Jordan Journal of Pharmaceutical Sciences, Volume 18, No. 1, 2025

In vitro Analysis of the Anticancer and Antidiabetic Effects of *Teucrium orientale* Leaf Hydrophilic Extract Grown in Two Palestinian Geographic Areas

Johnny Amer[1], Mustafa Ghanim[2*], Ahmad Salhab[2], Nidal Jaradat[3], Malik Alqub[2*], Maha Rabayaa[2] , Lana Hallak[3] , Tasneem Abdulhai[3] , Siba Dwayat[3] , Linda Issa[3]. Majdi Dwikat[1]

[1] Department of Applied and Allied Medical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine.
[2] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine.
[3] Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine.

ABSTRACT

Several studies have demonstrated that *Teucrium orientale* (*T. orientale*) species have therapeutic advantages, such as antioxidant, bacteriostatic, spasmolytic, and anti-inflammatory activity. This study aimed to assess the possible antidiabetic and anticancer activities of *T. orientale* leaf hydrophilic extracts collected from two distinct geographic regions in Palestine: Jerusalem and Ramallah. *T. orientale* hydrophilic extract was tested for its antidiabetic and anticancer properties on α-amylase activity and Lewis Lung Carcinoma (LLC) cells, respectively. The anticancer effect on LLC was evaluated by flow cytometry for cell proliferation and Annexin-V/propidium iodide (PI) staining for cell apoptosis. The *T. orientale* extract from Jerusalem had an IC50 of 7.43±0.84 µg/ml for inhibiting α-amylase enzyme activity, whereas the Ramallah extract had an IC50 value of 23.2±0.29 µg/ml. These values were compared to the positive control, Acarbose, which had an IC50 of 43.91±1.08 µg/ml. LLC cells were treated with one of the two extracts of *T. orientale* at different concentrations (0, 50, 100, 200, and 400 µg/ml) for 24 hours, and cell proliferation was assessed using an XTT assay. Total inhibition of LLC proliferation was achieved at 400 µg/ml in both extracts. The *T. orientale* extract from Jerusalem demonstrated a more efficient inhibitory effect at lower concentrations. Increasing concentrations of *T. orientale* (50, 100, 200, and 400 mg/ml) from the two geographic areas, Ramallah and Jerusalem, had no effect on the apoptosis rate in the control group. In contrast, elevated rates of apoptosis were observed following treatment with *T. orientale* extract in LLC cells at all tested concentrations, and this was positively associated with the late apoptosis marker Annexin-V+/PI+. Moreover, the *T. orientale* extract from Jerusalem exhibited an apoptotic rate of 90±3.4% at the highest concentration of 400 mg/ml, compared to 62.6±3.4% following treatment with the Ramallah extract. This suggests that the *T. orientale* extract from Jerusalem induced apoptosis in LLC cells more efficiently than the extract from Ramallah. The extracts derived from *T. orientale* show promising potential as a natural antidiabetic and anticancer agent, as evidenced by their ability to inhibit the α-amylase enzyme, impede the growth of LLC cells, and enhance apoptosis. Further in vivo and preclinical investigations are required to validate these effects.

Keywords: *Teucrium orientale;* α-Amylase; Lewis Lung Carcinoma; Cell proliferation; Apoptosis.

INTRODUCTION

The Mediterranean basin is home to over 300 species of *Teucrium* (Labiatae), a widespread flora genus. Traditional medicine widely uses Teucrium species as stimulants, tonics, diaphoretics, appetizers, and for treating gastric disorders and diabetes mellitus (1-3).

[*] ***Corresponding author:***
Malik Alqub, m.alqub@najah.edu
Mustafa Ghanim, mustafa.ghanim@najah.edu
Received: 20/03/2024 Accepted: 18/07/2024.
DOI: https://doi.org/10.35516/jjps.v18i1.2492

Teucrium (Labiatae) is a widespread flora genus with over 300 species, most of which are found in the Mediterranean basin regions. *Teucrium* species is widely used in traditional medicine as stimulants, tonics, diaphoretics, and appetizers, as well as for stomach problems and diabetes (4). *Teucrium orientale* L. is a wildly distributed woodland and shrubland species in Mediterranean countries' semiarid, arid, and steppe areas (5). Plant decoction has a role in herbal medicine for treating many conditions, such as diabetes, wounds, fever, insomnia, neurological disorders, abdominal cramps, gastrointestinal disorders, colds, diarrhea, and hypertension (2, 6).

Phytochemical investigations stated that *T. orientale* contained many chemical compounds with significant bioactivity, including neoclerodane diterpenoids, flavonoids, iridoids, and phenolic acids, and its major constituents in the essential oil are α-pinene (25.10%), and β-caryophyllene (56.01%) (6).

Cancer and diabetes mellitus are prevalent illnesses that significantly influence global and local health. Epidemiologic research indicates that people with diabetes have a greatly increased chance of developing various cancer types. While cancer and type 2 diabetes share several risk factors, the plausible biological connections between the two illnesses remain unknown (7). Furthermore, observational investigations' data revealed a correlation between some antidiabetic treatments and an elevated cancer risk (8-10). Cancer and diabetes mellitus type 2 are diagnosed more frequently within the same individual than would be predicted by chance, even when age is considered (11, 12).

Hundreds of active components found in herbs can be used to produce pharmacological agents. It has been established that the high biochemical specificity, phytochemical diversity, and other molecular properties of natural products make them valuable as model structures for drug production. Hundreds of active components found in herbs can be used to produce pharmacological agents (3-5). In recent years, there has been a significant increase in the demand for and use of dietary supplements and drugs extracted from plants. Microbiologists, pharmacologists, botanists, and natural products chemist's combine the earth for medicinal plants to treat various illnesses (13-15).

Many investigations approved that the medicinal plants' constituents are affected by many factors, including geographical locations, types of soil, rainfall, and many other factors, so there is a differentiation between Ramallah and Jerusalem regions in the kind of soil, so we compared their biological activities (16-19). Therefore, the study aimed to evaluate the antidiabetic and anticancer properties of *T. orientale* leaf extracts from Palestine's Jerusalem and Ramallah governorates.

MATERIALS AND METHODS

Plant collection, preparation, and extraction

The aerial parts of the *T. orientale* plant were collected in June 2021 from two Palestinian regions (Jerusalem and Ramallah) during the flowering period. A pharmacognosist, Professor N. Jaradat, identified the plant using a reference book (20), at the Department of Pharmacy at An-Najah National University in the Pharmacognosy Laboratory. A specimen (Pharm-PCT-2413A and Pharm-PCT-2413B, respectively) has been deposited in the same laboratory for both samples. The fresh aerial parts of *T. orientale* were washed with clean water and dried in ordinary conditions for two weeks. Then, the dried parts were powdered and stored in special glass jars for upcoming work. The hydrophilic extract was prepared by immersing 400 g of dried *T. oriental*e samples in 4 L of boiled water for 3 days. The resulting extracts were filtered twice to ensure clarity. Then, the filtered extracts were subjected to vacuum freeze-drying using a Stellar Laboratory freeze dryer (Millrock Technology Inc., NY, USA) for 48 h. The freeze-dried extracts were then stored in a closed container at 4 °C in the refrigerator (21).

Finally, using this formula, the fractionations yields was determined:

$$\% \text{ Yield} = \frac{\text{Weight of } T.orientale \text{ extract}}{\text{weight of a dry plant}} \text{ X } 100\% \qquad \text{Equation (1)}$$

Jerusalem and Ramallah *T. orientale* hydrophilic extract yields were 7.06 and 8.15%, respectively.

The α-Amylase enzyme inhibitory method

The inhibitory assay for α-amylase was performed as described by Dastjerdi et al. (21). The antidiabetic drug acarbose was used as a positive control. Control was performed similarly, with 1 ml of 10% DMSO replacing the extracts. The following concentrations were utilized in this study: 50, 70, 100, 200, and 500 µg/ml for the plant extracts and Acarbose. The plant extract working solution (1 mg/ml) was produced by dissolving 25 mg of each plant fraction in 10% DMSO, and then a buffer solution of up to 25 ml was added. The absorbance of the tested samples was measured at 540 nm by applying the spectrophotometer by which our blank was 10 % DMSO. The inhibitory potential of α-amylase was measured by the formula shown below.

$$\text{I } (\%) = \frac{\text{ABS control} - \text{ABS extract}}{\text{ABS control}} \text{ X } 100\% \qquad \text{Equation (2)}$$

Where I (%) is the α-amylase percent inhibition.

Cell culture

The Lewis Lung Carcinoma (LLC) cancer cell line and epithelial cells isolated from normal human bronchial epithelium derived from autopsies of noncancerous individuals (BEAS-2B cells; ATCC; CRL-3588) were used as a control. Cells were cultured in high-glucose DMEM with 100 U/ml penicillin G, 10% fetal bovine serum (FBS), and 100 µg/ml streptomycin in an atmosphere of 5% CO_2 at 37 °C. LLC cell line is resistant to 1,3-bis-(2-chloroethyl)-1-nitrosourea but is sensitive to methotrexate and DMSO (4). The cells are reported to be highly tumorigenic but weakly metastatic in mice. Therefore, we speculated that when performing the α-amylase activity and the proliferation assays, the *T. orientale* plant solubilized in DMSO could have better results in the selected cell line than in other cell lines (22).

XTT cell proliferation assay and cell viability

The Cell Proliferation Kit II (XTT) was used based on a colorimetric assay to quantify cellular proliferation, viability, and cytotoxicity (23). LLC and BEAS-2B cells were seeded at approximately 1×10^3 per well in a final 100 µL DMEM medium volume in 96-well flat-bottom microtiter plates. Following overnight incubation, LCC cells were treated with both extracts of *T. orientale* (Jerusalem's or Ramallah's extracts) at concentrations of (0, 50, 100, 200, and 400 µg/ml) for 24 hours. At the end of incubation, 100 µL of XTT (Merk, 11465015001) was added to each well, and plates were then incubated at 37°C for an additional 4 h. Absorbance was measured at 450 nm against a reference wavelength at 650 nm using a microplate reader (Beckman Coulter, DTX 880 Multimode Reader). We used the formula (A450–A670) of test cells/(A450–A650) of the blank to calculate the viability data. We used the Ceilometer automatic cell counter (Nexcelom Inc., USA) to verify the viability of LCC cells trypan blue dye exclusion test.

Detection of cellular apoptosis by flow cytometry

LCC and BEAS-2B cells (1×10^6 cells/well) were incubated in 6-well plates and treated with both extracts of *T. orientale* (Jerusalem's or Ramallah's extracts) at different concentrations of (0, 50, 100, 200, and 400 µg/ml) for 24 hours. For apoptosis and viability measurements, propidium-iodide (PI) and annexin V-conjugated to FITC (R&D Systems, Minneapolis, MN) were used to stain fragmented DNA and phosphatidylserine, respectively. Annexin-V (+) but propidium-iodide (-) defined early apoptosis, while annexin-V (+) but propidium-iodide (+) defined late apoptosis. Viable cells were annexin-V (-) and propidium-iodide (-). Unstained IgG isotype and FMO controls were used in every experimental setting. The analyses of cells were performed using BD LSR Fortessa cell analyzer, BD Biosciences, Mountain View, CA).

Statistical analysis

The data were presented as mean ± SD. Statistical

differences were analyzed by one-way analysis of variance (with Newman-Keuls post-tests among multiple groups) or with a two-tailed unpaired Student's t-test (for comparison between two groups). For the in vitro study, each experiment was repeated three times. The averages were then calculated and presented along with the standard deviation, and the results with $P<0.05$ were considered significant.

RESULTS

Antidiabetic α-amylase inhibitory activity

The inhibitory effect of *T. orientale* extract from Jerusalem and Ramallah governorates in Palestine on α-amylase was assessed at 50-500 µg/ml (Figure 1). Among the extractives, *T. orientale* extract from Jerusalem possessed higher α-amylase enzyme inhibitory activity. The α-amylase inhibitory activity of *T. orientale* extract from Jerusalem was 90.05±0.33% at a concentration of 500 µg/mL. In contrast, the standard Acarbose was 72.54±0.29% at the same concentration. At the same time, the *T. orientale* extract from the Ramallah region was 77.37±0.35%. The α-amylase enzyme inhibitory IC_{50} dose of *T. orientale* extract from Jerusalem was 7.43±0.84 µg/ml, while the Ramallah *T. orientale* sample IC_{50} dose was 23.2±0.29 µg/ml. In addition, the IC_{50} of Acarbose (positive control) was 43.91±1.08 µg/ml. The α-amylase enzyme inhibitory activity of *T. orientale* extracts and Acarbose were in the following order: Jerusalem extracts > Ramallah extract > Acarbose. These findings suggest that both extracts have potent α-amylase enzyme inhibitory activity and are more powerful than Acarbose (24, 25).

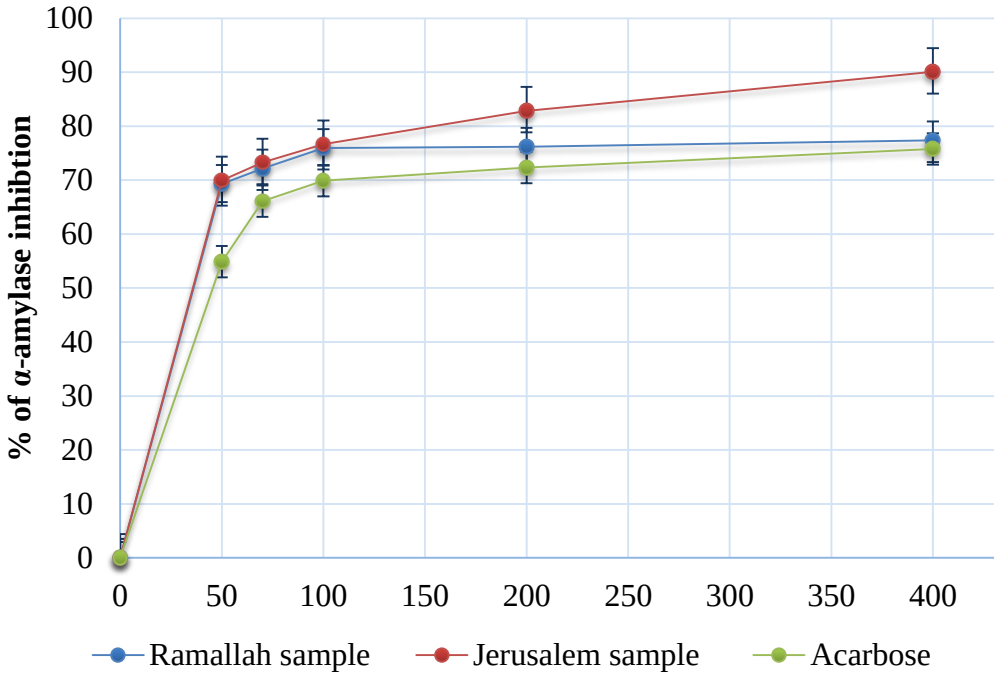

Figure 1. α-Amylase inhibitory effects of *T. orientale* extracts from Jerusalem and Ramallah regions of Palestine and Acarbose ($p< 0.05$)

***T. orientale* inhibits Lewis Lung Carcinoma (LLC) cell viability and induces apoptosis.**

To assess whether *T. orientale* could exert anticancer effects and influence lung cells viability, LCC and BEAS-2B cells were treated with one of both extracts of *T. orientale* (Jerusalem's or Ramallah's extract) at different concentrations of 0, 50, 100, 200, and 400 µg/ml for 24 h, and cell proliferation was inspected using XTT assay.

Figure 2 displays an inverse correlation obtained following increased concentrations of *T. orientale* on the LCC cell proliferation. Total inhibition of LCC proliferation was achieved at 400 µg/ml concentration in the Jerusalem (black line) and Ramallah (dash-dotted gray line) extracts. *T. orientale* of the Jerusalem extracts demonstrated a more efficient inhibitory effect at lower concentrations. *T. orientale* from both geographical areas of Ramallah and Jerusalem showed no significant effects on the viability of the lung cells of the control cells. Statistical analysis values of the t-test are presented in appendix 1.

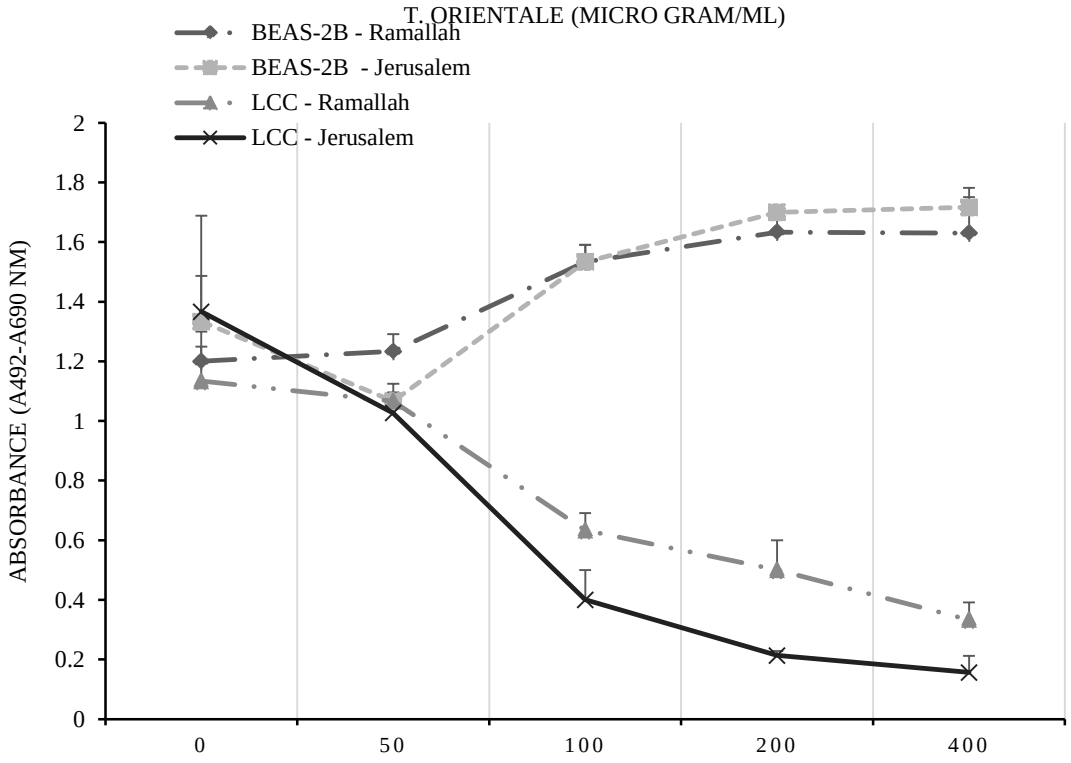

Figure 2. Effects of *T. orientale* on LCC cell proliferation

The decrease in LLC proliferation following *T. orientale* treatment was then evaluated to determine whether it was associated with changes in the apoptotic rate. Cells undergoing apoptosis exhibit cell surface phosphatidylserine (PS), which was estimated by staining with a fluorescent conjugate of Annexin-V. Necrotic cells were stained using propidium iodide (PI). Our results indicate that *T. orientale* treatment promoted late-stage apoptosis (Annexin V+/PI+) for both plant extracts (Figure 3).

The average percentage of apoptosis in all untreated *T. orientale* LLC and control cells was 9.30±2.8%. Increasing concentrations of *T. orientale* (50, 100, 200, and 400 µg/ml) from the two geographical areas, Ramallah and Jerusalem, did not affect the apoptosis rate of BEAS-2B cells (*P*=ns). In contrast, apoptosis rates significantly increased following treatment with *T. orientale* extract in LLC cells at all tested concentrations, which was positively associated with the late apoptosis marker Annexin-V+/PI+.

Moreover, the *T. orientale* extract from Jerusalem exhibited an apoptotic rate of 90±3.4% at the highest concentration of 400 µg/ml, compared to 62.6±3.4% following treatment with the Ramallah extract (*P*<0.05). This indicates that the *T. orientale* extract from Jerusalem mediates anticancer activity more efficiently than the extract from Ramallah. Statistical analysis values from the t-test are presented in Appendix 2.

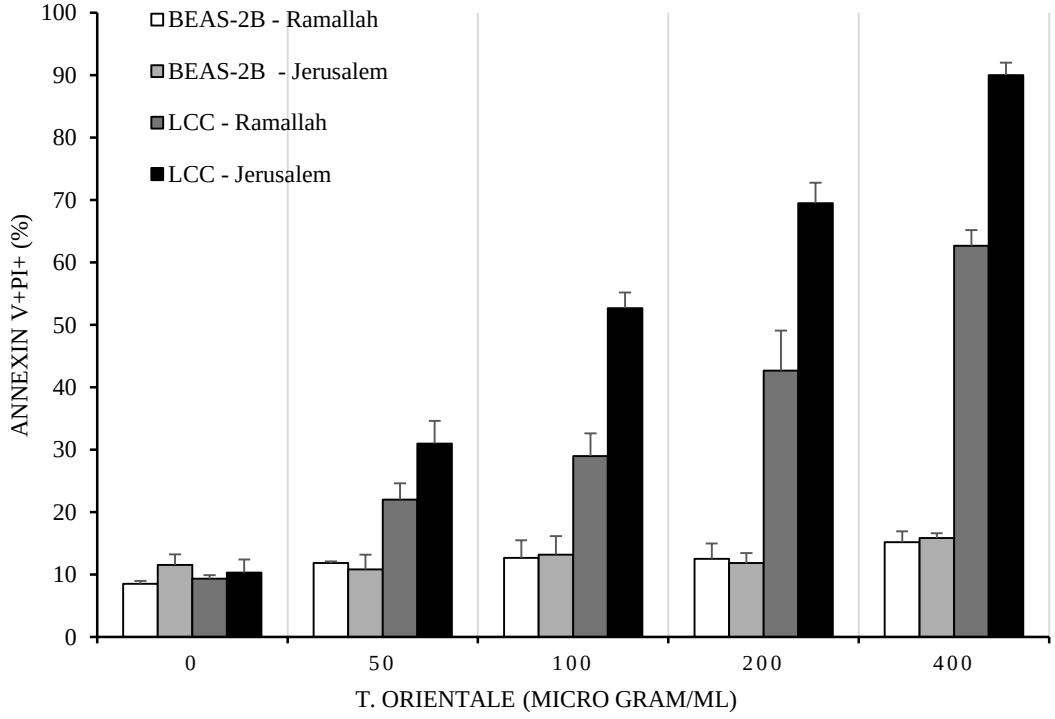

Figure 3. *T. orientale induced* apoptosis in LCC cells, measured by flow cytometry

DISCUSSION

Many bioactive compounds are derived from plants; more than 25% of available pharmaceutical forms are prepared from plant origin (26). Moreover, better therapeutic outcomes in clinical practice were reported for herbal medicine compared to chemically synthetic medications. Thus, identifying new herbal products with potential anticancer capacities has attracted notable attention in the pharmaceutical and cosmetics industries (27, 28).

Among several other Lamiaceae species, methanol extracts of *T. orientale* showed moderate to high inhibitory

effects on the enzymes α-amylase and α-glucosidase (29). In an earlier study, variable concentrations of hydroalcoholic, dichloromethane, and ethyl acetate were extracted from three *Teucrium* species and were tested for α-amylase inhibition using Acarbose as the standard inhibitor. All three studied extracts demonstrated potent inhibitory effects on the α-amylase activity. The IC_{50} for the dichloromethane, ethyl acetate, and hydroalcoholic were 22.59, 8.55, and 13.93 mg/ml, respectively (30). Previous research proved that acetone and methanol extracts of *T. orientale* had potent antioxidant activities (2, 5). Another study found that methanol extract led to a progressive increase in weight in diabetic rats and improved the associated hematological abnormalities. The authors suggested that weight might result from the fact that the extract leads to better utilization of nutrients in the diet (31). The possible role of *T. orientale* in the correction of hyperglycemia and, subsequently, in the prevention of diabetic complications was observed in another study (32). Acarbose is a hypoglycemic pharmaceutical formulation utilized to prevent and treat hyperglycemia and, in certain regions, used to treat prediabetes (33). Identifying a natural plant product with an antidiabetic effect that exceeds the potent effect of Acarbose is of great importance. Other plant species with in vitro anti-α-amylase and anti-α-glucosidase effects including *Achillea santolina*, *Coriandrum sativum*, *Teucrium barbeyanum***,** and *Teucrium polium* were also previously identified (34-36). Shown in Table 1.

Table 1. Amylase IC_{50} values of different plant extracts

Plant	IC_{50} of α- amylase inhibitory activity	Reference
Ethanol and hexane extracts of *Phyllanthus amarus*	36.05±4.01 µg/ml and 48.92±3.43 µg/ml, respectively	(37)
Ethyl acetate extract of *Phlomis bruguieri*	1.9 µg/ml	(38)
Teucrium polium	3.63 mg/ml	(39)
Teucrium oliverianum	3.86 mg/ml	(39)
Ethanol extracts of *Allium akaka*, *Allium sativum*, *Allium porrum*, and *Allium cepa*	16.74, 17.95, 15.73, and 16.36 mg/ml, respectively	(40)
Pomegranate leaves extract	43.24 µg/ml	(41)
Ethanolic extract of *Andrographis paniculata*	50.9±0.17 mg/ml	(42)
DMSO plant extract of *Teucrium Orientale*	13.93 mg/ml	(15)

One of the antidiabetic treatment protocols is to suppress carbohydrate metabolism in the gastrointestinal tract by inhibiting the action of enzymes like α-amylase involved in carbohydrate metabolism. Medicinal herbs delay glucose absorption by inhibiting the carbohydrate hydrolyzing enzymes such as pancreatic α-amylase. When this enzyme is inhibited, it slows down carbohydrate digestion. It extends the carbohydrate digestion period, reducing the rate of glucose absorption and, hence, reducing the postprandial plasma glucose level (43-45). Numerous studies demonstrated the inhibitory effects of the α-amylase enzyme of traditional medicinal herbs or their isolated products (37, 46, 47).

Most of the anticancer compounds were derived from natural sources (48). It is identified in the literature that more than 290 essential oils and non-volatile extracts are found in various species within the *Teucrium* taxa (3). *Teucrium* species were found to be rich in polyphenolic compounds, which might directly contribute to their antiproliferative and proapoptotic activity. Antioxidant, antiproliferative, and apoptotic effects of several *Teucrium* species, such as *T. chamaedrys*, *T. arduini*, and *T.*

montanum were previously identified (49). *T. persicum* showed a potent anti-tumor effect in a highly invasive prostate cancer cell line (50). *T. chamaedrys* methanol extract modulates apoptosis and biotransformation in colorectal carcinoma cells, causing apoptosis of SW480 cells, without affecting normal HaCaT keratinocytes (51). *Teucrium orientale* extract is found to be rich in chemical ccompounds such as aldehydes, hydrocarbons, monoterpene hydrocarbons, and other volatile components (52). However, the hydophilc leaf extract of *T. orientale ,* the anticancer effect of *T. orientale*, and the apoptotic potential of *T. oriental* was not previously studied to the best of our knowledge. The current study revealed the anti-diabetic, antiproliferative and apoptotic effects of *T. orientale* hydrophilic leaf extract on an in vitro model.

Besides habitat-related anatomic and morphological adaptation of *Teucrium* (Lamiaceae) species, total phenol quantity and essential oils identity were varied in different *Teucrium* species based on the geological substrate and habitat conditions such as soil mineral and water content (53-58). In the current study, the aerial parts of *T. orientale* from two regions in Palestine, Jerusalem and Ramallah were isolated and tested for their antidiabetic and anticancer effects, which are variable based on the geographical area. Although there are geographical similarities between the two regions regarding the "terra rossa" soil, Jerusalem's soil is more clay than Ramallah's. The characteristics of clay soils include that they are heavy, rich in nutrients, wet in winter, and dry in summer. Moreover, the temperature difference varies between the two cities; Jerusalem is 2 to 3 degrees less than Ramallah. Total annual rainfall in Ramallah is>700mm, while in Jerusalem ranges from 300-500 mm. These differences could contribute to the superior effects of *T. orientale* of Jerusalem concerning their antidiabetic and anticancer properties (59).

Presently, there are several antidiabetic drugs used for treating and managing diabetes. Several modes of action were proposed, including inhibiting the enzymes α-amylase, lipase, α-glucosidase, and DPP-IV. Our results indicated that the extract inhibited α-amylase even better than the Acarbose drug. Moreover, *T. orientale* caused cell death via apoptosis/necrosis of LCC in favor of the Jerusalem extract. Data was associated with reduced proliferation, indicating the ability of the *T. orientale* to prepare the cells to shift to necrosis.

CONCLUSION

The current study on the leaf hydrophilic extracts of T. orientale aerial parts from two Palestinian regions demonstrated potential α-amylase inhibitory activity compared to the antidiabetic drug Acarbose. Additionally, both T. orientale extracts inhibited LLC cell proliferation and induced cell death via apoptosis/necrosis, with the Jerusalem extract showing greater efficacy. Furthermore, this study highlights opportunities for future research in the search for novel, effective, natural antidiabetic and anticancer therapies. Further in vivo studies are needed to confirm these findings.

Appendix 1: The t-test analysis of the four tested groups of XTT assay

TTEST					
	0	**50**	**100**	**200**	**400**
AB	0.13728831	0.01205506	0.5	0.06003945	0.16830581
AC	0.2458835	0.00552825	2.2173E-05	3.5105E-05	3.7449E-05
AD	0.21975577	0.00269917	3.5105E-05	1.0388E-06	2.1848E-05
BC	0.072352	0.5	2.2173E-05	1.6146E-05	5.1662E-06
BD	0.5	0.00806504	0.03312991	0.06875769	0.05584542
CD	0.43949819	0.17432057	3.5105E-05	7.5794E-09	2.9524E-06

A=BEAS-2B – Ramallah B=BEAS-2B - Jerusalem
C=LCC – Ramallah D=LCC - Jerusalem

Appendix 2: The t-test analysis of the four tested groups of apoptosis assay

TTEST					
	0	**50**	**100**	**200**	**400**
AB	0.01976872	0.25357875	0.42232288	0.35871418	0.28950771
AC	0.06588878	0.0013528	0.00176113	0.00081438	5.7528E-06
AD	0.10608033	0.00039131	2.6529E-05	9.0208E-06	5.3181E-07
BC	0.04825265	0.00274891	0.00214858	0.00064378	3.2917E-06
BD	0.01673587	0.02388436	0.07745599	0.03815193	0.05527494
CD	0.23522311	0.00063056	3.1842E-05	5.3113E-06	2.31E-07

A=BEAS-2B – Ramallah B=BEAS-2B - Jerusalem
C=LCC – Ramallah D=LCC - Jerusalem

Data Availability

All data supporting the findings of the study are included in the manuscript.

Authors contribution

The authors performed all parts of this study.

Conflict of interest statement

The authors declare that they have no conflict of interest.

Financial support

This study received no funds.

Acknowledgment

The authors would like to thank An-Najah National University.

REFERENCES

1. Candela RG, Rosselli S, Bruno M, and Fontana G. A review of the phytochemistry, traditional uses and biological activities of the essential oils of genus Teucrium. *Planta Medica*. 2020; 87(06): 432-79.
2. Alviri M, Bahadori MB, and Bahadori S. Flavonoid and Diterpenoid Components from Teucrium orientale subsp. orientale and their Radical Scavenging Activity. *Biointerface Research in Applied Chemistry*. 2022; 12(1): 682-9.
3. Abdullah FO, Hussain FH, Sardar AS, Gilardoni G, Thu ZM, and Vidari G. Bio-Active compounds from Teucrium plants used in the traditional medicine of Kurdistan Region, Iraq. *Molecules*. 2022; 27(10): 3116.
4. Jaradat NA. Review of the taxonomy, ethnobotany, phytochemistry, phytotherapy and phytotoxicity of germander plant (Teucrium polium L.). *Asian Journal of Pharmaceutical and Clinical Research*. 2015; 8(2): 13 - 9.
5. Çakir A, Mavi A, Kazaz C, Yildirim A, and KÜFREVİOĞLU Öİ. Antioxidant activities of the extracts and components of Teucrium orientale L. var. orientale. *Turkish Journal of Chemistry*. 2006; 30(4): 483-94.
6. Reaisi Z, Yadegari M, and Shirmardia HA. Effects of Phenological Stage and Elevation on Phytochemical Characteristics of Essential Oil of Teucrium polium L. and Teucrium orientale L. *International Journal of Horticultural Science and Technology*. 2019; 6(1): 89-99.
7. Wang M, Yang Y, and Liao Z. Diabetes and cancer: epidemiological and biological links. *World journal of diabetes*. 2020; 11(6): 227. doi: 10.4239/wjd.v11.i6.227.
8. Hu J, Fan H-D, Gong J-P, and Mao Q-S. The relationship between the use of metformin and the risk of pancreatic cancer in patients with diabetes: a systematic review and meta-analysis. *BMC gastroenterology*. 2023; 23(1): 1-21.
9. Dąbrowski M. Diabetes, antidiabetic medications and cancer risk in type 2 diabetes: focus on SGLT-2 inhibitors. *International Journal of Molecular Sciences*. 2021; 22(4): 1680.

10. Abdalla B, Mansour M, Ghanim M, Aia B, and Yassin M. The growing burden of cancer in the Gaza Strip. *The Lancet Oncology*. 2019; 20(8): 1054-6.
11. Ballotari P, Vicentini M, Manicardi V, Gallo M, Ranieri SC, and Greci M, et al. Diabetes and risk of cancer incidence: results from a population-based cohort study in northern Italy. *BMC cancer*. 2017; 17(1): 1-8.
12. Zhu B. and Qu S. The relationship between diabetes mellitus and cancers and its underlying mechanisms. *Frontiers in Endocrinology*. 2022; 13: 800995.
13. Yuliana ND, Khatib A, Choi YH, and Verpoorte R. Metabolomics for bioactivity assessment of natural products. *Phytotherapy research: PTR*. 2011; 25(2): 157-69.
14. James Hamuel D. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In: Venketeshwer R, editor. Phytochemicals. *Rijeka: IntechOpen*; 2012. p. Ch. 1. In: Benzie IFF, Wachtel-Galor S, editors. Herbal Medicine: Biomolecular and Clinical Aspects. Boca Raton (FL): CRC Press/Taylor & Francis Copyright © 2011 by Taylor and Francis Group, LLC.; 2011.
15. Xu W, Cheng Y, Guo Y, Yao W, and Qian H. Effects of geographical location and environmental factors on metabolite content and immune activity of Echinacea purpurea in China based on metabolomics analysis. *Industrial Crops and Products*. 2022; 189: 115782.
16. Liu Y, Chen P, Zhou M, Wang T, Fang S, and Shang X, et al. Geographic variation in the chemical composition and antioxidant properties of phenolic compounds from Cyclocarya paliurus (Batal) Iljinskaja leaves. *Molecules*. 2018; 23(10): 2440.
17. Jaradat N, Qneibi M, Hawash M, Al-Maharik N, Qadi M, and Abualhasan MN, et al. Assessing Artemisia arborescens essential oil compositions, antimicrobial, cytotoxic, anti-inflammatory, and neuroprotective effects gathered from two geographic locations in Palestine. *Industrial Crops and Products*. 2022; 176: 114360.
18. Ghenabzia I, Hemmami H, Ilham B, Zeghoud S, Seghir BB, and Hammoudi R. Different methods of extraction of bioactive compounds and their effect on biological activity: A review. *International Journal of Secondary Metabolite*. 2023; 10(4): 469-94.
19. Townsend CC. Kew Bulletin. 1980; 35(3): 705-7.
20. Nishiyama J, Kuninori T, and Matsumoto H. The use of 1, 1-diphenyl-2-picrylhydrazyl for detecting free radicals in wheat flour dough. *Journal of the Science of Food and Agriculture*. 1978; 29(3): 267-73.
21. Bertram JS. and Janik P. Establishment of a cloned line of Lewis Lung Carcinoma cells adapted to cell culture. *Cancer letters*. 1980; 11(1): 63-73.
22. Assali M, Kittana N, Alhaj-Qasem S, Hajjyahya M, Abu-Rass H, and Alshaer W, et al. Noncovalent functionalization of carbon nanotubes as a scaffold for tissue engineering. *Scientific Reports*. 2022; 12(1): 12062.
23. Jaradat N, Khasati A, Hawi M, Hawash M, Shekfeh S, and Qneibi M, et al. Antidiabetic, antioxidant, and anti-obesity effects of phenylthio-ethyl benzoate derivatives, and molecular docking study regarding α-amylase enzyme. *Scientific Reports*. 2022; 12(1): 3108.
24. Jaradat N, Qneibi M, Hawash M, Sawalha A, Qtaishat S, and Hussein F, et al. Chemical Composition, Antioxidant, Antiobesity, and Antidiabetic Effects of Helichrysum sanguineum (L.) Kostel. from Palestine. *Arabian Journal for Science and Engineering*. 2021; 46(1): 41-51.
25. Rates SMK. Plants as source of drugs. *Toxicon*. 2001; 39(5): 603-13.
26. Karimi A, Majlesi M, and Rafieian-Kopaei M. Herbal versus synthetic drugs; beliefs and facts. *Journal of nephropharmacology*. 2015; 4(1): 27.
27. Jaradat N, Al-Lahham S, Abualhasan MN, Bakri A, Zaide H, and Hammad J, et al. Chemical constituents, antioxidant, cyclooxygenase inhibitor, and cytotoxic activities of Teucrium pruinosum boiss. Essential oil. *BioMed Research International*. 2018; 2018.

28. Eskandani M, Babak Bahadori M, Zengin G, Dinparast L, and Bahadori S. Novel natural agents from Lamiaceae family: An evaluation on toxicity and enzyme inhibitory potential linked to diabetes mellitus. *Current Bioactive Compounds*. 2016; 12(1): 34-8.
29. Dastjerdi ZM, Namjoyan F. and Azemi ME. Alpha amylase inhibition activity of some plants extract of Teucrium species. *European Journal of Biological Sciences*. 2015; 7(1): 26-31.
30. Tahmasebpour N, Dehghan G, Feizi MAH, and Esmaeili HA. Variation in body weight and some hematological parameters in streptozotocin-induced diabetic rats, treated with Teucrium orientale. *Pharmacology online*. 2013; 3: 32-6.
31. Dehghan G, Tahmasebpour N, Hosseinpourfeizii M, Sheikhzadeh F, and Banan Khojasteh S. Hypoglycemic, antioxidant and hepato-and nephroprotective effects of Teucrium orientale in streptozotocin diabetic rats. *Pharmacol online*. 2013; 1: 182-9.
32. Smith DL, Orlandella RM, Allison DB, and Norian LA. Diabetes medications as potential calorie restriction mimetics—a focus on the alpha-glucosidase inhibitor acarbose. *GeroScience*. 2021; 43: 1123-33.
33. Kasabri V, Afifi FU, and Hamdan I. In vitro and in vivo acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. *Journal of ethnopharmacology*. 2011; 133(2): 888-96.
34. Asghari AA, Mokhtari-Zaer A, Niazmand S, Mc Entee K, and Mahmoudabady M. Anti-diabetic properties and bioactive compounds of Teucrium polium L. *Asian Pacific Journal of Tropical Biomedicine*. 2020; 10(10): 433.
35. Eid A, Jaradat N, Issa L, Khraiwesh E, and Yaish Z. Qualitative analysis of the antioxidant, carbohydrates, and lipids enzymes inhibitory effects of Coriandrum sativum seeds; a member of Palestinian flora. *Palestinian Medical and Pharmaceutical Journal*. 2023; 8(2): 11.
36. Tamil IG, Dineshkumar B, Nandhakumar M, Senthilkumar M, and Mitra A. In vitro study on α-amylase inhibitory activity of an Indian medicinal plant, Phyllanthus amarus. *Indian journal of pharmacology*. 2010; 42(5): 280.
37. Safamansouri H, Nikan M, Amin G, Sarkhail P, Gohari AR, and Kurepaz-Mahmoodabadi M. et al. α-Amylase inhibitory activity of some traditionally used medicinal species of Labiatae. *Journal of Diabetes & Metabolic Disorders*. 2014; 13(1): 1-5.
38. Hussain SA, Hameed A, Fu J, Xiao H, Liu Q, and Song Y. Comparative in vitro analysis of anti-diabetic activity of Indo-Pak black cardamom (Amomum subulatum Roxb.) and Chinese black cardamom (Amomum tsao-ko Crevost et Lemaire). *Prog Nutri*. 2018; 20: 403-14.
39. Nikavar B. and Yousefian N. Inhibitory effects of six Allium species on α-amylase enzyme activity. 2009.
40. Yuniarto A, Sukandar EY, Fidrianny I, Setiawan F, and Ketut I. Antiobesity, Antidiabetic and Antioxidant Activities of Senna (Senna alexandrina Mill.) and Pomegranate (Punica granatum L.) Leaves Extracts and Its Fractions. *International Journal of Pharmaceutical and Phytopharmacological Research* (eIJPPR). 2018; 8(3): 18-24.
41. Subramanian R, Asmawi MZ, and Sadikun A. In vitro alpha-glucosidase and alpha-amylase enzyme inhibitory effects of Andrographis paniculata extract and andrographolide. *Acta Biochimica Polonica*. 2008; 55(2): 391-8.
42. Nakajima K. Low serum amylase and obesity, diabetes and metabolic syndrome: A novel interpretation. *World journal of diabetes*. 2016; 7(6): 112.
43. Kaur N, Kumar V, Nayak SK, Wadhwa P, Kaur P, and Sahu SK. Alpha-amylase as molecular target for treatment of diabetes mellitus: A comprehensive review. *Chemical Biology & Drug Design*. 2021; 98(4): 539-60.

44. Mukattash HK, Issa R, Abu Hajleh MN, and Al-Daghistani H. Inhibitory Effects of Polyphenols from Equisetum ramosissimum and Moringa peregrina Extracts on Staphylococcus aureus, Collagenase, and Tyrosinase Enzymes: In vitro Studies. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17(3): 530-48.
45. Jaradat N, Al-Maharik N, Abdallah S, Shawahna R, Mousa A, and Qtishat A. Nepeta curviflora essential oil: phytochemical composition, antioxidant, anti-proliferative and anti-migratory efficacy against cervical cancer cells, and α-glucosidase, α-amylase and porcine pancreatic lipase inhibitory activities. *Industrial Crops and Products*. 2020; 158: 112946.
46. Jaradat NA, Al-lahham S, Zaid AN, Hussein F, Issa L, and Abualhasan MN, et al. Carlina curetum plant phytoconstituents, enzymes inhibitory and cytotoxic activity on cervical epithelial carcinoma and colon cancer cell lines. *European Journal of Integrative Medicine*. 2019; 30: 100933.
47. Tan G, Gyllenhaal C, and Soejarto D. Biodiversity as a source of anticancer drugs. *Current drug targets*. 2006; 7(3): 265-77.
48. Stankovic MS, Curcic MG, Zizic JB, Topuzovic MD, Solujic SR, and Markovic SD. Teucrium plant species as natural sources of novel anticancer compounds: antiproliferative, proapoptotic and antioxidant properties. *International journal of molecular sciences*. 2011; 12(7): 4190-205.
49. Tafrihi M, Toosi S, Minaei T, Gohari AR, Niknam V, and Arab Najafi SM. Anticancer properties of Teucrium persicum in PC-3 prostate cancer cells. *Asian Pacific Journal of Cancer Prevention*. 2014; 15(2): 785-91.
50. Milutinović MG, Maksimović VM, Cvetković DM, Nikodijević DD, Stanković MS, and Pešić M, et al. Potential of Teucrium chamaedrys L. to modulate apoptosis and biotransformation in colorectal carcinoma cells. *Journal of ethnopharmacology*. 2019; 240: 111951.
51. Yildirmiş S, Aliyazicioglu R, Emre Eyupoglu O, Ozgen U, and Alpay Karaoglu S. Biological Activity and Characterization of Volatile Compounds of Teucrium orientale Var. glabrescens by SPME and GC-FID/MS. *Journal of Food Biochemistry*. 2017; 41(1): e12284.
52. DÖNMEZ İ. VOLATILE OIL COMPOSITION OF TEUCRIUM SPECIES OF NATURAL AND CULTIVATED ORIGIN IN THE LAKE DISTRICT OF TURKEY. *Applied Ecology & Environmental Research*. 2022; 20(3).
53. Zlatić NM, Stanković MS, and Simić ZS. Secondary metabolites and metal content dynamics in Teucrium montanum L. and Teucrium chamaedrys L. from habitats with serpentine and calcareous substrate. *Environmental monitoring and assessment*. 2017; 189: 1-15.
54. Afifi F, Abu–Irmaileh B, and Al-Noubani R. Comparative analysis of the essential oils of Teucrium polium L. grown in different arid & semi arid habitats in Jordan. *Jordan Journal of Pharmaceutical Sciences*. 2009; 2(1).
55. Lakušić B, Stevanović B, Jančić R, and Lakušić D. Habitat-related adaptations in morphology and anatomy of Teucrium (Lamiaceae) species from the Balkan peninsula (Serbia and Montenegro). *Flora-Morphology, Distribution, Functional Ecology of Plants*. 2010; 205(10): 633-46.
56. Alhalaseh L, Issa R, Said R, and Al-suhaimat R. Antioxidant Activity, Phytochemical Screening published. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17.
57. Al-Gharaibeh MM, Maslat AO, Kanakr NAA, and Jarra YB. In Vivo Evaluation of Genotoxicity and Antioxidant Capacity of Ajuga Orientalis L. (Lamiaceae) Leaf Extracts. *Jordan Journal of Pharmaceutical Sciences*. 2024; 17(4): 755-66.
58. Richard M. and Issac J. Analysis of climatic variability and its environmental impacts across the occupied Palestinian territory. Water and Environment Research Department, Applied Research Institute–Jerusalem Bethlehem, Palestine. 2012.

تحليل مختبري للتأثيرات المضادة للسرطان والسكري لمستخلص أوراق Teucrium orientale المحب للماء المزروع في منطقتين جغرافيتين في فلسطين

جوني عامر[1]، مصطفى غانم[2*]، احمد سلهب[2]، نضال جرادات[3]، مالك القب[2*]، مها ربايعه[2]، لانا حلاق[3]، تسنيم عبد الحي[3]، صبا دويات[3]، ليند عيسى[3]، مجدي دويكات[1]

[1] دائرة العلوم الطبية التطبيقية والمساندة، كلية الطب وعلوم الصحه، جامعة النجاح الوطنية، نابلس، فلسطين.

[2] دائرة العلوم الطبية الحيوية، كلية الطب وعلوم الصحه، جامعة النجاح الوطنية، نابلس، فلسطين.

[3] دائرة الصيدلة، كلية الطب وعلوم الصحه، جامعة النجاح الوطنية، نابلس، فلسطين.

ملخص

أظهرت العديد من الدراسات أن أنواع Teucrium orientale تمتلك خصائص علاجية مهمة، بما في ذلك النشاط المضاد للأكسدة، والمضاد للجراثيم، والمضاد للالتهابات، إضافةً إلى تأثيرها المزيل للتشنجات. تهدف هذه الدراسة إلى تقييم الأنشطة المحتملة المضادة لمرض السكري والسرطان لمستخلصات أوراق Teucrium orientale المحبة للماء، التي جُمعت من منطقتين جغرافيتين في فلسطين: القدس ورام الله. تم اختبار المستخلص المائي للنبات لتحديد تأثيره المضاد لمرض السكري عبر قياس تثبيط نشاط إنزيم α-amylase، بينما تم تقييم تأثيره المضاد للسرطان على خلايا سرطان الرئة Lewis Lung (LLC)باستخدام قياس التدفق الخلوي لفحص تكاثر الخلايا، بالإضافة إلى تلطيخ Annexin-V / بروبيديوم يوديد (PI) للكشف عن موت الخلايا المبرمج. أظهر مستخلص Teucrium orientale من القدس قدرة تثبيطية قوية لإنزيمα-amylase بقيمة IC50 تبلغ 7.43 ± 0.84 ميكروغرام/مل، مقارنة بـ IC50 لمستخلص رام الله الذي بلغ 23.2 ± 0.29 ميكروغرام/مل. كانت هذه القيم أفضل من الشاهد الإيجابي Acarbose، الذي سجل IC50 =43.91 ± 1.08ميكروغرام/مل. في اختبار التأثير المضاد للسرطان، تم تعريض خلايا LLC لتركيزات مختلفة من مستخلصي T. orientale (0، 50، 100، 200، و400 ميكروغرام/مل) لمدة 24 ساعة، وتم قياس تكاثر الخلايا باستخدام اختبار XTT لوحظ التثبيط الكامل لنمو الخلايا عند 400 ميكروغرام/مل في كلا المستخلصين، إلا أن مستخلص القدس أظهر كفاءة أكبر في التثبيط عند التركيزات المنخفضة. من ناحية أخرى، لم تؤثر الزيادة في تراكيز المستخلصات من المنطقتين (50، 100، 200، و400 ميكروغرام/مل) على معدل موت الخلايا المبرمج في العينات الضابطة، بينما أدى العلاج بمستخلص Teucrium orientale إلى ارتفاع ملحوظ في معدل موت الخلايا المبرمج في خلايا LLC بجميع التراكيز المختبرة. وكان هذا التأثير مرتبطًا بزيادة علامة موت الخلايا المبرمج المتأخر Annexin-V+/PI- علاوةً على ذلك، أظهر مستخلص القدس معدل موت خلايا مبرمج بلغ 90 ± 3.4% عند أعلى تركيز (400 ميكروغرام/مل)، مقارنة بـ 62.6 ± 3.4% لمستخلص رام الله، مما يشير إلى كفاءته الأعلى في تحفيز موت الخلايا المبرمج لخلايا LLC بناءً على هذه النتائج، تمتلك مستخلصات Teucrium orientale إمكانات واعدة كعوامل طبيعية مضادة لمرض السكري والسرطان، نظرًا لقدرتها على تثبيط إنزيم α-amylase، وتقليل تكاثر الخلايا السرطانية، وتعزيز موتها المبرمج. إلا أن هناك حاجة إلى مزيد من الدراسات في النماذج الحية والتجارب قبل السريرية للتحقق من هذه التأثيرات.

الكلمات الدالة: Teucrium orientale؛ ألفا الأميليز؛ سرطان الرئة لويس؛ تكاثر الخلايا؛ موت الخلايا المبرمج.

---

* ***المؤلف المراسل:***

مالك القب m.alqub@najah.edu

مصطفى غانم mustafa.ghanim@najah.edu

تاريخ استلام البحث 2024/03/20 وتاريخ قبوله للنشر 2024/07/18.