Jordan Journal of Pharmaceutical Sciences, Volume 14, No. 4, 2021

Evaluation of phytochemical and pharmacological activities of *Taraxacum syriacum* and *Alchemilla arvensis*

Hazar Ali[1]; Raed Alkowni[1*]; Nidal Jaradat[2]; Motasem Masri[3]

[1] Department of Biology and Biotechnology, An-Najah National University, Nablus, Palestine
[2] Department of Pharmacy, An-Najah National University, Nablus, Palestine
[3]Division of Immunology, Microbiology, and Pathology, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, Palestine

ABSTRACT

Oxidative stress, obesity, and multidrug resistance to pathogenic microorganisms are major challenges in the health care systems and pharmaceutical industries that prompt scientists to search for alternative sources with maximum efficacy and few side effects. Therefore, this study aimed to screen phytoconstituents, and estimate total phenols, flavonoids contents, antioxidant, antilipase, and antimicrobial activities of two selected plants, *Taraxacum syriacum* and *Alchemilla arvensis* four extracts. Conventional phytochemical assays were utilized for qualitative and quantitative determinations of the major phytochemical classes, total phenol, and flavonoids contents of methanol, hexane, acetone, and water extracts of both plants. While the antioxidant activity was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. In addition, the antilipase activity was conducted using the porcine pancreatic lipase inhibitory test which was conducted by using a UV-visible spectrophotometer. Moreover, the antimicrobial activity of both plants' four extracts was established utilizing agar diffusion and micro-broth dilution methods against six microbial strains. The results revealed that the highest total phenol content was observed in the *T. syriacum* acetone extract (272.0 mg gallic acidE/g), while the highest total flavonoid content was detected in *A. arvensis* methanolic extract (83.3 mg rutinE/g). Actually, *T. syriacum* water extract has the best antioxidant potential among other extracts with an IC_{50} value of 95.5 µg/ml while *A. arvensis* acetone extract has the best antioxidant activity among other plant extracts with an IC_{50} dose of 4.9 µg/ml. Regarding antilipase activity, *A. arvensis* water extract showed a potent porcine pancreatic lipase inhibitory effect with an IC_{50} value of 21.4 µg/ml. However, most of the evaluated *T. syriacum* and *A. arvensis* plants' extracts showed broad-spectrum antibacterial and antifungal activities. This study recommended targeting these potentially medicinal plants in antioxidants and anticipate drugs for further *in-vivo* and preclinical studies.

Keywords: *Taraxacum syriacum; Alchemilla arvensis*; antioxidant; antilipase; antimicrobial.

INTRODUCTION

Plants provide endless sources of active therapeutic agents for the treatment of several diseases; in contrast to chemical medications which may be more expensive and more harmful [(1)]. Nowadays, many medical practitioners are looking at herbal remedies for common ailments, and these have gained momentum in the medical field, which are becoming more popular [(2; 3; 4)]

Taraxacum syriacum Boiss. is an annual herbaceous plant from the *Compositae* family [(5; 6)], whose roots have been used for treating hepatic diseases, anemia, gout, rheumatism, gastric ulcers and skin diseases, such as

[*] ***Corresponding author: Raed Alkowni***
ralkowni@najah.edu
Received on 3/7/2020 and Accepted for Publication on 18/10/2021.

eczema. *T. syriacum* was used in traditional herbal medicine for the treatment of jaundice, liver disorders, and gallstones, as it lowering the total cholesterol, triglyceride, insulin, and fasting glucose levels, as well as insulin resistance induced by a high-fat diet. Therefore, *T. syriacum* extracts may show promise for both the prevention and treatment of fatty liver disease triggered by obesity**.** Morphologically, the plant has a rosette leaf arrangement with pinnate-type leaves and dentate leaf margins, although it does not have stipules. It has yellow flowers which appear in April [7].

Alchemilla arvensis (L.) Scop. belongs to the *Rosaceae* family and used to treat various health problems, such as kidney, bladder stones, renal edema and hepatic disorders. *A. arvensis* is an edible plant, particularly the leaves which are consumed as a salad. It has 2–20 cm in height with fan-shaped leaves. The flowers are less than 2 mm in length, occur in dense clusters in leaf-axils, surrounded by cups formed by leaf stipules, and bloom in March till mid of summer. Compositions of *Alchemilla* are rich in tannins and other minor components of therapeutic interest. It has been used in the treatment of diarrhea since it has important astringent properties. *A. arvensis* has also been used to treat stomach aches and gastrointestinal inflammation [8].

Nowadays, free radicals, obesity, and microbial resistance to antibiotics represented serious health care problems. For that, many studies were established to solve these complexities by providing natural herbal plants medicine and cure forms. Thus, this study was aimed to screen phytochemical constituents of two recommended medicinal plants (*A. arvensis* and *T. syriacum),* to estimate their potential total phenols and flavonoids contents as well as to assess their antioxidant, anti-lipase, and antimicrobial properties.

MATERIALS AND METHODS

Collection and identification of plant materials

The roots of *T. syriacum* and leaves of *A. arvensis* were collected in April 2017 from the Nablus region in Palestine. The collected plants were identified by An-Najah National University experts before they were air-dried at room temperature for later use.

Extraction of plant materials

The air-dried plant materials (leaves and roots) were ground to a uniform powder, to be used for two extractions: organic and aqueous (crude) ones. Organic extraction was performed using the Soxhlet extraction method [9]. This extraction was established by taking 20 g of dried plant powders in a glass thimble using 250 ml of each solvent separately (methanol and acetone). The extraction processing was continued till the solvent in the siphon tube of the Soxhlet apparatus became colorless. Then extracts were incubated in a hot water bath at 35ºC until the solvent had completely evaporated. The dried plant crude extract was stored in the refrigerator at 4ºC for later use. While, the aqueous extraction was performed from 5 g of the plant powder that mixed with 200ml of distilled water and then heated to reach 30–40ºC with continuous stirring for 20 min [10]. The mixture was filtered using Whatman filter paper and used for phytochemical analysis.

Phytochemical screening

Phytochemical screenings were performed using standard procedures according to the methods reported by Trease and Evans [11].

Phenols and flavonoids tests

Total phenolic contents (TPC) in plant extracts were determined using a spectrophotometric method with some modifications [12]. The aqueous solutions of methanolic extracts (1 mg/ml) were prepared for the analysis. The reaction mixture was prepared by mixing 0.5 ml of plant extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, and 2.5 ml of 7.5% of $NaHCO_3$ aqueous solution. Samples were incubated in a thermostat at 45ºC for 45 min. The absorbance was determined by a

spectrophotometer at 765 nm wavelength. The samples were prepared in triplicate for each analysis and the mean value of absorbance was reported. The same procedure was repeated for the standard solution of gallic acid and a calibration curve was constructed. Based on the measured absorbance, the concentration of phenol content was expressed in terms of gallic acid equivalent (mg GAE/g).

Total flavonoid content (TFC) was determined from the calibration curve of rutin (reference substance) and was expressed as milligram of rutin equivalent per gram of extract (mg RUE/g extract) [13]. Total flavonoid content was determined according to the modified procedure of Chang *et al.* [13], and validated by Nugroho *et al*. [14]. Rutin (100 mg) was dissolved in 10 ml distilled water and diluted in a final volume of 100 ml. Subsequently, the stock solution was diluted to provide a series of concentrations (5, 10, 20, 40, and 100 mg/ml). Aliquots of each solution (0.5 ml) were mixed with 3 ml methanol, 0.2 ml of 10% $AlCl_3$, 0.2 ml of 1 M potassium acetate, and 5 ml distilled water and then incubated at room temperature for 30 min. Absorbance was measured at 415 nm wavelength using a spectrophotometer. Distilled water with methanol, 10% $AlCl_3$, and potassium acetate were used as a blank. The total flavonoids contents of both screened plants extracts were expressed as rutin equivalents (mg of RUE/g plant extract).

Antioxidant DPPH- test

Stock solutions of plant extract and Trolox (the reference substance) were prepared at a concentration of 0.1 mg/ml in methanol. Working solutions at concentrations of 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, and 100 µg/ml were prepared by serial dilution of the respective stock solution in methanol.

DPPH was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above-prepared working solutions in a ratio of 1:1:1, respectively. Methanol was used as a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 min at room temperature before the absorbance readings were recorded at 517 nm [15]. The percentage of antioxidant activity of the plants and the Trolox standard was calculated using the following formula:

DPPH activity (%) = (A-B)/A ×100

where A = optical density of the blank, and B = optical density of the sample.

Antilipase test

The porcine pancreatic lipase inhibitory assay was adapted from the published method of Zheng *et al*. [16], with some modifications. Plant extract stock solution (1 mg/ml) was used to prepare five different solutions in 10% DMSO at concentrations of 200, 400, 600, 800 and 1000 µg/ml. A stock solution of pancreatic lipase enzyme (Riedeldehan, Germany) (1 mg/ml) in Tris-HCl buffer was prepared immediately before use.

A stock solution of *p*-nitrophenyl butyrate (PNPB) was prepared by dissolving 20.9 mg in 2 ml of acetonitrile. For each working test tube, 0.1 ml of porcine pancreatic lipase (1 mg/ml) was added to a test tube containing 0.2 ml plant extract from each diluted solution series for each studied plant. The resulting mixture was then made up to 1ml by adding Tris-HCl solution and was incubated at 37°C for 15 min. After the incubation period, 0.1 ml of PNPB solution was added to each test tube. The mixture was incubated for a further 30 min at 37°C.

Pancreatic lipase inhibitory activity was determined by measuring the hydrolysis of p-nitrophenolate to p-nitrophenol at 405 nm using a spectrophotometer. The same procedure was repeated for Orlistat which was used as a positive control.

Antimicrobial assays

Antibacterial test

Antibacterial activity was tested by simple agar diffusion [17], against *Staphylococcus aureus* [ATCC 25923], *Escherichia coli* [ATCC 25922] *Pseudomonas aeruginosa* [ATCC 27853], and *Shigella sonnie* [ATCC 25931]. Agar media plates were gently swabbed with

turbidity-adjusted bacterial suspension before 50 µl of plant extracts were added to the wells. Plant extracts were obtained by different extraction methods (methanol, acetone, hexane, and aqueous crude extracts) and dissolved in 1 ml of 10% DMSO, before filling agar gel wells. Plates were then incubated for 16–18 h at 37°C and the antibacterial activity was evaluated by measuring the diameter of clear zones surrounding the wells.

The minimal inhibitory concentration (MIC) of the plant extracts against bacteria was determined using the micro-broth dilution method [18]. Mueller-Hinton broth (MHB) was used for this test in a polystyrene panel containing approximately 96 wells including a positive and negative growth control. The plant extract (100 µl) was added to the first wells, which already contained 100 µl MHB and then serially diluted with MHB in the remaining wells. The microorganisms (bacteria) were added to the MHB in a tube using a loop at a concentration of 1.5 $\times 10^8$ CFU/ml, compared to McFarland standard. The bacterial suspension was then diluted 1:3 with 4ml MHB to a concentration of 5×10^7 CFU/ml. Then the bacterial suspension (1 µl) was applied to all wells except the negative control one. The panel was covered and incubated at 35˚C for 16–20 hrs.

Several antibiotics (Azithromycin; Clarithromycin; Levofloxacine; Doxycycline; Cefuroxime; and Ciprofloxacin (Birzeit Pharmaceutical company, Palestine) were used to determine bacterial resistance. These were dissolved in their corresponding solvents according to the solubility tests; and prepared as a 1:10 dilution.

Antifungal test

Antifungal activity was examined against two pathogenic fungi *Epidermophyton floccosum* [ATCC52066] and *Candida albicans* [ATCC 90028] that were cultured on Potato Dextrose Agar (PDA) media. The antifungal activity of plant extracts against *Candida albicans* was determined using the micro-broth dilution method, similar to the previously reported procedure for MIC determination of bacterial isolate, with some modifications in inoculums preparation [19]. The *Candida* concentration in a broth with turbidity similar to 0.5 McFarland was $1\times10^6$to 5×10^6CFU\ml. This was diluted twice, 1:50 and 1:20, first in MHB and then in RPMI media, resulting in 1×10^3 to 5×10^3CFU/ml before the aliquots of (100 µl) were added to each well containing a defined concentration of plant extract. On the other hand, the antifungal activity against *Epidermophyton floccosum* was determined using the agar dilution method [20]. In this method, the plant extract was serially diluted with Sabouraud's Dextrose Agar (SDA). The fungus was prepared by adding sterile distilled water with 0.05% Tween 80 onto the surface growth. Spores and hyphae were then scraped off using a sterile scalpel. The turbidity of the resulting suspension was adjusted to be equivalent to 0.5 McFarland (absorption 0.08 to 1 at 600 nm). Later, 20 µl of the fungal solution was added to each tube and incubated at 25 ºC for 14 days. MIC was measuring the lowest concentration of plant extract that caused visible inhibition of fungal growth. Two types of antifungal drugs (Terbenafin and Tinidazole); which mixed in their suitable solvents, were diluted in a 1:10 dilution and used to determine the resistance of fungi [21].

RESULTS & DISCUSSION

Phytochemical screening

The two selected medicinal plants (*T. syriacum* and *A. arvensis*) (Figure 1) were collected from Palestinian territories and subjected to four different extraction methods.

(a) **(b)** **(c)**

Figure 1: The collected plants *Taraxacum syriacum* (a,b) and *Alchemilla arvensis* (c) from Palestinian territories.

Medicinal plants are containing phytochemical compounds include tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids. They are widely used in human therapy, veterinary, agriculture, and scientific research. There are large numbers of phytochemicals belonging to chemical classes, which have been shown to have inhibitory effects on many types of microorganisms in vitro.

Standard phytochemical tests were utilized to screen *T. syriacum* and *A. arvensis* four extracts. The results showed that *T. syriacum* methanolic and acetone extracts contained phenols, tannins, flavonoids, glycosides, and steroids. While hexane extract contains only cardiac glycosides. Moreover, the water extract contained phenols, tannins, flavonoids, and cardiac glycosides. In addition, *A. arvensis* methanolic extract contained phenols, tannins, glycosides, steroids, and cardiac glycosides. In fact, the results showed that the hexane extract did not contain any of screened phytochemicals while the acetone extract contained glycosides, steroids, and cardiac glycosides. The aqueous extract was containing only flavonoids.

Methanol extract yield was found the best one for phytochemical extraction from *T. syriacum* (12.5%) while water extract was the best yield for *A. arvensis* (25.6%) as shown in Table 1. Nine phytochemical tests were used to determine the presence of organic materials and the results revealed that *T. syriacum* and *A. arvensis* were rich in phytochemicals as presented in Table 2.

Total phenol and flavonoid contents

Phenolic compounds are reactive metabolites in a wide range of plant-derived foods and work as chelators of metal ions that are capable of catalyzing lipid oxidation. Besides, they have many beneficial properties, such as antioxidant effects, and anti-mutagenic activities, as well as they, can prevent cardiovascular diseases.

The total phenol and flavonoid contents were calculated in 1 g of plant extract (Table 3 and 4). Total phenol content was higher (272 mg GAE/g plant extract) in *T. syriacum* than in *A. arvensis* (151.5 mg GAE/g plant extract). In this study, *A. arvensis* revealed high total phenol content in the methanolic extract (151.51 mg GAE/g) compared with a similar one conducted by Kiselova *et al.*. [(22)], who found the total polyphenolic content between 88.00 and 112.33 µg/ml. In *T. syriacum,* the highest content of phenol was detected in acetone extract, equal to 271.95 mg GAE/g followed by water and methanol plant extracts with total phenolic contents of 143.7 and 120.4 mg GAE/g of plant extract. To the best of our knowledge, no previous studies have been conducted on total phenolic contents of *T. syriacum*.

Flavonoids are used in the treatment of eczema such as

quercetin. They also influence heart disease by reducing the risk of developing atherosclerosis by increasing the release of nitric oxide causing vasodilation. Also, they reduce allergic responses and stimulate the immune system.

This study revealed that flavonoid content was higher in *A. arvensis* (83.3 mg of RUE/g plant extract) than in *T. syriacum* (27.1 mg RUE/g plant extract). Aqueous extracts of *T. syriacum* were found to contain a high quantity of flavonoids (27.13 mg RUE/g plant extract), followed by acetone and methanolic extracts with total flavonoids contents of 17.8 and 8.4 mg RUE/g plant extract. To the best of our knowledge, no previous studies have been conducted on the total flavonoid contents of the *T. syriacum* plant. A study by Liu *et al.* [23] found that the total flavonoids content in *Taraxacum mongolicum* was 20.57±1.12 mg/g in methanol extract and 6.55±1.20 mg/g in water extract [23]. Aqueous extract of *A. arvensis* contained a high quantity of flavonoid and in 1 g of plant water extract, the quantity was 83.31 mg.

Antioxidant test-DPPH

Using DPPH assay, the antioxidant IC_{50} was calculated for each plant while the potent antioxidant drug Trolox was used as a positive control. In this study, *A. arvensis* has a potential antioxidant effect in methanol, hexane, and acetone extracts with IC_{50} values of 97.72, 11.22, and 4.86 µg/ml, respectively. These results showed that *A. arvensis* acetone extract had the most potent antioxidant effect among other plant extracts, While, *T. syriacum* aqueous extract has the highest antioxidant potential with an IC_{50} dose of 95.5 µg/ml among other plant extracts (Table 5).

In fact, the acetone extract exhibited potent antioxidant activity compared with Trolox which has an antioxidant IC_{50} value of 2.2 µg/ml. A study conducted by Nedyalkov *et al.* [24] found that *Alchemilla mollis* has also potent antioxidant capacity which was determined using four different assays (FRAP, CUPRAC, DPPH and ABTS).

Regarding, *T. syriacum* antioxidant activity, the best was detected in water extract which has an antioxidant IC_{50} dose of 95.49 µg/ml.

Antioxidants are bioactive molecules that inhibit the oxidation of free radicals which lead to chain reactions that can cause damage to cells and organs [25; 26].

Anti-lipase activity

The anti-lipase activity was detected using porcine pancreatic lipase inhibitory assay while Orlistat anti-obesity medication was utilized as a positive control. Table 6 depicts that both studied plants have an anti-lipase effect.

The results of the current study showed that water, methanol, acetone, and hexane extracts of *A. arvensis* had the highest antilipase activity with IC_{50} values of 21.37, 30.90, 45.70, and 72.44 µg/ml, respectively. To the best of our knowledge, no previous studies had been conducted on *A. arvensis* antilipase effect. Even though, a study of *Alchemilla vulgaris* was reported to show the inhibitory activity of pancreatic lipase [27; 28].

In *T. syriacum* plant, the best antilipase effect was shown in the water and hexane extracts, with IC_{50} values of 154.88 and 218.77 µg\ml, respectively. While the used positive control Orlistat has antilipase effect win an IC_{50} value of 20.4 µg\ml. To the best of our knowledge, no previous studies have been conducted on the *T. syriacum* as an antilipase agent.

Orlistat, as a lipase inhibitor, was used in this study as a positive control. The lipase inhibitors that are used to reduce the activity of lipases found in the intestine prevent the hydrolysis of dietary triglycerides to monoglycerides and fatty acids, so no absorption takes place. This mechanism could be used for the treatment of obesity [29].

Antimicrobial activity

Antimicrobials are medicinal products that kill or inhibit the growth of living microorganisms, usually called antibiotics because they act against bacterial infections [30]. These also include antimycobacterial, antiviral, antifungal, and antiparasitic drugs. Some bacteria are resistant to certain antibiotics and others can acquire resistance

through mutations in some of their genes when they are exposed to an antibiotic.

Bacteria causing a wide range of infections may become resistant to one or many antibiotics, such as those causing urinary tract infection, pneumonia, skin infection, and bloodstream infections. For example, a high proportion of resistance to third-generation cephalosporins was reported for *E. coli* and *K. pneumonia*.

Antibacterial effects of *Taraxacum syriacum* and *Alchemilla arvensis* plants were firstly verified with simple agar diffusion tests as shown in Table 7. The MIC values were also determined for each plant extract against each different bacteria, as shown in Table 8. Besides, drug resistances were tested for these selected bacteria with different antibiotics to reveal that Cefuroxime 250 mg had a low MIC value (2.35 µg/ml) for all tested bacteria; meanwhile, the most inhibitory antibiotic was recorded for Levofloxacin 500 mg and Ciprofloxacin (< 10 ng/ml).

The MIC values for different plant extracts against two types of fungi were determined as shown in Table 9. In addition, drug resistance was tested for these selected fungi against different antifungals to found that Terbinafine 250 mg has advantages in killing *C. albicans* over Tinidazole 500 mg

This study showed that *A. arvensis*, had antimicrobial effects for bacteria and fungi in different extracts, and MIC values for different types of bacteria and fungi as shown previously. To the best of our knowledge, no previous studies were conducted on *A.arvensis*. These results were in accordance with other ones on *Alchemilla vulgaris* [31] and *Alchemilla mollis* [32; 33] which resulted in presence of antibacterial activity in these plants.

Taraxacum syriacum has an antimicrobial effect on bacteria and fungi in different extracts, with different MIC values. To the best of our knowledge, no previous studies were conducted on *T. syriacum* antimicrobial effects. Even though studies on *Taraxacum mongolicum* [34] and *Taraxacum officinale* [35] revealed strong antimicrobial activity in vitro.

Finally; this study was the first to report the antioxidant, antilipase, antibacterial and antifungal activities of the *Taraxacum syriacum* and *Alchemilla arvensis* herbal plants; as well the total phenols and total flavonoids constituents that were not estimated before.

CONCLUSION

The phytochemical constituents, total phenols, and flavonoids contents of *A. arvensis* and *T. syriacum* were successfully reported in the current study. Both plants expressed high potentiality in their antioxidant, anti-lipase, and antimicrobial tests. The obtained results recommended the use of *A. arvensis* and *T. syriacum,* for the treatment of oxidative stress, obesity, and infectious diseases. Further phytochemical and pharmacological studies on *T. syriacum* and *A.arvensis* are required to isolate the bioactive compounds from these plants, elucidate their chemical structures and estimate their therapeutic activity *in vivo* besides investigating their toxicity and side effects.

Acknowledgment

The authors would like to acknowledge the Faculty of Graduate Studies (MSc program in Biology) at An-Najah National University.

Table 1: The percentage yields of selected herbs from each different extraction solvents

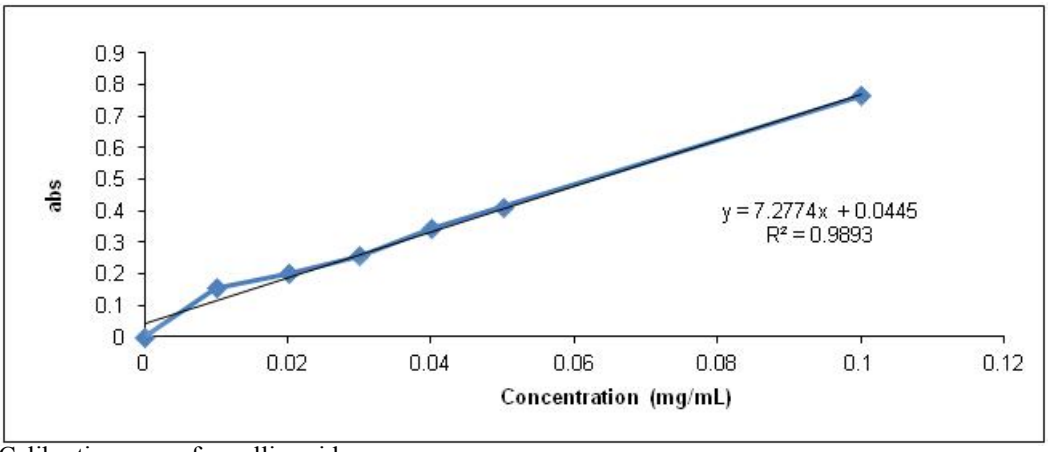
Extraction Method	Yields	
	T. syriacum	***A. arvensis***
Methanol	**12.5%**	14.2%
Hexane	2.07%	3.9%
Acetone	3.2%	2.8%
Water	2.2%	**25.6%**

Table 2: Phytochemical screening test results for *T. syriacum* and *A. arvensis*

Taraxacum syriacum				
Test \ Extract solvent	**Methanol**	**Hexane**	**Acetone**	**Water**
1. Protein (Millon's test)/(Ninhydrine)	-	-	-	-
2. Carbohydrate tests (Fehling's)/(Benedict's) /(Iodine)	-	-	-	-
3. Phenol and tannin ($FeCl_3$)	+	-	+	+
4. Flavonoid (Shinoda) /(Alkaline)	+	-	+	+
5. Saponins	-	-	-	-
6. Glycosides (Liebermann's) /(Salkowski's)	+	-	+	-
7. Cardiac steroidal glycoside	-	+	-	+
8. Steroid	+	-	+	-
9. Terpenoids	-	-	-	-
Alchemilla arvensis				
Test \ Extract solvent	**Methanol**	**Hexane**	**Acetone**	**Water**
1. Protein (Millon's test)/(Ninhydrine)	-	-	-	-
2. Carbohydrate (Fehling's)\ (Benedict's)\(Iodine)	-	-	-	-
3. Phenol and tannin ($FeCl_3$)	+	-	-	-
4. Flavonoid (Shinoda) /(Alkaline)	-	-	-	+
5. Saponins	-	-	-	-
6. Glycosides (Liebermann's)/(Salkowski's)	+	-	+	-
7. Cardiac steroidal glycoside	+	-	+	-
8. Steroid	+	-	+	-
9. Terpenoids	-	-	-	-

Table 3: Total phenol content results.

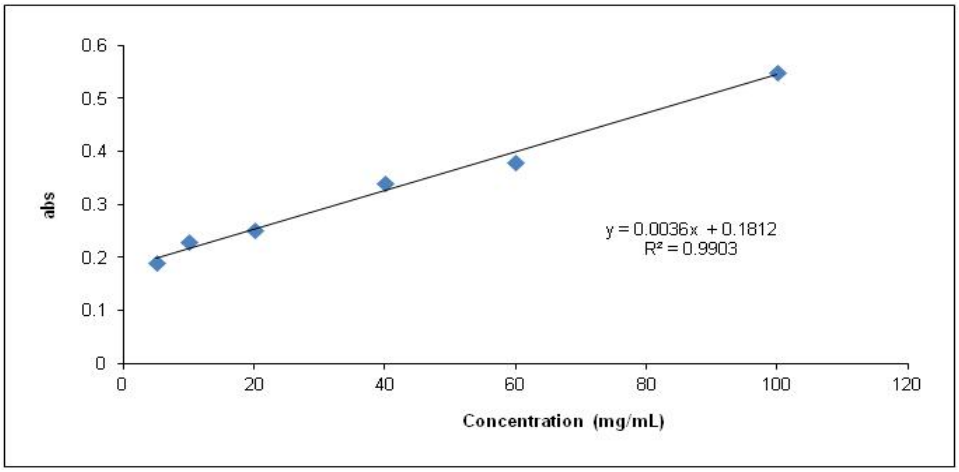
Plant	Extract solvent	Total phenol content (mg GAE/g)
T. syriacum	Methanol	120.4
	Acetone	272.0
	Water	143.7
A. arvensis:	Methanol	151.5



Calibration curve for gallic acid

Table 4: Total flavonoid content in *T. syriacum* and *A. arvensis* plants

Plant	**extract solvent**	**Total flavonoid content (**RUE/g plant extract**)**
T. syriacum	Methanol	8.4
	Acetone	17.8
	Water	27.1
A. arvensis	Methanol	83.3



Calibration curve for rutin

Table 5: Antioxidant IC_{50} values for *T. syriacum* and *A. arvensis* and DPPH inhibitory activity curves by both plants

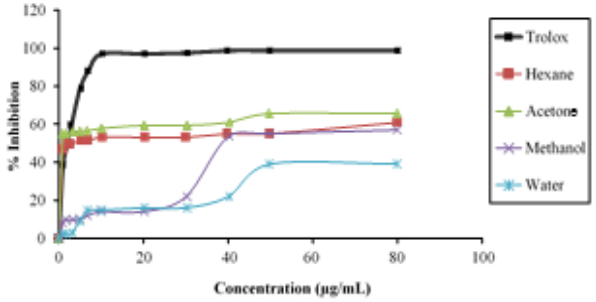
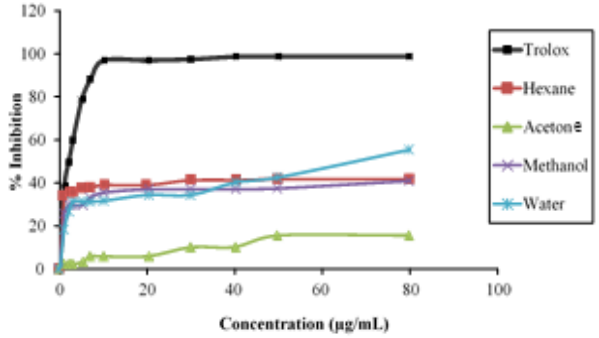
Plant	Extracts	IC_{50} (µg/ml)	
T. syriacum			
	Hexane	177.8	
	Methanol	281.8	
	Acetone	7079457.8	
	Water	95.5	
			DPPH inhibitory activity curves by *T. syriacum*
A. arvensis			
	Hexane	11.2	
	Methanol	97.7	
	Acetone	4.9	
	Water	724.4	
			DPPH inhibitory activity curves by *A. arvensis*
	Trolox	2.2	

Table 6: Antilipase IC_{50} values of *T. syriacum and A. arvensis and* porcine lipase inhibitory curve by both plants

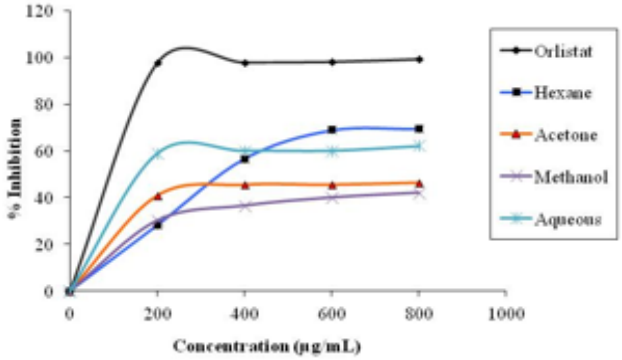
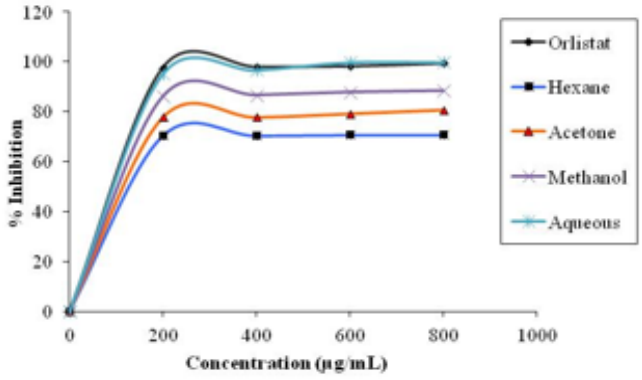
Plant	Extracts	IC_{50} (µg/ml)	
T. syriacum			
	Hexane	218.8	
	Methanol	3311.3	
	Acetone	977.2	
	Water	154.9	Antilipase curve for *T. syriacum*
A. arvensis			
	Hexane	72.4	
	Methanol	30.9	
	Acetone	45.7	
	Water	21.4	Antilipase curve for *A. arvensis*
	Orlistat	20.4	

Table 7: Results of simple agar diffusion method

T. syriacum plant					
	Plant extract solvent	**Water**	**Acetone**	**Methanol**	**Hexane**
	Bacteria name	**Inhibition zone diameter (mm)**			
	Escherichia coli	-	-	-	-
	Pseudomonas aeruginosa	-	10	-	-
	Staphylococcus aureus	-	-	-	10
	Shigella sonnie	-	-	-	-
A. arvensis plant					
	Escherichia coli	20	10	10	-
	Pseudomonas aeruginosa	-	-	-	-
	Staphylococcus aureus	15	10	10	-
	Shigella sonnie	-	20	15	10

Table 8: MIC values for *T. syriacum* and *A. arvensis* with bacteria

T. syriacum plant						
	Plant extract solvent	**Water**	**Acetone**	**Methanol**	**Hexane**	**DMSO**
	Bacterial Name	**MIC values (mg/ml)**				
	Staphylococcus aureus	6.3	12.5	12.5	No inhibition	6.3
	Pseudomonas aeruginosa	25	12.5	6.3	No inhibition	6.3
	Escherichia coli	25	12.5	6.3	No inhibition	6.3
	Shigella sonnie	25	12.5	12.5	No inhibition	6.3
A. arvensis plant						
	Staphylococcus aureus	25	25	3.1	No inhibition	6.3
	Pseudomonas aeruginosa	12.5	No inhibition	3.1	No inhibition	6.3
	Escherichia coli	12.5	No inhibition	3.1	No inhibition	6.3
	Shigella sonnie	12.5	25	3.1	12.5	6.3

Table 9: MIC values for *T. syriacum*, and *A. arvensis* with fungi

T. syriacum plant						
	Plant extract solvent	**Water**	**Acetone**	**Methanol**	**Hexane**	**DMSO**
	Fungus Name	**MIC values (mg/ml)**				
	Candida albicans	25	3.1	6.3	6.3	3.7
	Epidermophyton floccosum	1.6	0.8	0.8	0.8	6.3
A.arvensis plant						
	Candida albicans	12.5	6.3	6.3	12.5	3.7
	Epidermophyton floccosum	0.78	0.78	0.78	0.78	6.3

REFERENCES

1. Hamburger M., Hostettmann K. 7. Bioactivity in plants: the link between phytochemistry and medicine. *Phytochemistry*, 1991; 30(12):3864-3874
2. Shawarb N., Jaradat N., Abu-Qauod H., Alkowni R. and Hussein F. Investigation of antibacterial & antioxidant activity for methanolic extract from different edible plant species in Palestine. *Moroccan Journal of Chemistry*. 2017; 5 (4):573-579
3. Alkowni R., Jodeh S., Hussein F., Jaradat N. Phytochemical Screening and Antibacterial Activity of (Cyclamen Persicum Mill) Tuber Extracts. *Pak. J. Pharm. Sci*. 2018; 31(1):187-192
4. Eid A M, Jaradat N. A., Elmarzugi A., Alkowni R, Hussein F., Abu Ayyash L., Sawafta M., Danaa H. Development of Nigella Sativa Oil Colloidal Emulgel Using Heat Inversion Technique and Evaluation of Its Anti-Bacterial Activity. *Letters in Drug Design & Discovery*. 2019; 16 (4): 408 – 416.
5. Tristram, H. *The Fauna and Flora of Palestine* (Cambridge Library Collection - Botany and Horticulture). Cambridge: Cambridge University Press. 2013; doi:10.1017/CBO9781139162340
6. Younis N, Abu-Mallouh S, Almasri I, Issa A, Bustanji Y.. Pancreatic Lipase Inhibition by Edible Plants Used in Three Middle East Countries: *Jordan Journal of Pharmaceutical Sciences*, 2021; 14(2): 179-188
7. Savadkoohi F, Nejadsattari T, Assadi M, Jafari E, Mehregan I.. Application of achene morphology in systematics of some Iranian *Taraxacum* (*Asteraceae* tribe *Cichorieae*) species. *Iran. J. Bot.* 2012; 18(2):249-261
8. Trouillas P, Calliste C-A, Allais D-P, Simon A, Marfak A, Delage C, Duroux J-L.. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chemistry*, 2003; 80(3):399-407.
9. Ganzler K., Salgó A., Valkó K. Microwave extraction: A novel sample preparation method for chromatography. *J. Chromatogr. A*, 1986; 371, 299–306.
10. Parekh J, Jadeja D, Chanda S. Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology*, 2006; 29(4):203-210.
11. Evans W. *Trease and Evans' Pharmacognosy*, 16th Ed. *Saunders Ltd*; 2009, P600
12. Ainsworth E.A., Gillespie K.M. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. *Nature protocols* 2007; 2(4):875.
13. Chang C-C., Yang M-H., Wen H-M., Chern J-C. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *Journal of food and drug analysis*, 2002; 10(3): 178-182.
14. Nugroho A.E., Malik A, Pramono S. Total phenolic and flavonoid contents, and in vitro antihypertension activity of purified extract of Indonesian cashew leaves (Anacardium occidentale L.). *International Food*

Research Journal, 2013; 20(1): 299-305

15. Rashed, K & Lai, C-S & Pan, M-H & Feitosa, C. Study of the antioxidant and anti-inflammatory potential of the aerial parts of ficus nitida L. (Moraceae) and its phytochemical composition. *Jordan Journal of Pharmaceutical Sciences*. 2018; 11. 79-83.
16. Zheng C-D, Duan Y-Q, Gao J-M, Ruan Z-G. Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs. Journal of the Chinese Medical Association, 2010; 73(6):319-324.
17. Abualhasan M., Jaradata N., Abu-Hasanb N., Almasrib M., Tahac A.A., Rabbaaa A., Natsheha N., Shalalfeha S., Najibd M. Bioactivity of Viscum album extracts from Olive and Almond host plants in Palestine. *Pharmacogn. J*. 2014; 6(2):117-123.
18. Baron E.J., Finegold S.M. Methods for testing antimicrobial effectiveness. In Diagnostic Microbiology. Stephanie, M. (Ed.). *Baltimore, Mosby*. 1990; 171–194.
19. Zgoda J, Porter J.. A convenient microdilution method for screening natural products against bacteria and fungi. *Pharmaceutical Biology*, 2001; 39(3):221-225.
20. Therese K, Bagyalakshmi R, Madhavan H, Deepa P.. In-vitro susceptibility testing by agar dilution method to determine the minimum inhibitory concentrations of amphotericin B, fluconazole and ketoconazole against ocular fungal isolates. *Indian journal of medical microbiology,* 2006; 24(4):273.
21. Darwish R & Aburjai T. Antimicrobial activity of some medicinal plants against different Candida species. Jordanian. *Jordan Journal of Pharmaceutical Sciences*. 2011; 4(1): 70-80.
22. Kiselova Y., Ivanova D., Chervenkov T., Gerova D., Galunska B., Yankova T. Correlation between the in vitro antioxidant activity and polyphenol content of aqueous extracts from Bulgarian herbs. *Phytotherapy research*, 2006; 20(11):961-965.
23. Liu H, Qiu N, Ding H, Yao R. Polyphenols contents and antioxidant capacity of 68 Chinese herbals suitable for medical or food uses. *Food Research International*, 2008; 41(4):363-370.
24. Nedyalkov P, Kaneva M, Mihaylova D, Kostov G, Kemilev S.. Influence of the ethanol concentration on the antioxidant capacity and polyphenol content of Alchemilla mollis extracts. *Comptes rendus de l'Académie bulgare des Sciences*, 2015; 68(12): 1491-502.
25. Cheeseman K, Slater T. An introduction to free radical biochemistry. *British medical bulletin*, 1993; 49(3):481-493.
26. Ivanov I.G. Polyphenols content and antioxidant activities of Taraxacum officinale FH Wigg (dandelion) leaves. *International journal of pharmacognosy and phytochemical research*, 2014; 6(4):889-893.
27. Slanc P., Doljak B., Mlinaric A., Strukelj B. Screening of wood damaging fungi and macrofungi for inhibitors of pancreatic lipase. *Phytother. Res*., 2004; 18: 758-762.
28. Altameme H.J., Hameed I.H., Abu-Serag N.A.: Analysis of bioactive phytochemical compounds of two medicinal plants, Equisetum arvense and Alchemila valgaris seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Malays. Appl. Biol* 2015; 44(4):47-58.
29. Birari RB, Bhutani KK. Pancreatic lipase inhibitors from natural sources: unexplored potential. *Drug discovery today*, 2007; 12(19-20):879-889.
30. Nelson DC, Schmelcher M, Rodriguez-Rubio L, Klumpp J, Pritchard DG, Dong S, Donovan DM.. Endolysins as antimicrobials. *In*: *Advances in virus research*. edn. Elsevier, 2012; 83:299-365.
31. Hamid K.H.A., Azman N.A.B.M., Md Shaarani S., Zain N., Ahmad N., Sulaiman A.Z., Chik S.S.T., Ishak W.F.W., Almajano Pablos M.P. Alchemilla vulgaris and Filipendula ulmaria extracts as potential natural preservatives in beef patties. *Malaysian Journal of Analytical Sciences*, 2017; 21:986-986.
32. Duckstein SM, Lotter EM, Meyer U, Lindequist U, Stintzing FC. Phenolic constituents from Alchemilla vulgaris L. and Alchemilla mollis (Buser) Rothm. at

different dates of harvest. *Z. Naturforsch C.* 2012; 67: 529-40.

33. Denev P, Kratchanova M, Ciz M, Lojek A, Vasicek O, Blazheva D, Nedelcheva P, Vojtek L, Hyrsl P. Antioxidant, antimicrobial and neutrophil-modulating activities of herb extracts. *Acta Biochimica Polonica*, 2014; 61(2): 13-22.
34. Zhang H, Xiong Y., Zhao H., Yi Y., Zhang C., Yu C., Xu C..An antimicrobial compound from the endophytic fungus Phoma sp. isolated from the medicinal plant Taraxacum mongolicum. *Journal of the Taiwan Institute of Chemical Engineers*, 2013; 44(2):177-181
1. 35. Ghaima K.K., Hashim N.M., Ali S.A. Antibacterial and antioxidant activities of ethyl acetate extract of nettle (Urtica dioica) and dandelion (Taraxacum officinale). *J. Appl. Pharm. Sci*., 2013; 3(05): 096-099.

تقييم النشاط الكيميائي النباتى والدوائى لنباتين طبيين (*Alchemilla arvensis* و *Taraxacum syriacum*)

هزار علي[1]، رائد الكوني[1] *، نضال جرادات[2]، معتصم مصري[3]

[1] قسم الأحياء والتكنولوجيا الحيوية، جامعة النجاح الوطنية، نابلس، فلسطين

[2] قسم الصيدلة، جامعة النجاح الوطنية، نابلس، فلسطين

[3] قسم المناعة والأحياء الدقيقة وعلم الأمراض، قسم العلوم الطبية الحيوية، كلية الطب والعلوم الصحية، جامعة النجاح الوطنية، نابلس، فلسطين.

ملخص

يعد الإجهاد التأكسدي والسمنة ومقاومة الأدوية المتعددة للكائنات الدقيقة المسببة للأمراض من التحديات الرئيسية في أنظمة الرعاية الصحية والصناعات الدوائية والتي تدفع العلماء للبحث عن مصادر بديلة بأقصى قدر من الفعالية وآثار جانبية قليلة. لذلك هدفت هذه الدراسة إلى فحص المكونات النباتية وتقدير إجمالي محتوى الفينولات والفلافونويدات ومضادات الأكسدة ومضادات اللايبيز والأنشطة المضادة للميكروبات ل أربعة مستخلصات من نباتين مختارين هما Taraxacum syriacum و Alchemilla arvensis. تم استخدام فحوصات كيميائية نباتية تقليدية للتحديد النوعي والكمي للفئات الكيميائية النباتية الرئيسية، ومحتوى الفينول الكلي، ومحتويات الفلافونويد من الميثانول، والهكسان، والأسيتون، والمستخلصات المائية لكلا النباتين. بينما تم تقييم نشاط مضادات الأكسدة باستخدام مقايسة DPPH (2،2-Diphenyl-1-picrylhydrazyl). . بالإضافة إلى ذلك، تم إجراء نشاط مضادات اللايبيز باستخدام اختبار مثبط لايبيز بنكرياس الخنازير والذي تم إجراؤه باستخدام مقياس الطيف الضوئي المرئي للأشعة فوق البنفسجية. علاوة على ذلك، تم إنشاء النشاط المضاد للميكروبات للمستخلصات الأربعة للنباتين باستخدام طريقة الانتشار بالأجار وطرق التخفيف الدقيق للبيئة المغذية ضد ستة سلالات ميكروبية. أظهرت النتائج أن أعلى محتوى إجمالي للفينول لوحظ في مستخلص T. syriacum acetone (272.0 مجم حمض جاليك / جم)، بينما تم الكشف عن أعلى محتوى إجمالي من الفلافونويد في مستخلص A. arvensis الميثانولي (83.3 مجم روتين إي / جم). في الواقع، يحتوي المستخلص المائي من T. syriacum على أفضل إمكانات مضادات الأكسدة من بين المستخلصات الأخرى بقيمة IC50 تبلغ 95.5 ميكروغرام / مل بينما يحتوي المستخلص الاسيتوني من A. arvensis على أفضل نشاط مضاد للأكسدة من بين المستخلصات النباتية الأخرى بجرعة IC50 تبلغ 4.9 ميكروغرام / مل. فيما يتعلق بنشاط مضادات اللايبيز، أظهر المستخلص المائي من A. arvensis تأثيرا مثبطا قويا للايبيز بنكرياس الخنازير بقيمة IC50 بقيمة 21.4 ميكروغرام / مل. ومع ذلك، أظهرت معظم مستخلصات نباتات T. syriacum و A. arvensis التي تم تقييمها أنشطة واسعة النطاق كمضادات للبكتيريا والفطريات. أوصت هذه الدراسة باستهداف هذه النباتات الطبية المحتملة في مضادات الأكسدة والأدوية الاستباقية ولمزيد من الدراسات على الجسم الحي وما قبل السريرية.

الكلمات الدالة: Taraxacum syriacum؛ Alchemilla arvensis، مضادات الأكسدة، مضادات اللايبيز، مضادات الميكروبات

---

* ***المؤلف المراسل: رائد الكوني***

ralkowni@najah.edu

تاريخ استلام البحث 2020/7/3 وتاريخ قبوله للنشر 2021/10/18.