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Research paper

Total phenol, flavonoids, and tannin contents, antimicrobial, antioxidant, vital digestion enzymes inhibitory and cytotoxic activities of *Verbascum fruticulosum*

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ABSTRACT

Introduction: In Palestine, *Verbascum fruticulosum* is one of the most frequently used traditional medicinal plants. The objective of this study was to analyze the contents of the *V. fruticulosum* extract and evaluate its biological properties.

Methods: Plant total phenol, flavonoid, and tannin contents were estimated using Folin–Ciocalteu's, aluminum chloride, and vanillin-hydrochloride colorimetric assays, respectively. Reference biomedical assays were performed to evaluate the cytotoxic, antimicrobial, DPPH free radical scavenging, antilipase, α-amylase, and α-glucosidase inhibitory effects of the extracts.

Results: The methanol and acetone extracts exhibited the highest antibacterial activity against *Pseudomonas aeruginosa* with a Minimum Inhibitory Concentration (MIC) value of 1.56 mg/mL for both extracts. The n-hexane extract demonstrated the most potent activity against *Epidermophyton floccosum* fungi, with a MIC of 3.13 mg/mL. The aqueous extract exhibited excellent antioxidant activity with a Half-maximal Inhibitory Concentration (IC_{50}) of 0.71 ± 0.31 µg/mL. The acetone extract displayed the highest inhibitory activities against lipase and α-amylase, with IC_{50} values of 129.05 ± 0.48 µg/mL and 9.01 ± 1.76 µg/mL, respectively. The plant methanolic extract presented the highest inhibitory effect against α-glucosidase (IC_{50} = 75.46 ± 0.66 µg/mL) and the acetone extract exhibited the highest cytotoxic effect (IC_{50} =3.79 ± 0.01 mg/mL).

Conclusion: This study is the first to investigate the phytochemistry and inhibitory activity of *V. fruticulosum* against lipase, α-amylase, and α-glucosidase. The results indicate that V. fruticulosum extracts exhibit potential biological activities. Further, *in vivo* trials are necessary to validate these bioactivities.

1. Introduction

Since prehistoric times, people have employed numerous plants and their extracts for medicinal reasons, including the treatment of infectious illnesses [1]. Over two hundred thousand different plants' secondary metabolites have been isolated and characterized, and most of them are extensively utilized in the food, cosmetic, and pharmaceutical industries [2]. Previous investigations demonstrated that plant extracts play an important role in the treatment of cancer, obesity, diabetes, and infectious diseases due to their bioactive contents, which have medicinal properties and can be used to treat these conditions [3].

Verbascum fruticulosum Post (Scrophulariaceae) is a perennial herbaceous plant about 1 m in height and found in steppes, deserts, and areas with calcareous soil. In traditional medicine, *Verbascum* species are used to treat inflammation, bacteria, fungi, cancer, coughs, and spasms. In addition, they are used to treat tuberculosis, asthma, ear infection, itching, eczema, and sinusitis [4–6].

This study aims to explore the multifaceted properties of *V. fruticulosum* through the evaluation of its n-hexane, acetone, methanol, and aqueous extracts, including their potential as antimicrobial, antioxidant, anti-obesity, anti-diabetic, and cytotoxic agents.

Abbreviations: ATCC, American type culture collection; CA, catechin; DMSO, dimethyl sulfoxide; DPPH, 2,2-diphenyl-1-picryl-hydrazyl-hydrate; GA, Gallic Acid; IC50, half maximal inhibitory concentration; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant staphylococcus aureus; RU, rutin; SD, standard deviation.

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2. Materials and methods

2.1. Plant materials collection and classification

V. fruticulosum aerial parts were collected from the Nablus region of Palestine (32°13′20″N 35°15′40″E) during the flowering period in June 2019. A taxonomic description was provided by pharmacognosist Dr. Nidal Jaradat, and the voucher specimen (Pharm-PCT-2593) was deposited in the Department of Pharmacy at An-Najah National University. The collected leaves were air-dried in shade for two weeks at an average temperature of 20–30 °C and then were coarsely pulverized for later use.

2.2. Preparation of plant extracts

Fifty grams of air-dried plant material were macerated in n-hexane for 2 days at room temperature with occasional agitation. The produced extract was filtered and dried by a rotary evaporator at temperatures not exceeding 35 °C. The remaining plant material was then macerated in acetone and treated in the same manner, followed by maceration in methanol and finally extraction with distilled water. The filtered extracts were dried in a freeze-dryer and stored in a refrigerator at 4 °C before testing.

2.3. Qualitative phytochemical analysis

Standard qualitative analyses, as described by Trease and Evans [7], were conducted to perform phytochemical screening tests for plant metabolites, including protein, carbohydrates, phenol, tannin, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids

2.4. Determination of total tannin content

For the determination of total tannin contents, the method described by [8] was used. The absorbance of each sample was measured using spectrophotometry at 500 nm, against a methanol blank. Total tannin contents were expressed as mg catechin equivalent/g of the plant extract (mg of CAE/g of the extract).

2.5. Determination of total phenol content

The total phenolic content in the plant extracts was determined using the spectrophotometric method [9]. Stock aqueous solutions (1 mg/mL) of extracts were prepared. The reaction mixture was prepared by mixing 0.5 mL of plant extract solution, 2.5 mL 10% Folin–Ciocalteu's reagent dissolved in water, and 2.5 mL 7.5% $NaHCO_3$ aqueous solution. The absorbance of each mixture was determined using a spectrophotometer at a wavelength of 765 nm. Based on the measured absorbance, the concentration was expressed in terms of gallic acid equivalents (mg of GAE/g of the extract).

2.6. Determination of total flavonoids content

The total flavonoid contents of the obtained extracts were detected using the method proposed by Chang et al. which was validated by Nugroho [10,11]. The absorbance of each dilution was measured at a wavelength of 415 nm; distilled water with methanol, 10% $AlCl_3$, and potassium acetate was used as a blank. The total flavonoid content of each plant extract was determined from the calibration curve of rutin and was expressed in rutin equivalents (mg RUE/g extract).

2.7. Antioxidant test

The DPPH test was performed as described by Jaradat et al. [12]. Stock solutions of plant extract and Trolox (Positive control) were prepared at a concentration of 1 mg/mL in methanol. Methanol serial dilutions of 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, and 100 μg/mL were also prepared as working concentrations. The absorbance readings of these solutions were recorded at 517 nm.

The antioxidant activity of the plant and Trolox standard was calculated using the following formula:

$$\text{DPPH inhibition activity } (\%) = \frac{(A-B)}{\text{A}} X100$$

Where A = Absorbance of the blank and B = Absorbance of the sample.

2.8. Antimicrobial tests

2.8.1. Microbial species

Methanol, acetone, *n*-hexane, and aqueous extracts of *V. fruticulosum* were investigated for their antibacterial and antifungal activities using broth microdilution and agar dilution methods [13]. Antibacterial activities of the extracts were examined against a clinical isolate of multidrug-resistant *S. aureus* (MRSA) and 6 referenced bacterial strains obtained from the American Type Culture Collection (ATCC). The tested strains included gram-positives like *Staphylococcus aureus* (ATCC 25, 923), *Enterococcus faecium* (ATCC 700,221), in addition to gram-negative species *vis Escherichia coli* (ATCC 25,922), *Pseudomonas aeruginosa* (ATCC 27,853), and *Shigella sonnei* (ATCC 25,931). The antifungal activity was examined against *Candida albicans* (ATCC 90, 028) and *Epidermophyton floccosum* (ATCC 52,066). Aqueous Extract was dissolved in distilled water at a concentration of 50 mg/mL for antifungal tests and 100 mg/mL for antibacterial tests. Each of the prepared organic extracts was dissolved in 50% dimethyl sulfoxide (DMSO) at concentrations of 25 mg/mL, using a sonicator to aid dissolution. Solutions were then sterilized by filtration through a 0.45 μm pore size syringe filter. The minimum inhibitory concentration (MIC) of the plant extracts against bacterial and fungal strains was determined using the microdilution method (Supplementary material presented full details of the method).

2.9. Lipase inhibitory activity

A working solution (1 mg/mL) was done by dissolving 100 mg of *V. fruticulosum* in 100 mL of 10% dimethyl sulfoxide (DMSO) (Riedeldehan, Seelze, Germany). Then, the obtained solution was diluted to produce the following concentrations: 400, 300, 200, 100, and 50 μg/mL. The absorbance was measured utilizing a UV– Vis-spectrophotometer at 405 nm. However, the lipase enzyme inhibitory potential was measured utilizing the following equation:

$$\text{I}(\%) = \frac{(\text{ABSblank} - \text{ABStest})}{\text{ABSblank}} X100$$

where I (%) is the percent inhibition of the lipase enzyme [12].

2.10. α-Amylase inhibitory activity

A stock solution (1 mg/mL) was prepared by dissolving 25 mg of the *V. fruticulosum* extracts in 0.1 mL of 10% DMSO. Then, a buffer solution (buffer (0.02 M Na_2HPO_4/NaH_2PO_4, 0.006 M NaCl, pH 6.9) was added to 25 mL. The absorbance was assessed at 540 nm using a UV–Vis-spectrophotometer. The α-amylase inhibitory potential was calculated by the following formula:

$$\text{I}(\%) = \frac{(\text{ABSblank} - \text{ABStest})}{\text{ABSblank}} X100$$

where I (%) is the α-amylase inhibitory percentage [12].

2.11. α-Glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the *V. fruticulosum* extracts

was determined according to the standard protocol, with some modifications [12]. The absorbance of the released p-nitrophenol was measured by a UV–Vis spectrophotometer at 405 nm. The inhibition percentage of the *V. fruticulosum* extracts was calculated using the following equation:

$$\alpha - \text{Glucosidase } (\%) = \frac{(\text{Ab} - \text{As})}{\text{Ab}} X100$$

Where Ab is the absorbance of the blank and AS is the absorbance of the tested sample or control.

2.12. Cytotoxicity assay

The cytotoxic effect of the obtained *V. fruticulosum* extracts against the Human cervical (HeLa) cell line was determined by MTT assay using the method suggested by [14]. The cells were cultivated in RPMI-1640 media (Sigma, Norwich, UK) and treated with 1% L-glutamine (Sigma, London, UK), 1% penicillin/streptomycin antibiotics (BI, New Delhi, India), and 10% fetal bovine serum. Cancer cells were grown at 37 °C in a humidified atmosphere with 5% CO_2. Cells were seeded at 2.6 × 104 cells/well in a 96-well plate. After 48 h, cancer cells were incubated with various concentrations (500, 120, 60, 30, and 10 µg/mL) of the *V. fruticulosum* extracts and Doxorubicin (positive control) for 24 h. Cell viability was evaluated by the CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's directions (Promega Corporation, Madison, WI, USA). At the end of the treatment, 20 µL of MTS solution per 100 µL of media was added to each well, and the solutions in the well plates were incubated at 37 °C for 2 h. The absorbance was measured at 490 nm

2.13. Statistical analyses

All the conducted tests on *V. fruticulosum* extracts were carried out in triplicate. Results were presented as means ± standard deviation (SD). Only *p*-values less than 0.005 were considered statistically significant. Data were compared using unpaired *t*-tests.

3. Results

3.1. Phytochemical screening

Phytochemical screening tests for *V. fruticulosum* extracts showed that the aqueous extract contains protein, carbohydrates, phenol, tannin, flavonoids, saponins, glycosides, and steroids. In addition, the methanolic extract contains protein, carbohydrates, phenol, tannin, glycosides, steroid, terpenoids, and alkaloids. Moreover, acetone *V. fruticulosum* contains protein, carbohydrates, phenol, tannin, flavonoids, glycosides, terpenoids, and steroids. Besides, its n-hexane extract contains alkaloids, carbohydrates, glycosides, terpenoids, and steroids are presented in Supp. Table 1.

3.2. Total phenols, tannins, and flavonoids

For the determination of the content of total phenols, tannins, and flavonoids, standard calibration curves were constructed using different concentrations of gallic acid (mg of GAE/g), catechin (mg of CAE/g), and rutin (mg of RUE/g), respectively. From these curves, the equations $y = 0.1112x + 0.0176$ ($R^2 = 0.9956$), $y = 0.0009x + 0.077$ ($R^2 = 0.9596$), and $y = 0.0032x + 0.0086$ ($R^2 = 0.994$), respectively, were obtained. Table 1 shows that the V. fruticulosum acetone extract has the highest total phenol and flavonoid contents, and the methanolic plant extract has the highest content of tannins.

Table 1
Total phenols, tannins, and flavonoids in V. fruticulosum extracts.

V. fruticulosum	Total phenols content (mg of GAE/g) ± SD	Total tannin content (mg of CAE/g) ± SD	Total flavonoid content (mg of RUE/g) ± SD
Aqueous extract	35.06 ± 0.52	14.07 ± 1.29	20.88 ± 0.63
Acetone extract	64.70 ± 1.60	87.41 ± 8.41	59.84 ± 0.36
Methanol extract	58.87 ± 2.38	120.74 ± 7.14	–
Hexane extract	–	–	–

3.3. Antioxidant activity

The aqueous and acetone extracts of *V. fruticulosum* demonstrated the highest free radical scavenging activities followed by the methanol extract, which demonstrated moderate activity. The free radical scavenging activity of the tested samples was compared with the antioxidant drug Trolox, which was used as a positive control in our experiment. The free radical scavenging activity IC_{50} values of *V. fruticulosum* extracts and Trolox are illustrated in Table 2.

3.4. Porcine pancreatic lipase, α-amylase, and α-glucosidase inhibitory activities

As presented in Table 3, the *V. fruticulosum* acetone and methanol extracts inhibited the pancreatic lipase activity in a dose-dependent manner at the concentrations of 100–800 µg/mL in the porcine pancreatic lipase inhibition procedure. The IC_{50} dose of *V. fruticulosum* acetone extract was 129.05 ± 0.48 µg/mL, which was slightly higher than that of the anti-obesity drug Orlistat which inhibited the lipase with an IC_{50} value of 91.33 ± 0.33 µg/mL. At the same time, the methanolic extract showed weak lipase inhibitory activity (IC_{50} = 589.71 ± 0.38) while the *V. fruticulosum* aqueous and hexane extracts were inactive.

As presented in Table 2, the *V. fruticulosum* extracts exhibited potential α-amylase and α-glucosidase inhibitory activities. *V. fruticulosum* acetone extract at the 80 µg/mL concentration, reached the highest inhibitory activities on α-amylase (94.65%), which were higher than the antidiabetic drug Acarbose (82.54%). Furthermore, at a concentration of 500 µg/mL, *V. fruticulosum* methanolic extract had the highest inhibitory activity on α-glucosidase (96.26%), which was slightly higher than Acarbose (92.22%).

The α-amylase inhibitory effect IC_{50} value of *V. fruticulosum* extract acetone was 9.01 ± 1.76 µg/mL followed by n-hexane and aqueous extracts with IC_{50} values of 36.86 ± 0.90 and 37.91 ± 1.16 µg/mL, respectively, whereas the IC_{50} value of Acarbose was 9.61 ± 1.22 µg/mL. Besides, the highest α-glucosidase inhibitory effect was noticed in

Table 2
IC_{50} values (µg/mL) for *V. fruticulosum* extracts and positive controls drugs against DPPH and basal metabolic enzymes.

Tests	IC_{50} (µg/mL)				
	Positive control	*n*-Hexane	Acetone	Methanol	Aqueous
Lipase	91.33 ± 0.33[a]	NI	129.05 ± 0.48	589.71 ± 0.38	NI
α-Amylase	9.61 ± 1.22[b]	36.86 ± 0.90	9.01 ± 1.76	43.36 ± 0.55	37.91 ± 1.16
α-Glycosidase	44.81 ± 0.33 [b]	85.76 ± 0.26	392.09 ± 0.33	75.46 ± 0.66	334.01 ± 1.12
Antioxidant	0.87 ± 0.71[c]	NI	4.81 ± 0.77	>100	0.71 ± 0.31

[a] : Orlistat
[b] : Acarbose
[c] : Trolox; NI: No Inhibition; IC_{50}: Half-maximal Inhibitory Concentration.

Table 3
Microbial Minimal inhibitory concentrations (mg/mL) for *V. fruticulosum* extracts.

Microbial strains	MIC (mg/mL)				DMSO MIC%
	Aqueous extract	Methanol extract	Acetone extract	*n*-hexane extract	
Staphylococcus aureus	25	12.5	6.25	12.5	25%
E. coli	25	12.5	6.25	12.5	25%
Pseudomonas aeruginosa	25	1.56	1.56	3.13	12.5%
Enterococcus faecium	12.5	3.13	UN	3.13	6.25%
Shigella sonnei	12.5	3.13	6.25	3.13	25%
Staphylococcus aureus (MRSA Positive)	3.13	6.25	6.25	6.25	25%
Candida albicans	UN	3.13	6.25	3.13	25%
Epidermophyton floccosum	UN	3.13	3.13	1.56	12.5%

UN: undermined because the first well-showing inhibition of microbial growth contains an inhibitory concentration of DMSO; MIC: Minimum Inhibitory Concentration; MRSA: Methicillin-resistant *Staphylococcus aureus*; DMSO: Dimethyl sulfoxide.

the *V. fruticulosum* methanol extract (IC_{50}= 75.46 ± 0.66 μg/mL), followed by *n*-hexane (IC_{50}=85.76 ± 0.26 μg/mL), whereas Acarbose was 44.81 ± 0.33 μg/mL.

3.5. Antimicrobial activity

The antibacterial MIC results showed that V. fruticulosum organic extracts (acetone, methanol, and n-hexane) were more effective than the aqueous extract against all the bacteria under study, with a range of MIC values of 1.56–12.5 mg/mL. The exception was MRSA, where the aqueous extract inhibited the bacteria at a MIC dose of 3.125 mg/mL. All the organic extracts inhibited MRSA at 6.25 mg/mL (Table 3).

In the antifungal MIC tests, all organic extracts inhibited fungal growth at 3.125 mg/mL, except the acetone extract on C. albicans, where the MIC was 6.25 mg/mL. The n-hexane extract was the most effective one against E. floccosum with a MIC of 1.56 mg/mL, while the aqueous extract showed no antifungal activity (Table 3).

Antibacterial tests showed that the highest inhibition activities (the lowest MIC) were for the methanol and acetone extracts against P. aeruginosa, with MICs of 1.56 mg/mL in both cases. In addition, the acetone extract showed the highest inhibition activity against S. aureus and E. coli, with MICs of 6.25 mg/mL. However, it showed the lowest inhibition activity against E. faecium. Notably, the aqueous extract showed the highest inhibition activity against S. aureus (MRSA positive) with a MIC of 3.125 mg/mL. The best antibacterial activities for the methanol and n-hexane extracts were against E. faecium and S. sonnei with MICs of 3.125 mg/mL in all cases. The best antifungal inhibition activity was for n-hexane against E. flacosum with a MIC of 1.56 mg/mL. Unfortunately, the aqueous extract was inactive against the tested fungal strains used in this study.

3.6. Cytotoxicity

To understand the effect of V. fruticulosum extracts on the HeLa tumor cell line, it was carried out utilizing cultured. The viability of cells is decreased by increasing the concentration of the extracts. The highest cytotoxic effect was observed by using acetone, followed by methanol, n-hexane, and aqueous extracts, with IC_{50} values of 3.79 ± 0.01, 7.69 ± 0.03, 10.77 ± 0.04, and 49.17 ± 0.12 mg/mL, respectively. The results arising from the cytotoxic activity are shown in Supp. Fig. 1.

The highest cytotoxic effect was observed with using acetone extract, with an IC_{50} of 3.79 ± 0.01 mg/mL compared with the positive control doxorubicin, which has an IC_{50} of 0.84 ± 0.03 mg/mL.

4. Discussion

The preliminary phytochemical screening tests showed that the *V. fruticulosum* extracts contain phenol, tannin, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids besides carbohydrates and proteins in various extracts. In addition, the obtained results are presented in Table 2 which showed the total phenols, flavonoids, and tannins contents. However, the acetone V. fruticulosum extract has the highest content of total phenols, followed by the methanolic extract, representing 64.70 ± 1.60 and 58.87 ± 2.38 mg of GAE/g, respectively. Besides, the highest total tannin content was observed in the methanol and acetone extracts, with 120.74 ± 7.14 and 87.41 ± 8.41 mg of CAE/g, respectively. Moreover, the acetone extract has the highest amount of flavonoids (59.84 ± 0.36 mg of RUE/g). From these facts, it was noticed that the acetone and the methanolic extracts have the highest amounts of phenols and tannins (64.70 ± 1.6 mg of GAE /g; 59.84 ± 0.36, respectively), while the aqueous extract has moderate amounts of these components. The methanolic extracts did not have flavonoids, and the hexane extracts did not contain phenols, flavonoids, or tannins.

Alali et al.'s study [15] found that the total phenolic contents of *V. fruticulosum* aqueous and methanolic extracts were 13.4 and 11.9 mg GAE/g^{-1} of dry weight, respectively. While the total phenolic contents in *V. jordanicum* aqueous and methanolic extracts were 9 and 8.7 mg GAE/g^{-1} of dry weight, respectively.

The DPPH free radical scavenging test results showed that the aqueous and acetone extracts have strong antioxidant activity with IC_{50} values of 0.71 ± 0.31 and 4.81 ± 0.77 μg/mL, respectively, compared with Trolox, which has an antioxidant IC_{50} dose of 0.87 ± 0.71 μg/mL.

V. fruticulosum aqueous and acetone extracts have a mixture of total phenols, flavonoids, and tannins, which may be attributed to their high free radical scavenging activity. The methanolic *V. fruticulosum* plant extract contains only phenol and tannin contents without flavonoids, which makes it a weak free radical scavenging extract.

In a study conducted in Jordan by Alali et al. [15], the antioxidant activities of aqueous and methanolic *V. fruticulosum* extracts were 68.4 and 46.6 μmol of Trolox equivalents g^{-1} dry weight, respectively. For *V. jordanicum* they equaled 38.0 and 38.4 μmol of Trolox equivalents g^{-1} dry weight, respectively. In addition, Moustapha et al. from Egypt screened the antioxidant activities of V. fruticulosum methanol extracts *in* vitro using the DPPH assay and obtained a value of 28.9% of DPPH inhibition [16].

The ability of phenolic compounds to act as antioxidants is dependent on factors such as the arrangement and number of phenolic substituents, positions of hydroxyl groups, and their molecular weight. For example, flavonoids with fewer hydroxyl groups are less easily reduced and consequently poorer oxidizing agents [17–20].

In this work, *V. fruticulosum* extracts exhibited potential inhibitory capacity toward key enzymes including lipase, α-amylase, and α-glucosidase linked to obesity and diabetes. The phenolic contents of *V. fruticulosum* extracts, including simple phenols, flavonoids, and tannins, suppressed the actions of pancreatic lipase, α-amylase, and α-glucosidase enzymes primarily due to their bioactive constituents, which allows them to interact with these enzymes and blocked their effects [21,22]. Several investigations demonstrated the *in vitro* inhibition of lipase, α-amylase, and α-glucosidase by phenolic compounds [23, 24].

The antimicrobial MIC results showed that the V. fruticulosum organic extracts were more effective against all the screened bacterial strains than the aqueous extract. Abdallah and Omar reported that *V. fruticulosum* ethanol extract inhibited the growth of MRSA I and II clinical isolates with inhibition zones diameters of 10 mm, while the methanolic extract inhibited the growth of MRSA I and II clinical isolates with inhibition zones diameters of 13 and 10 mm, respectively [25].

Moreover, Abdallah and Ismail's investigation found that V. fruticulosum aqueous extract inhibited the growth of multidrug-resistant E. coli with a MIC value of 50 mg/mL [26].

Plants extracts are considered a source of phytochemical compounds that could inhibit microbial growth by employing many mechanisms, and many studies demonstrated the relationship between the chemical structure of phenolic compounds and their antimicrobial action [27,28].

In fact, *Verbascum* species have antimicrobial activity due to containing biologically active compounds such as polyphenols, terpenes, alkaloids, flavonoids, phenylethanoids, neolignan, saponins, and iridoid glycosides [29,30].

Cytotoxicity activity of *V. fruticulosum* extracts against the HeLa tumor cell line was observed by using acetone extract. A study conducted by Sathiyamoorthy et al. found that *V. fruticulosum* aerial parts aqueous extract exhibited cytotoxic activity against BG and GA melanoma cell lines with 4.7 and 16.1%, respectively [31].

4.1. Limitations

In this study, there was no attempt to separate the active compounds of the *V. fruticulosum* plant. This is due to the fact that column chromatography necessitates a large amount of plant material, which was not available at the time. Therefore, chemical structure elucidation and determination of the most bioactive molecules from *V. fruticulosum* extracts are limitations in this investigation. Nonetheless, we will pursue these experiments in the future.

5. Conclusion

The findings demonstrated the potent antioxidant properties of *V. fruticulosum's* aqueous and acetone extracts, while the methanol and acetone extracts exhibited remarkable antibacterial activity against *P. aeruginosa*, and the n-hexane extract demonstrated the strongest effect against *E. floccosum* fungi. Notably, the plant's acetone extract exhibited the highest cytotoxicity and significant inhibition of lipase and α-amylase activities. Furthermore, the methanol extract was found to be the most effective in inhibiting α-glucosidase activity. Nonetheless, to validate these pharmacological benefits of *V. fruticulosum*, further *in vivo* investigations are needed.

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CRediT authorship contribution statement

Raed Alkowni : Conceptualization, Methodology, Software, Data curation, Writing original draft, Supervision, Software, Validation, Writing review & editing. **Nidal Jaradat :** Conceptualization, Methodology, Software, Data curation, Writing original draft, Supervision, Software, Validation, Writing review & editing. **Saleh Fares :** Visualization, Investigation.

Declaration of Competing Interest

Dr. Nidal Jaradat is an Associate Editor of the European Journal of Integrative Medicine. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data generated or analyzed during this study are included in this manuscript.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.eujim.2023.102256.

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