concomitant decrease in the level of full length 116 kDa PARP after MDA-MD-231 treatment with 100 µg.mL^{-1} of Baalbeck and Boday extracts for 24 h (Figure 4).

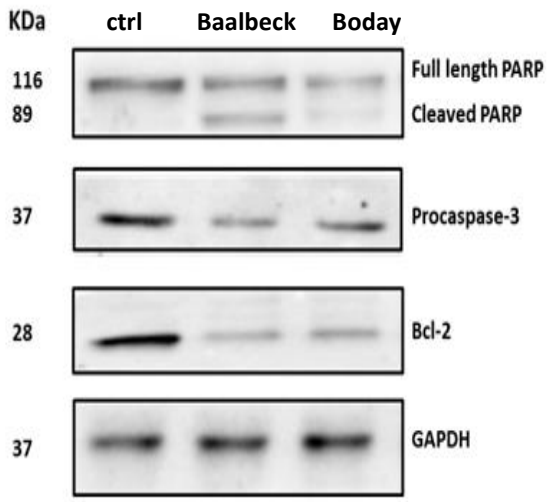

Figure 4: Induction of apoptosis in MDA-MB-231 cancer cells. MDA-MB-231 cells were incubated with 100 µg/ml of Baalbeck and Boday extracts for 24 h. Total protein extracts were made and subjected to western blot analysis using the indicated antibodies (anti-PARP; anti-procaspase-3 and anti –Bcl2). Expression levels were normalized to GAPDH protein content.

Caspases are aspartate-specific cysteine proteases that play critical roles in apoptosis. Activation of caspases results in cleavage and inactivation of key cellular proteins, including the DNA repair enzyme PARP. Since PARP cleavage was observed in Baalbeck/ Boday extracts treated MDA-MB-231 cells, we reasoned that Baalbeck/ Boday extracts induced apoptosis might involve caspases. We explored this possibility by determining the effect of Baalbeck and Boday extracts treatment on activation of caspase 3 by western blotting using antibody that recognizes the full-length (pro-caspase-3). Treatment of MDA-MB-231 cells with 100 µg.mL^{-1} Baalbeck and Boday extracts resulted in cleavage of procaspase-3.

The effect of Baalbeck and Boday extracts treatment on expression of anti- apoptotic (Bcl-2) protein was also determined to gain insights into the mechanism for Baalbeck and Boday extracts induced cell death. A marked decrease in Bcl-2 expression was observed in Baalbeck and Boday extracts treated cells at 24 h.

Previous study conducted by many research teams on other plants revealed that TOF extracts afforded antioxidant and anticancer activities[38–40]. Among secondary metabolites families detected in *N. tabacum* TOF extracts, phenolic compounds which are powerful antioxidants were considered as interesting anti-inflammatory, antimutagenic and anticancer molecules[41].

Thus the cytotoxic effect is in agreement with the TPC and TFC content in Baalbeck and Boday extracts, however the antioxidant effect is not. Such result was obtained recently in a study covering fifty-seven medicinal and edible plant extracts[42]. The interpretation of this observation led us to classify the oligomeric flavonoids of *N. tabacum* into two classes; the ones that are produced in the normal state of plant growth and the others produced under a state of stress. The first class is the ones that contain compounds with potential antioxidant activity. Therefore, the extracts of plants from regions where normal climatic conditions prevail are rich in the oligomers with antioxidant effect. While in extracts from regions with severe climatic conditions, these antioxidant active compounds become mixed with other inactive emerged compounds, which influence the richness of the extract with the active compounds. It is evident that the TOF extract from *N. tabacum* has dose and time dependent *in vitro* cytotoxic activity against the cell line studied. Another explanation can be provided is that the difference in the cytotoxic activity is not mainly due to the level of antioxidants but could also be associated with the inhibitory effects via other signaling pathways. Further studies are needed to isolate and chemically identify the active compounds responsible for the above activities. Moreover, the validity of the conclusions of this study must be tested on other cancer cell-lines.

CONCLUSION

According to the present study, results have demonstrated that *N. tabacum* TOF extracts from some Lebanese regions possess potent antioxidant in DPPH radical assay and anticancer activities. Those activities could be ascribed to the synergistic action of bioactive compounds present in it. The observed variation between regions may be due to the difference in the environmental conditions and geographical distribution, which impact the constituents of the plant. All these results suggest that TOF extracts can be a rich source of highly bioactive substances, especially with

cytotoxic potential. Further studies are required to identify the active compounds that confer the antioxidant and/or anticancer activities of the extract. Once such compounds are identified, the mechanisms by which they exert their effects can be characterized. In addition, *in vivo* evidence for the biological activities is needed. So we hope to succeed in transferring the tobacco plant from source of carcinogen compounds to factory of anticancer compounds.

Acknowledgements: The authors are grateful to the Lebanese University (Faculty of Pharmacy and Faculty of Science-I) - Lebanon- for providing all chemicals necessary to carry out this project.

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Source of Support: Nil, **Conflict of Interest:** None.