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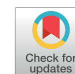
Research paper

Antioxidant, antimicrobial and cytotoxic properties of four different extracts derived from the roots of *Nicotiana tabacum L.*



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ABSTRACT

Introduction: Few studies have investigated the biological activities of *Nicotiana tabacum* L. and most of these studies have focused on its leaves. This is perhaps due to the fact that tobacco smoking is a major risk factor for lung cancer. Therefore, we aimed to investigate, for the first time, the antioxidant, anticancer and antimicrobial activities of extracts derived from the roots of *N. tabacum.*
Methods: Antioxidant activity was assessed employing 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) method. Antimicrobial activity was tested using broth-microdilution, agar-dilution and Agar-well diffusion methods. Anti-proliferative and apoptotic activities were evaluated utilizing colorimetric methods.
Results: Hexane, acetone and methanol extracts and trolox exhibited a potent and significant antioxidant activity with IC_{50} values of 2, 6, 21 and 2 μg/ml respectively. Acetone extract had the strongest antimicrobial activity followed by hexane and then methanol extracts. Both hexane and acetone extracts had strong and significant anti-proliferative effects followed by methanol extract. This could be due to a growth arrest or cell death. To clarify this, we investigated the effect of acetone extract on caspase-3 activity and it appeared to have a 2 times stronger apoptotic effect than the chemotherapeutic medicine, doxorubicin.
Conclusion: In the current study we demonstrate for the first time that organic extracts derived from the roots of *N. tabacum* possessed antioxidant, anticancer and antimicrobial properties. Our data draws attention to *N. tabacum* as a promising source of bioactive molecules that can be utilized in pharmaceutical industry, despite the fact that tobacco smoking is a major risk factor for lung cancer.

1. Introduction

Historically, medicinal plants and their secondary metabolites have been used in medicine synthesis [1]. More than 50,000 plant species (over a tenth of the plant species) are used in pharmaceutical and cosmeceutical industries [2]. The Eastern region of the Mediterranean (Lebanon, Jordan and Palestine) is the home of at least 3600 plant species; many of them are endemic only to this region. Approximately, 450–550 species of these plants have been used as medicinal herbs [3]. Therefore, there is a need to screen the remaining uninvestigated plants for their potential applications in medicine.

N. tabacum is an annual herbaceous plant belonging to the Solanaceae family. It grows to up to 2.5 m with large ovate green leaves and terminal clusters of tubular white-pinkish flowers. It is a worldwide cultivated plant, but its native land is tropical America [4]. Tobacco is made from the leaves of *N. tabacum.* Tobacco smoke is a serious threat to health; since it is the most important risk factor for lung cancer. Lung cancer is the most common cancer in men and the most common cause of cancer deaths worldwide [5], responsible for approximately 22 % of cancer deaths [6]. This is due to the carcinogens produced when tobacco is burned during smoking [7]. On the other hand, *N. tabacum* has a long history in folk medicine in some Asian countries. Their leaves have been reported to activate biological mechanisms such as antibacterial, anthelmintic, anti- Alzheimer and antinociceptive activities [8].

Perhaps because tobacco smoke is a major risk factor for lung cancer; it is less attractive to investigate its biological properties, such as antibacterial, anticancer and antioxidant properties. Therefore, few studies have investigated the biological activities of extracts obtained from *N. tabacum* and most of these studies focused on the leaves of *N.*

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tabacum. Hardly any evidence is available on the biological properties of other parts of the plant. As a consequence, the current study aims to investigate for the first time the biological activities of extracts derived from the roots of *N. tabacum* plant. To achieve this, we prepared four extracts from *N. tabacum* using aqueous, methanol, hexane and acetone solvents and we investigated their antioxidant, antibacterial, anti-proliferative and apoptotic properties.

2. Methods

2.1. Collection and preparation of N. tabacum roots

The roots of *N. Tabacum* were collected in October 2016 from Tobacco farms in Kufur Ra'i village, Jenin-Palestine. The plant was botanically identified by pharmacognosist Dr. Nidal Jaradat from the Pharmacy Department at An-Najah National University. A voucher specimen PharmPCT-2805 has been retained in the herbarium of the Laboratory of Pharmacognosy. The plant was washed well several times with distilled water and then dried in the shade for four weeks at room temperature. After drying, the roots were ground into a fine powder and placed into airtight containers with proper labeling for future use.

2.2. Extraction procedure

The extraction process was conducted using aqueous, methanol, hexane and acetone solvents, which have a wide range of polarity [9]. Roots of *N. tabacum* were cut into small pieces and then ground into a fine powder. Approximately, 50 g of the obtained powder was soaked in 50 ml of each of the used four solvents and kept in a shaker (100 RPM at room temperature) for 72 h. Later on, each extract was filtered through filter papers and Buchner funnel. Filtered extracts were vaporized under high pressure by a rotary evaporator at a maximal temperature of 35 °C. The obtained dry extracts were stored at 4 °C for further use [10,11].

2.3. In vitro evaluation of antioxidant activity (DPPH radical method)

Antioxidant activity of each extract was evaluated using the DPPH radical method as described previously [12,13]. Stock solutions (1 mg/ml) of each extract and trolox (reference compound) were prepared in methanol. Then, serial dilutions in methanol were prepared as following 100, 80, 50, 40, 30, 20, 10, 7, 5, 3, 2, 1 µg/ml. By using a spectrophotometer, the optical density of each of these working solutions (prepared in triplicates) was measured at 517 nm. DPPH radical inhibition was calculated by using the following formula:

DPPH radical scavenging activity = $((A0 - A1) / A0)) \times 100$ %, where, A0 and A1 are the optical densities of Trolox standard and the working solution at 30 min, respectively.

The antioxidant activity of a substance is expressed as IC_{50} (µg/ml), which represents the concentration of that substance that causes a 50 % decrease in the optical density at 517 nm [14].

2.4. Antimicrobial test

Antimicrobial activities against bacterial isolates were carried out using a broth micro-dilution method and to validate results agar-well diffusion assay was applied. On the other hand, antimicrobial activity against yeast and mold were determined using broth micro-dilution and agar dilution method, respectively [15,16].

2.4.1. Microbial isolates

The antibacterial activities of organic and aqueous extracts were examined against 5 types of reference bacterial strains obtained from the American Type Culture Collection (ATCC), i.e. *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922), *Shegilla sonnie* (ATCC 25931), *Enterococcus faecium* (ATCC 700221), *Staphylococcus aureus* (ATCC 25923) and *Methicillin Resistance Staphylococcus aureus* (*MRSA*) clinical isolate. In addition, antifungal activity was determined against *Candida albicans* (ATCC 90028) and *Epidermatophyton floccosum* (ATCC 52066).

2.4.2. Preparation of plants extracts

25 mg/ml solution of the aqueous extract was prepared by dissolving the powder in sterile distilled water. Organic extracts were dissolved in 100 %, 50 % or 10 % of Dimethyl sulfoxide (DMSO) to achieve a concentration of 25 mg/ml. The resulting solutions were sterilized by filtration (0.45 µm).

2.4.3. Inoculums preparations

A fresh bacterial isolate inoculum was suspended in sterile normal saline until turbidity was equivalent to 0.5 McFarland standard (1.5×10^8 CFU/ml), after that the suspension was diluted and the final concentration was 5×10^5 CFU/ml. For *C. albicans*, the same procedure was followed, but the final concentration was 0.5×10^3 to 2.5×10^3 CFU/ml [17]. Preparation of *E. floccosum* mold inoculum was done by suspending its spores and hyphae with 0.05 % Tween 20 in sterilized distilled water until turbidity matched the 0.5 McFarland standard.

2.4.4. Broth Micro-dilution method

Solutions of plant extract were two-fold serially diluted with Mueller-Hinton II Broth (Becton Dickinson, France) 11 times in a microplate (wells 1–11) and well number 12 contained only Mueller-Hinton II broth and was free of plant extract and used as a positive control of microbial growth. For yeast *C. albicans*, RPMI1640 medium was used instead of Mueller-Hinton II Broth. Various types of microbes (bacteria or yeast) were inoculated in the wells of plates except well number 11, which was considered as a negative control of microbial growth. Inoculated plates were covered and incubated at 35 ºC for about 24 h for bacteria and 48 h for yeast. The lowest concentration of plant extract that did not allow any visible growth of microbe in the test broth was considered the minimal inhibitory concentration (MIC). Each plant extract was examined in duplicate.

2.4.5. Agar-well diffusion assay

Mueller-Hinton agar medium was prepared and poured in Petri dishes. Next day, from appropriate concentration (1.5×10^8 CFU/ml), the bacterium was subcultured on the media by streaking the bacterial suspension using a cotton swab. Wells (each 6 mm in diameter) were made in agar plates. A volume of 80 µl of each plant extracts was added to each well. The inoculated dishes were covered and incubated at 35 ºC for 24 h, then the diameter of the bacterial inhibition zone was measured to evaluate the antibacterial activity [16]. Each plant extract was examined in duplicate.

2.4.6. Determination of the anti-mold activity of plant extracts

Agar dilution method was used to evaluate the inhibition of *E. floccosum* mold by plant extracts [18]. Sabouraud dextrose agar (SDA) was prepared and distributed in tubes (1 ml/tube) then sterilized by autoclave and melted in a 40 °C water bath so that dilutions of the plant extracts could be placed in 6 tubes. The prepared plant extracts concentrations ranged from 0.4–12.5 mg/ml for aqueous and organic extracts. The prepared tubes were allowed to solidify at room temperature in a slanted position. A suspension equivalent to 0.5 McFarland standard turbidity was prepared from *E. floccosum* fresh culture. Then 20 µl of the *E. floccosum* prepared suspension was added to all tubes except the 6th tubes, which were the negative control of fungal growth. Whereas the 7th tube that contained SDA alone was the positive control for the mold. After 14 days of incubation at 25 °C the results were read. The minimal inhibitory concentration (MIC) was the lowest concentration of plant extract that did not allow any visible growth of *E. floccosum* on the SDA.

2.5. Cell culture, proliferation and caspase-3 activity assays

HeLa cervical adenocarcinoma cancer cells were cultured in RPMI-1640 media, which was supplemented with 10 % fetal bovine serum, 1 % Penicillin/Streptomycin antibiotics and 1 % L-glutamine. Cells were grown in a humidified atmosphere with 5 % CO_2 at 37 °C. Cells were treated as described earlier [19,20], but with slight modifications. Cells were seeded in 96-well plates in their corresponding culture media (about 1×10^5 cells in 100 µl volume/well in triplicate) and incubated for 24 h. After that the culture media was replaced with the same fresh media that contain various concentrations (2.5, 1.25, 0.625 and 0.3125 mg/ml) of each extract and further incubated for 4, 24 and 48 h. A concentration of 10 µM of doxorubicin was used as a positive control for anti-proliferative activity. The types of extracts that were investigated in this study were acetone, hexane and methanol and aqueous extracts. Then the anti-proliferative effect of the plant extracts was assessed by Cell Tilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20 µl of MTS solution per 100 µL of media was added to each well and incubated at 37 °C for 2 h. Absorbance was measured at 490 nm. The apoptosis-inducing effect of acetone extract and doxorubicin (positive control) was determined on the basis of the level of caspases-3 according to the manufacturer guidelines (biovision).

2.6. Statistical analysis

Statistical analysis was performed employing GraphPad Prism software version 6.01. Comparison between 3 groups or more belong to one factor (as in antioxidant and caspase activity results) was analyzed via one-way ANOVA followed by Bonferonni's post hoc test. Nonlinear regression test was used to determine the IC50. Comparison between multiple groups belong to two factors (as in cytotoxicity results) was analyzed via two-way ANOVA followed by Tukey's post hoc test. Results were considered to be statistically significant at $P < 0.05$.

3. Results

3.1. Antioxidant activity

DPPH radical scavenging activities induced by various tested concentrations of aqueous, methanol, acetone, and hexane extracts derived from the roots of *N. tabacum* are demonstrated in Table 1. There was a significant (P-value < 0.0001) dose–dependent increase in the percentage of antioxidant activities for all tested concentrations of all tested extracts, however, with a different extent. IC_{50} values (Table 2) of aqueous, methanol, acetone, and hexane extracts were 1257.0, 21.0, 6.0 and 2.0 µg/ml respectively, while the IC_{50} value of trolox, the standard material, was 2 µg/ml.

Table 1
Antioxidant activity of trolox standard and extracts derived from *N. tabacum.*

% of Inhibition (mean ± SEM)					Conc. µg/ml
Trolox*	N-hexane*	Acetone*	Methanol*	Aqueous*	
97.0 ± 0.0	88.1 ± 1.4	78.8 ± 2.6	60.6 ± 1.6	47.8 ± 0.9	100.0
97.0 ± 0.0	87.4 ± 1.6	76.9 ± 1.7	56.6 ± 0.8	45.7 ± 0.2	80.0
97.0 ± 0.0	81.2 ± 0.7	73.1 ± 0.7	47.3 ± 1.3	43.3 ± 0.4	50.0
97.0 ± 0.0	74.7 ± 1.3	63.9 ± 2.6	41.9 ± 1.1	42.8 ± 0.3	30.0
97.0 ± 0.0	68.8 ± 1.6	62.4 ± 2.1	40.8 ± 0.9	42.2 ± 0.9	20.0
96.8 ± 0.25	59.6 ± 1.1	55.5 ± 1.9	39.5 ± 0.3	41.8 ± 0.6	10.0
96.4 ± 0.3	56.4 ± 1.6	49.8 ± 0.9	39.1 ± 0.2	40.6 ± 0.2	7.0
92.9 ± 0.2	53.9 ± 1.6	49.1 ± 0.7	36.8 ± 0.5	40.1 ± 0.3	5.0
76.3 ± 0.3	52.1 ± 0.8	47.6 ± 1.2	36.8 ± 0.5	40.0 ± 0.3	3.0
68.6 ± 0.1	50.8 ± 0.2	44.2 ± 1.3	36.8 ± 0.5	39.3 ± 0.4	2.0
61.8 ± 0.6	49.7 ± 0.2	44.2 ± 1.3	36.8 ± 0.5	38.6 ± 0.1	1.0
0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 (C)

SEM: standard error of the mean; C: Control; *: P < 0.0001 (tested concentrations vs. control).

Table 2
IC50 of trolox standard material and extracts derived from *N. tabacum.*

N. tabacum extract / Trolox	IC50 (µg/ml) (mean ± SEM)
Aqueous	1257 ± 823.5
Methanol	21 ± 9.6
Acetone	6 ± 0.1
Hexane	2 ± 0.2
Trolox standard reagent	2 ± 0.01

SEM: standard error of the mean.

Table 3
Antimicrobial activity of extracts derived from *N. tabacum* determined by minimum inhibitory concentration method (mg/ml).

Type of microbes	MIC mg/ml			
	Aqueous	Methanol	Acetone	*n*-hexane
S. aurous	0	0	1.6	3
MRSA	0	0	3	0
E. faecium	0	1.6	3	0
E. coli	0	3	3	3
S. sonnei	0	1.6	6.3	0
P. aeruginosa	0	0	3	1.6
C. albicana	0	3	3	3
E. floccosum	0	0	0.8	0.8

0: No inhibition.

3.2. Antimicrobial activity

As shown in Table 3, aqueous fraction of *N. tabacum* had no antimicrobial activity against the tested microbes. Methanol fraction had antibacterial activity against *E. faecium, E. coli* and *S. sonnie* with MIC values of 1.6, 3, and 1.6 mg/ml, respectively. In addition, methanol fraction possessed antifungal activity against *C. albicans* with MIC value of 3 mg/ml. Acetone fraction had potent antimicrobial activity against all tested microbes; *S. aureus,* MRSA, *E. faecium, E. coli, S. sonnie, P. aeruginosa, C. albicans* and *E. floccosum* with MIC values of 1.6, 3, 3, 3, 6.3, 3, 3 and 0.8 mg/ml, respectively. Hexane extract had antimicrobial activity against *S. aureus, E. coli, P. aeruginosa, C. albicans* and *E. floccosum* with MIC values of 3, 3, 1.6, 3 and 0.8 mg/ml, respectively. These results were validated using the agar diffusion method. Aqueous fraction of *N. tabacum* did not produce inhibition zones against any type of tested bacteria; whereas methanol fraction produced inhibition zone diameter of 13, 15, 9 and 13 mm against MRSA, *E. faecium, E. coli* and *S. sonnie,* respectively. Acetone fraction produced inhibition zone diameter of 1, 1, 6, 1, 1 and 6 mm against *S. aureus*, MRSA, *E. faecium*, *E. coli*, *S. sonnie* and *P. aeruginosa*, respectively. Hexane fraction produced inhibition zone diameter of 4, 6 and 8 mm against *S. aureus*, *E. coli and P. aeruginosa*, respectively.

3.3. Anti-proliferative activity

In the present study, the anti-proliferative potential of extracts derived from the roots of *N. tabacum* was investigated on HeLa cervical adenocarcinoma. Hela cells were treated with. Hela cells were exposed to increasing concentrations of aqueous, methanol, acetone and hexane extracts (0.3125, 0.625, 1.25, and 2.5 mg/ml). We investigated the effect at various time points 4, 24 and 48 h, to determine whether it has a time-dependent effect. 4 h incubation with 2.5 mg/ml of acetone and hexane extracts (Fig. 1A and B), decreased cell viability significantly (p-value < 0.0001) to 13 % and 25 % respectively, compared to doxorubicin (positive control) that had relative cell viability of 38 %.

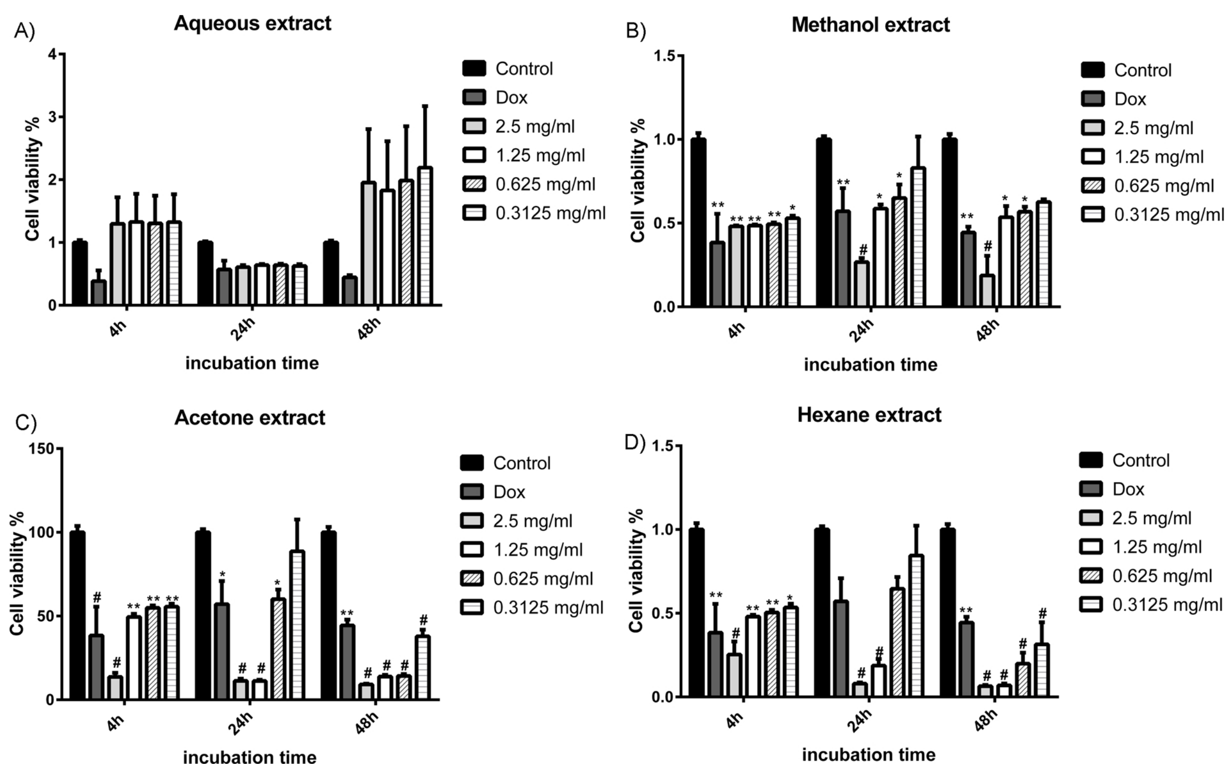

Fig. 1. Anti-proliferative effect of (A) Aqueous, (B) methanol, (C) acetone, (D) hexane and extracts derived from *N. tabacum*. Hela cells were treated with 2.5, 1.25, 0.625 and 0.3125 mg/ml of Aqueous, acetone, hexane and methanol extracts obtained from *N. tabacum* and incubated for 4, 24 and 48 h. Proliferation was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (with only media; C). *#P < 0.0001*, ***P < 0.01* and **P < 0.05*. Error bars represent SD.

Treatment with the rest of concentrations of both extracts decreased significantly (p-value < 0.01) cell viability by approximately 50 %. All tested concentrations of methanol extract (Fig. 1C) decreased significantly (p-value < 0.01 and < 0.05) cell viability by approximately 50 %. As shown in Fig. 1A-C, 24 h incubation with 2.5 mg/ml of acetone, hexane and methanol extracts decreased cell viability significantly (p-value < 0.0001) to 11 %, 8 % and 27 % respectively, compared to doxorubicin that had relative cell viability of 57 %. Treatment with 1.25 mg/ml of acetone, hexane and methanol extracts decreased significantly (p-value < 0.0001) cell viability to 11 %, 19 % and 60 % respectively. Treatment with 0.625 mg/ml of acetone, hexane and methanol extracts decreased significantly (p-value < 0.05) cell viability to approximately 60 %, while 0.3125 mg/ml of all extracts had no significant effect. After 48 h treatment with 2.5 mg/ml of acetone, hexane and methanol extracts, cell viability decreased significantly (p-value < 0.0001) to 9 %, 6 % and 19 % respectively, compared to doxorubicin that had relative cell viability of 44 %. Treatment with 1.25 mg/ml of acetone, hexane and methanol extracts decreased significantly (p-value < 0.0001) cell viability to 13 %, 7 % and 53 % respectively. Treatment with 0.625 mg/ml of acetone, hexane and methanol extracts decreased significantly (p-value < 0.0001 and < 0.05) cell viability to approximately 14 %, 20 % and 57 % respectively. Treatment with 0.3125 mg/ml of acetone and hexane but not methanol extracts decreased significantly (p-value < 0.0001) cell viability to approximately 38 % and 31 % respectively (Fig. 1A-C).

3.4. Caspase-3 activity

As acetone extract of *N. tabacum* possessed the strongest anti-proliferative effect with relative cell viability of 14 % at 0.625 mg/ml dose, caspase activity was evaluated at this concentration and doxorubicin was used as a positive control at a concentration of 10 μM. As demonstrated in Fig. 2, treatment with doxorubicin (dox) and acetone extract increased the activity of caspase-3 significantly (p < 0.05) by 2 and 4 folds respectively.

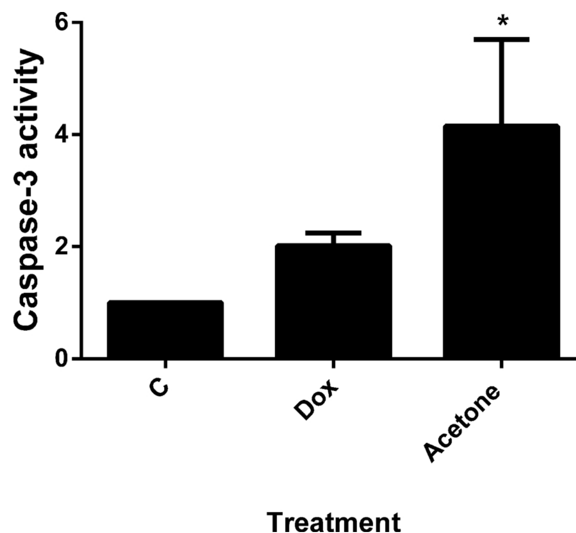

Fig. 2. Effect of acetone extract of *N. tabacum* on Caspase-3 activity. Hela cells were treated with 0.6215 mg/ml of acetone extract derived from *N. tabacum* for 48 h. Caspase-3 activity was determined using caspase-3 /CPP32 Colorimetric Protease Assay kit. Results were depicted as relative quantities (RQs) compared to the control (with only media; C). **P < 0.05*. Error bars represent SD. Dox: doxorubicin.

4. Discussion

Although tobacco smoking is a major risk factor for lung cancer, few studies have demonstrated that extracts derived from the leaves of *N.*

tabacum possess beneficial effects [8]. Extracts derived from the roots have never been investigated earlier. Therefore, in the current study, four types of extracts were derived from the roots of *N. tabacum* and were tested for their biological activities, i.e. anti-oxidant and anticancer and antimicrobial activities.

Reactive oxygen species (ROS) are produced as a result of normal cellular metabolism and environmental factors, such as alcohol, radiation and tobacco smoking. Normal cells have anti-oxidant defense systems that can inactivate the generated ROS. However, an imbalance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress. Oxidative stress contributes to many pathological conditions, including cancer, due to oxidative DNA damage. This causes mutations in oncogenes and tumor suppressor genes that affect both the control of cells division and apoptosis and consequently causes cancer. Exogenous antioxidants intake would ameliorate the damage caused by oxidative stress [21]. Therefore, we checked the antioxidant activity of *N. tabacum's* roots and it appeared that hexane extract was the strongest organic extract with an IC50 of 2 μg/ml. This was as strong as trolox, the standard reference molecule, followed by acetone (6 μg/ml) and methanol (21 μg/ml) extracts, however, aqueous (1257 μg/ml) extract had almost no antioxidant effect. The IC50 we observed are higher than what was reported earlier in extract obtained from roots (95 μg/ml) and leaves (29 μg/ml) [22,23].

Natural antioxidants exhibit a wide range of biological effects, such as antimicrobial and anticancer [24]. Therefore we investigated the anticancer property of *N. tabacum* employing proliferation and apoptotic assays. We found that both hexane and acetone had a strong antiproliferative effect followed by methanol, while aqueous extract had no effect. This could be due to a growth arrest or cell death. To reveal this we investigated the effect of acetone extract on caspase-3 activity, as it was one of the strongest anti-proliferative stimulants. Interestingly, acetone extract had a stronger apoptotic effect than the chemotherapeutic medicine, doxorubicin. Caspase-3 is known as an executioner caspase in apoptosis because of its role in coordinating the destruction of cellular structures such as DNA fragmentation or degradation of cytoskeletal proteins [25]. Our data clearly suggest that *N. tabacum* inhibits growth of cells through at least apoptosis and therefore it has an anticancer property. Furthermore, this suggests that the root of *N. tabacum* is a potential source of strong anticancer medicine(s). The studies concerning this effect are scarce and they focus on the leaves and there is no earlier studies investigated the anticancer effect of the roots. In one study, the cytotoxic effect has been shown at 10,000 μg/ml of leaves' extract after 48 h [26], which is way lower than what we observed. This suggests that the roots might be a better potential source of anticancer medicine than leaves.

Infectious diseases are the top and second leading causes of death in low- and high-income countries. There is even a growing association between communicable (infectious) and non-communicable diseases [27]. The health problems related to microbial infections are seriously exacerbated by the widespread of antibiotic resistance and the lack of effective new therapeutics. Each year, more than 400,000 people develop multidrug-resistant tuberculosis (MDR-TB). Other examples of known bacteria that develop antibiotic resistance are Methicillin-resistant *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella* species [28]. Therefore, we investigated the antimicrobial activity of extracts obtained from *N. tabacum* as a natural alternative of synthetic antibiotics and we found that acetone extract had the most potent and broad spectrum antimicrobial activity followed by hexane and then methanol extracts, while aqueous extract had no effect at all. The observed aqueous effect is in agreement with an earlier study, while acetone effect we observed had a stronger antimicrobial effect [29]. In another study, acetone had no antimicrobial effect [30], which is contradictory to our finding, but this could be due to the fact that the investigated microbes were not similar to ours. In another study, it has been shown that methanol and water extracts had weaker anti-*C. albicana* activity in comparison to our study [31]. In contrast to our study, methanol extract had no effect on *E.coli*, while had anti-*P. aeruginosa* and *S. aureus* effects [32]. These discrepancies most probably are due to the fact that the investigated extracts in these studies were derived from the leaves of *N. tabacum*, in contrast to our study where we used the roots.

The separation of active compounds of *N. tabacum* roots has not been attempted in this study, as separation with column chromatography requires huge amounts of the plant, which was not available at this time. In addition, isolation, chemical structure elucidation and determination of structure activity relationship of the most therapeutic active compounds from the extracts of *N. tabacum* roots are limitations in this study. However, these experiments will be our future goal. Once we determine the active compounds, it will be way easier to investigate them in an *in vivo* setup. Although DPPH method is fast, accurate and widely used method in many studies, it will be better to validate DPPH results by other methods in the future.

5. Conclusion

In the current study we found for the first time that all extracts, except water extract, derived from the roots of *N. tabacum* possessed antioxidant, anticancer and antimicrobial properties. Our data suggest that the potency of all tested biological activities is higher in the nonpolar extracts (acetone and hexane). This study draws attention to *N. tabacum* (especially nonpolar extracts) as a potent source of bioactive molecules that can be further utilized as a prominent bioresource in drug discovery efforts, despite the fact that its smoke is a major risk factor for lung cancer.

Authors' contributions

Saad Al-Lahham: conceptualized and designed the study, drafted and revised the manuscript, analyzed data and supervised the research project. Reem Sbieh: conducted the research, optimized methods and participated in conceptualizing the study, drafting and revising the manuscript. Nidal Jaradat: participated in conceptualizing and supervising the study and revising the manuscript. Motasem Almasri: participated in investigating antimicrobial activity, collecting and entering the data and revising the manuscript. Ahmed Mosa: helped in conducting research methods, entering data and revising the manuscript. Abdallah Hamayel and Faris Hammad: participated in entering data and revising the manuscript. All authors had read and approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Authors

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No.

Declaration of Competing Interest

None.

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