An-Najah National University

Faculty of Graduated Studies

"Phytochemical Screenings and Pharmacological Activities of two Medicinal Plants (*Alchemilla arvensis* and *Taraxacum syriacum)*"

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Dedication

I dedicate my thesis to my God that help me to complete my research, and giving me a chance to prove and improve myself.

I would like to dedicate my thesis to my wonderful husband Engineer Nihad Hindia who support me in my studies and had given me the dreams to look forward, thank you very much.

To my kids Hala, Batool, Jana, and Osama, they have made me more stronger, better and more fulfilled than I could have ever imagined, I love them to the moon and back.

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الإقرار

أنا الموقع أدناه و مقدم الرسالة التي تحمل العنوان:

Phytochemical Screenings and Pharmacological Activities of two Medicinal Plants (*Alchemilla arvensis* and *Taraxacum syriacum)*

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Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالبة:**

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Date: **التاريخ:**

List of contents

List of Tables

List of Figures

List of Abbreviations

TPC	Total Phenol Content
TFC	Total Flavonoid Content
DPPH	2,2-diphenyl-picrylhydrazyl
DMSO	Dimethyl sulfoxide
PNPB	P-nitrophenyl butyrate
ATCC	American Type Culture Collection
MIC	Minimum Inhibitory Concentration
MHB	Mueller Hinton Broth
CFU	Colony Formation Unit
PDA	Potato Dextrose Agar
SDA	Sabouraud's Dextrose Agar
RPMI	Rose Well Park Memorial Institute
MOPS	3-N-Mopholino Propane Sulfonic Acid Buffer
IC_{50}	Half Maximal Inhibitory Concentration
Tris-HCl	Tromethamine –HCl

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Abstract

Background: Oxidative stress, obesity problems and multidrug-resistant microorganisms represent major challenges for pharmaceutical industries. These problems have prompted scientists to screen for alternative substances that act as strong antioxidant, antilipase and antimicrobial agents with maximum efficacy and few side effects. From the beginning of human history, different herbal remedies and other natural products have become important as biological sources of antioxidant, antilipase and antimicrobial agents. Therefore, the aims of this study were to investigate the antioxidant, antilipase and antimicrobial activities of two plant species, *Alchemilla arvensis* and *Taraxacum syriacum.* In addition, plants were screened for phytochemicals, specifically the total content of phenols and flavonoids.

Methods: Antioxidant activity was examined by preparing a stock solution of plant extract at a concentration of 0.1mg/ml in methanol. A similar stock solution of 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), the reference substance, was also prepared. Solutions of different concentrations were prepared from the stock solutions by serial dilutions using methanol. The 2,2-diphenyl-picrylhydrazyl (DPPH) solution was

mixed with methanol and the previously mentioned concentrations. The percent of antioxidant activity of the plant extracts and the trolox standard was calculated using the following formula:

DPPH activity (%) = (A-B)/A ×100%

A: Optical density of blank, B: Optical density of sample.

Antilipase activity was examined by preparing a stock solution of *p*-nitrophenyl butyrate (PNPB). Pancreatic lipase 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 (is a drug designed to treat obesity.) which was used as a reference compound.

The antimicrobial activities were examined using micro-broth dilution, agar dilution and agar-well diffusion methods. The tested strains, which were obtained from the American Type Culture Collection (ATCC), included *Pseudomonas aeruginosa, Escherichia coli, Shigella sonnie, Staphylococcus aureus, Candida albicans, and Epidermophyton floccosum.*

Results: Trolox was used as reference drug in the detection of antioxidant activity and showed that the aqueous extract of *Taraxacum syriacum* was a more potent antioxidant (95.49µg/ml) than the methanol extract (281.83µg/ml). The acetone extract of *Alchemilla arvensis* was more potent antioxidant (4.86µg/ml) than the hexane extract (11.22µg/ml).

Orlistat drug was used as reference to detect antilipase activity and showed that aqueous extract of *Taraxacum syriacum* more potent (154.88µg/ml) than the hexane extract (218.77µg/ml). The aqueous extract of *Alchemilla*

arvensis was more potent(21.37µg/ml) then the methanol extract (30.90µg/ml).

With regard to antimicrobial activity against bacteria and fungi, *Taraxacum syriacum* was evaluated against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Shigella sonnie* using aqueous and acetone extracts. The same were noticed against *Candida albicans* and *Epidermophyton floccosum,* in all extracts were used.

Alchemilla arvensis was assessed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Shigella sonnie,* and *Escherichia coli* in aqueous and methanol extracts. *Alchemilla arvensis* was only potent against *Epidermophyton floccosum* fungus for all extracts were used.

Conclusion: Extracts of *Alchemilla arvensis* and *Taraxacum syriacum* were tested for antioxidant, antilipase and antimicrobial activities. This study recommends that these plants could be used as cancer treatment drugs, also for obesity, in addition to used as antibacterial and antifungal drugs, or could be used as prevention against these diseases.

Chapter one

Introduction

1.1 Introduction

Plants provide an endless source of active therapeutic agents for treatment of several diseases. Herbal medicines generally have few side-effects and involve low cost, in contrast to currently used chemical medication which may be more expensive and more harmful[1]. Ancient Chinese and Egyptian writings described the medicinal use of plants as early as 3000 BC[2]. Then, chemists began making their own version of plant compounds and, over time, the use of herbal medicines declined in favor of conventional drugs. In fact, herbal medicines are not necessarily harmless, they are drugs, as such they should be prescribed by a qualified, registered practitioner. However today, many medical practitioners are beginning to look at herbal remedies for some common ailments. Some physicians use herbs to offset the side-effects of pharmaceuticals, and these have gained momentum in the medical field, with herbs are becoming more popular[3]. There appears to be a move away from some pharmaceutical medicines due to concerns about harmful side-effects and potential addiction. The pursuit of alternatives to modern medical interventions has resulted in increased cultivation of medicinal herbs, as few herbs are available for harvesting from the wild [3].

In otherwise oxidative stress, obesity problems, and multidrug resistant micro-organisms represent major challenges for pharmaceutical industries. These problems have prompted scientists to screen for alternative substances that act against these problems.

Alchemilla arvensis and *Taraxacum syriacum* were selected to extract the active constituents from them and doing the different tests that show the activity of the plant against free radicals and lipase enzyme, also reference types of bacteria and fungi were used

1.2 Background

Literature review

***Alchemilla arvensis*(L.)Scop.** Is a synonym of *Aphanes arvensis* L. which belongs to the *Rosaceae* family. *Aphanes* is a genus of plants consisting of about 20 species distributed in temperate regions across the world, with the majority of the plants native to Arab countries and sandy soil [4].

A.arvensis is an edible plant, especially the leaves which are consumed as salad, and it is used to treat various health problems, such as kidney, bladder stones, renal edema and hepatic disorders. In addition it shows antitumor activity by attacking free radicals in the body, which cause cell damage, also protein and DNA, With regard to the morphology of *arvensis*, it is 2–20cm high with leaves that are fan-shaped, have a short corner, are divided into three segments with short stalks, have three deep-toothed main lobes and are only 2–10mm long. The flowers are less than 2mm length[5], occur in dense clusters in leaf-axils, are surrounded by cups formed by leaf-stipules and begin to bloom from March and continue well into the summer. Composition of the plant is similar to other plants of the same genus, such as *Alchemilla alpina* (Lady's Mantle), being rich in tannins and other minor components of therapeutic interest[6]. The volatile fraction

from the aerial part of the plant which has been obtained by hydrodistillation, was found to contain compounds from several chemical groups, such as aldehydes, alcohols, terpenes, esters, acids and hydrocarbons.[7] .

Taraxacum syriacum **Boiss.** is an annual herbaceous plant from the *Compositae* family[8], whose roots have been used for treating hepatic diseases, anemia, gout, rheumatism, gastric ulcers and skin diseases, such as eczema. *T.syriacum* was used in traditional herbal medicine for the treatment of jaundice, liver disorders and gallstones, as it lowered total cholesterol, triglyceride, insulin and fasting glucose levels, as well as insulin resistance induced by a high-fat diet [7]. With regard to its morphology, 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[9].

Chemical constituents of T.syriacum Boiss. roots, including 1,1-dimethyldiborane, 1-propene 3-ethoxy, 3,5-octadien-2-one, nonanal, decanal, nonanoic acid and carvacrol[12], were identified and extracted.

In one study was designed to investigate the effect of the ethanolic extract of the root of T. syriacum on acetaminophen-induced nephrotoxicity[10]. The roots of T. syriacum Boiss. were analyzed using solid-phase micro extraction It shows that T.syriacum decrease the chance of toxicity with acetaminophen in the body [11], [12] .

1.3 Problem statements

Oxidative stress, obesity, and antimicrobial resistance

Oxidative stress occurs when the production of reactive oxygen is greater than the body's ability to detoxify these reactive intermediates. The resulting imbalance leads to damaged proteins, molecules and genes within the body. When oxygen is metabolized, it creates 'free radicals' which steal electrons from other molecules causing damage[13].

Oxidative stress can occur when cells use glucose to make energy, as well as when inflammation takes place because the immune system is fighting off bacteria. It may also occur when our bodies detoxify pesticides, cigarette smoke and pollutants in general. In addition, oxidative stress is associated with important health conditions, including chronic fatigue syndrome, diabetes, Alzheimer’s disease and cancer. In fact, reducing unnecessary oxidation in our body by avoiding sugar and processed foods, while balancing blood sugar levels, also prevents infections and avoids toxin formation[14].

In other wise, Obesity is linked to increasing risk of heart disease, diabetes, hypertension and cancer, in addition to social and emotional effects, such as depression, and other chronic conditions. Fortunately, weight loss can reduce the risk of developing some of these problems.

Obesity also leads to other illnesses, acute and chronic. Some experts believe that obesity ranks as the second leading cause of cancer-associated death after cigarette smoking[15].

The study conducted by Wei Zheng et al. by the American Cancer Society, published in The New England Journal of Medicine[16], involved follow-up of more than 900,000 people for a period of 16 years. The study showed a link between excess bodyweight and many different cancers. The findings showed that among people aged 50 years and older, overweight condition, obesity in particular may account for 14% of all cancer deaths in men and 20% of all cancer deaths in women. In men, excess weight also increased the risk of dying from stomach or prostate cancer. In women, deaths from cancer of the breast, uterus, cervix or ovary were elevated in those with higher body mass.

In previously conducted studies showed that the larger the woman, the more likely she was to delay having a pelvic examination. This was largely because of negative experiences with doctors and office staff. In men, screening tests, such as prostate examination, may be physically difficult if people are very overweight, particularly if fat is stored on hips, buttocks or thighs. Losing weight can make one feel physically, as well as emotionally, better and can help one live longer. In cases of hypertension, weight loss can result in hypertension medicines being unnecessary in some people. In cases of diabetes, weight loss can result in the risk of diabetes –related diseases being cut by nearly 60%[17].

Also Antimicrobial resistance occurs when microorganisms, such as bacteria, fungi, viruses and parasites, are exposed to antimicrobial drugs, such as antibiotics and antifungal, antiviral, antimalarial and anthelmintic drugs[18].

Resistance occurs when an antibiotic has lost its ability to effectively control or kill bacterial growth, so bacteria become resistant and continue to multiply in the presence of therapeutic levels of an antibiotic.

There are many facts that should be known, such as when antimicrobial resistance happen it will be serious threat to global public health that requires action across all government sectors and society. Also, without effective antibiotics, the success of surgery and cancer would be compromised. In addition, the cost of health care for patients with resistant infections is higher than for patients with non-resistant infections, because of increased duration of the illness and the use of more expensive drugs[19]. Further, new mechanisms of resistance are spreading globally, threatening our ability to treat common infectious diseases. It is important to recognize that, without effective antimicrobials for treatment of infections, medical procedures, such as organ transplantation and cancer chemotherapy, become very high risk. Finally, genetic changes, misuse or overuse of antimicrobials, poor infection control, inadequate sanitary conditions and inappropriate food-handling all contribute to the acceleration of the resistance process.

Examples of antimicrobial resistance are as follows:

i. Resistance of *Klebsiella pneumonia,* common intestinal bacteria that can cause life-threatening infections, to a last resort treatment (carbapenem antibiotics) has spread to all regions of the world. *K. pneumonia* is a major cause of hospital-acquired infections, such as pneumonia[20].

ii. Resistance of *Escherichia coli* to one of the most widely used medicines for treatment of urinary tract infections (fluoroquinolone antibiotics) is very widespread[21]. There are countries in many parts of the world where this treatment is now ineffective in more than half of patients.

iii. Treatment failure to the last resort medicine for gonorrhoea (third generation cephalosporin antibiotics) has been confirmed in at least 10 countries (Australia, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden and the United Kingdom)[22].

iv. Resistance to first-line drugs for treating infections caused by *Staphlylococcus aureus*, a common cause of severe infections in health facilities and the community, is widespread. People with methicillin-resistant *Staphylococcus aureus* (MRSA) are estimated to be 64% more likely to die than people with a non-resistant form of the infection[23].

1.4 Aims and objectives of the study

The free radicals, obesity, and resistance to drugs represented serious problems nowadays, and cost of treatment for these problems was very high.

So many studies established to solve this complexity and facilitating people by trying to make medicine and cure from natural herbal plants.

So it would examined its ability to protect our cells from damage, in governorate ideal weight, also destroyed microbial cells, and eliminated them, by low side effects, and less cost needed.

Chapter Two

Methodology

2.1 Materials and methods

2.1.1 Chemicals and reagents

Chemicals and reagents used in the experiments are shown in Table 1.

Table 1: Chemicals and reagents used

Chemicals and reagents	Supplier	Supplier Country
Ethyl alcohol 99.9%	Sun Farm	Nablus, Palestine
Methanol	Backing Self	Israel
Nutrient agar 28g/L	Himedia Laboratories	Mumbai, India
Mannitol salt agar 111g/L	Himedia Laboratories	Mumba, India
MacConkey agar 49.53g/L	Himedia Laboratories	Mumbai, India
Sabouraud's dextrose agar 65g/L	Oxoid	England
Mueller-Hinton broth 21g/L	Himedia Laboratories	Mumbai, India
Sodium hydroxide 40g/mole	Sun Farm	Nablus, Palestine
3-(N-morpholino) propane sulfonic acid (MOPS) buffer34.53g/L	Sigma-Aldrich	United Kingdom
RPMI 1640 medium with L-glutamine without sodium bicarbonate 0.165mol/L (Rose well Park Memorial Institute)	Sigma-Aldrich	United Kingdom USA
DMSO 100% - 10%	Carlo ERBA	Germany
Drugs: donation from military medical services(MMS) Tinogyn (tablet)		

Tinidazole 500mg	Jerusalem	Ramallah, Palestine
Lamirase (tablet) Terbinafine 250mg	Birzeut	Birzeut, Palestine
Ciprox (tablet) Ciprofloxacin 250mg	Birzeit	Birzeit, Ramallah-Palestine
Azicare(capsule) Azithromycine 250mg	Pharmacare	Ramallah, Palestine
Laricid (tablet) Clarithromycine 500mg	Birzeit	Ramallah, Palestine
Zinaxim (tablet) Cefuraxime as axetil 250mg	Jerusalem company	Ramallah, Palestine
Doxypharm (tablet) Doxycycline hyclate 100mg	Jerusalem	Ramallah, Palestine
Voloxal (tablet) Levofloxacin 500mg	Birzeit	Birzeit, Ramallah
Hexane	Self packing	Israel
Acetone	Self packing	Israel
DPPH	Sigma-Aldrich	USA
Trolox	Sigma-Aldrich	USA
TrisHCl buffer	Sigma-Aldrich	USA
PNPB	Sigma-Aldrich	USA
Pancreatic lipase	Sigma-Aldrich	USA
Acetonitrile	Carlo ERBA	France
Orlistat	Sigma-Aldrich	USA
Phytochemical screening reagents: Millon’s reagent	Prepared in the laboratory	
Ninhydrin reagent	Sigma 151173	USA
Fehling’s reagent	Fluka 46203\46202	USA
Benedict’s reagent	Prepared in the laboratory	
Iodine reagent	Prepared in the laboratory	
$FeCl_3.6H_2O$	Riedel-de Haen	Germany
HCl	SDFCL	India
Liebermann’s reagent	Prepared in the	

	laboratory	
H_2SO_4	Merck	Germany
Salkowski's reagent	Prepared in the laboratory	
Glacial acetic acid	Frutaron	Israel
Chloroform	S.D.F CL	India
Rutin	Alfa Aesar	Israel
$AlCl_3$	Biomedicals	Germany
Potassium acetate	Sigma-Aldrich	USA
Gallic acid	Sigma-Aldrich	USA
Folin-Ciocalteu's reagent	Sigma-Aldrich	USA
$NaHCO_3$	Self packing	Israel

2.1.2 Equipments:

Equipments used in the study are shown in Table 2.

Table 2: Equipments used

Instrument	Supplier	Supplier Country
Spectrophotometer	Jenway	U.K
Water bath	Arij-Levy Memert	Israel Germany
Shaker	Memert	Germany
Sonicater	Mrc-lab-equipment	Israel
Balance	Sartorius max 300g Radway max 220g BECO	Canada Poland Germany
PH meter	Jenway	UK
Oven	Arilevy	Israel
Freeze dryer	Mill rock technology	China
Rotary evaporator	Heidolph OB2000,VV2000	Germany
Syringe filtration: Syringe 10/5mL	Changzhou Hekang Medic	Jiangsu,China
Needle	KDL	Shanghai,China
Sterile filter syringe 25mm	KDL	Shanghai, China
Incubator	ARij-Levy	Israel
Autoclave	ARij-Levy	Israel
Bunsen burner	Ningbo IGI Gas Industry Co.	United EN
Refrigerator	Ariston	Italia
Multichannel 30–300 µL micropipette	Mrc	Israel
Multichannel 1–10 µL micropipette	Eppendorf Research	Germany
Single micropipette 100–1000 µL	Microlit	India
Tubes, plates, swaps, loops,96-well culture plates	Cell Star	USA
Vortex	VELP Scientific	Europe
Heater	Lab Tech	Korea
Aluminium foil	Diamond(Reynolds Consumers)	USA
Sterile pipette 5/10mL	Nichipet EX	Japan
Tips (white, blue, yellow)	Labcon	USA
Blender(mixer)	IKA	Germany

2.2 Plant collection

The leaves of *A.arvensis* and the roots of *T.syriacum* were collected from Palestine and classified by experts in the field of botany from An-Najah National University.

The plant parts under study were washed with distilled water, dried in the shade at an average temperature of 20–30 °C for 72 h and then stored in a dry place for further use.

Photographs of plants are shown in Figure 1, 2 and 3.

Figure 1*:* Taraxacum syriacum

Figure 2: *A.arvensis*

Figure 3: *Alchemilla and Taraxacum in the laboratory*

2.3 Extraction methods

a) **Organic extraction:** Organic extraction was performed using the Soxhlet extraction method[24]. This extraction was established by taking 20g of dried plant powder, placing this in a glass thimble and

extracting using 250mL of each solvent separately (hexane, methanol and acetone). The extraction process was continued until the solvent in siphon tube of Soxhlet apparatus became colorless. The extract was then heated in a hot water bath at 35ºC until the solvent had completely evaporated. The dried plant crude extract was stored in the refrigerator at 2–8ºC for future use.

b) Aqueous (crude) extraction: Aqueous extraction[25] was performed by taking 5 g of the plant powder and mixing it with 200mL of distilled water in a beaker. The mixture was heated (not boiled) on a hot plate at 30–40ºC with continuous stirring for 20 minutes. The mixture was filtered using Whatman filter paper then used freeze dryer, finally the filtrate was used for further phytochemical analysis.

2.4 Phytochemical screening tests

Medicinal plants contain some organic compounds which can have physiological action on the body. These substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. They are widely used in human therapy, veterinary, agriculture, and scientific research. There are a large number of phytochemicals belonging to chemical classes which have been shown to have inhibitory effects on many types of microorganisms in vitro.

Phytochemical screening tests were conducted according to the methods reported by Trease and Evans (1996)[26].

The percent of yields of phytochemicals from the studied plants using methanol, hexane, acetone and water extraction solvents are shown in Table.

Table 3: The percentage yields from plants using different extraction solvents

T.syriacum:	Yield
Methanol	12.5%
Hexane	2.07%
Acetone	3.15%
Water	2.19%
A.arvensis:	
Methanol	14.17%
Hexane	3.87%
Acetone	2.83%
Water	25.55%

2.5 Determination of total phenols and flavonoids

Total phenolic content (TPC) in the plant extracts was determined using spectrophotometric method with some modifications[27]. Aqueous solutions of methanolic extracts (1 mg/mL) were prepared in 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.

The samples were incubated in a thermostat at 45ºC for 45 min. The absorbance was determined using a spectrophotometer at a wavelength of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. 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 of GA/g of extract).

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 RU/g extract)[28]. Total flavonoid content was determined according to the modified procedure of Chang et al.(2002)[28],validated by Nugrohoet al. (2011)[29]. Rutin (100 mg) was dissolved in 10 mL distilled water and then 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 1M potassium acetate and 5 mL distilled water, and then incubated at room temperature for 30 min.

Absorbance was then measured at 415 nm wavelength using a spectrophotometer. Distilled water with methanol, 10% $AlCl_3$ and potassium acetate were used as a blank.

Total flavonoid content of extracts was expressed as rutin equivalents (mg of RU/g plant extract).

2.6 Antioxidant methods

2.6.1 Plant extract preparation (crude extract)

Approximately 10g of ground plant material was soaked in 1 L of methanol (99%) and placed in a shaker device (100 revolutions per min) for 72 h at room temperature. The resulting solution was stored in a refrigerator for four days. The extracts were filtered using filter paper and then

concentrated under vacuum using a rotator evaporator. The crude extract was stored at 4ºC for further use.

2.6.2 Antioxidant test

Stock solutions of plant extract and trolox (the reference substance) were prepared at a concentration of 0.1mg/ml in methanol. Working solutions at concentrations of 1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80 and 100µg/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 a blank solution. The first solution of the series concentration was DPPH with methanol only. The solutions were incubated in dark for 30 minute at room temperature before the absorbance readings were recorded at 517nm. The percentage of antioxidant activity of the plants and the trolox standard was calculated using the following formula:

$$\text{DPPH activity (\%)} = (A-B)/A \times 100$$

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

2.7 Antilipase methods

2.7.1 Lipase stock solution

The porcine pancreatic lipase inhibitory assay was adapted from the published method of Zheng et al. (2010)[30], with some modifications.

Plant extract stock solution (1mg/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(1mg/mL)in tris-HCl buffer was prepared immediately before use.

2.7.2 Lipase substrate stock solution

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 pocrine 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.

2.7.3 Lipase activity

Pancreatic lipase 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 reference compound.

2.8 Antimicrobial assays

2.8.1 Antibacterial test

Antibacterial activity was examined against references strains of *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*, *Shigella sonnie.* These bacterial strains were obtained from the American Type Culture Collection (ATCC).

Antibacterial activity was tested using simple agar diffusion[31], by culturing bacteria on nutrient agar then made wells put 50µl of plant extract. Results were collected the following day by recording the zone of inhibition, if present. Plant extracts that were obtained using different extraction methods (methanol, acetone, hexane and aqueous [crude] extracts) were used.

Briefly, 10 mg of each plant material was dissolved in 1 mL of 10% DMSO, which is an organic solvent. After adjusting the turbidity of bacterial suspension, a sterile cotton swab was dipped into the adjusted suspension and gently streaked three times on the agar media. The wells were then filled with the plant extracts.

Each well was filled with 50µL of plant extract. Each plate had positive and blank control. Then the plate was inverted and incubated for 16–18 h at 37°C. The antibacterial activity was then observed on the plate, represented by a clear zone surrounding the well.

The minimal inhibitory concentration (MIC) of the plant extracts against bacteria was determined using the micro-broth dilution method[32].

Mueller-Hinton broth (MHB) was used for this test. This broth is considered as the best medium for routine susceptibility tests as it has good reproducibility and enables satisfactory growth of most bacterial pathogens. The media was tested to ensure a pH of between 7.2 and 7.4 at room temperature (25˚C). MIC testing was performed in a polystyrene panel containing approximately 96 wells including a positive and negative growth control. Briefly, 100 µL MHB was added to each well. The plant extract (100 µL) was added to the first wells and then serially diluted with MHB in the remaining wells. The microorganisms (bacteria) were added to the MHB using a swab 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 serial dilutions were made by inoculating 1µl of each concentration of 5×10^5CFU/mL in the first well. The panel was covered and was incubated at 35˚C for 16–20 h before analysis of the results.

In this study, many types of media were prepared for culturing bacteria according to manufacturer's instructions. These media are as follows:[33]

i. MHB: 8.4g of MHB was dissolved in 400 mL distilled water (21g in 1000mL), and then 5mL was put in each test-tube and then sterilized by autoclave. The solution was kept in the refrigerator until being used for direct determination of MIC in wells and assessment of the turbidity of bacterial growth compared to the McFarland reference.

ii. Mannitol agar: 11.1g of agar was dissolved in 100mL distilled water (111g/L), and then placed in an autoclave for sterilization. Finally,

the agar was poured into a large plate to cool. This medium was used to culture gram-positive bacteria.

iii. MacConkey agar: 5.15g of agar was dissolved in 100 mL distilled water (51.5g/L). After autoclaving, the agar was poured into large plates (25mL) and allowed to cool. This medium was used to culture gram-negative bacteria.

iv. Nutrient agar: 5.6g of agar was dissolved in 200 mL distilled water (28g/L). This was autoclaved and then poured into large plates. This medium was used to culture gram-positive and -negative bacteria.

McFarland 0.5 solution: (Preparation of McFarland Turbidity Standards - microbe online)[34]. A 1% solution of anhydrous barium chloride ($BaCl_2$) and 1% solution of sulfuric acid (H_2SO_4)was prepared. The two solutions were combined and mixed well to form a turbid suspension, containing $BaSO_4$The resulting mixture was stored in a foil-covered screw-cap tube, together with the McFarland standard, at room temperature (25 °C).When not in use, a fresh standard solution was prepared every 6 months. Absorbance is 0.08-0.1 at 600 nm.

Many types of antibiotics were used to determine bacterial resistance. These were dissolved in a specific volume of each solvent according to the solubility tests.

The concentrations of antibiotics and solvents that were used are shown in Table 4.

Table 4:Concentration of antibiotics in respective solvents that were tested

Antibiotic and solvent	Concentration in each well (µg/mL)
Azithromycine : Ethanol 95%	Well 1= 22.7 Well 2= 11.4 Well 3= 5.9 Well 4=2.8 Well 5=1.4 Well 6=0.71 Well 7=0.35 Well 8=0.18 Well 9=0.09 Well 10=0.045
Clarithromycine: Methanol	Well1=2.13 Well2=1.06 Well3=0.53 Well4=0.27 Well5=0.13 Well6=0.07 Well7=0.03 Well8=0.016 Well9=0.008 Well10=0.004
Levofloxacine: Distilled water	Well1=0.16 Well2=0.08 Well3=0.04 Well4=0.02 Well5=0.01 Well6=0.005 Well7=0.003 Well8=0.001 Well9=0.0006 Well10=0.0003
Doxycyline: DMSO 100%	Well1=0.31 Well2=0.16 Well3=0.08 Well4=0.04 Well5=0.02 Well6=0.009 Well7=0.005 Well8=0.003 Well9=0.001 Well10=0.0006

Cefuroxime: DMSO100%	Well1=18.9 Well2=9.4 Well3=4.7 Well4=2.4 Well5=1.18 Well6=0.6 Well7=0.3 Well8=0.15 Well9=0.07 Well10=0.04
Ciprofloxacin: Distilled water	Well1=1.9 Well2=0.96 Well3=0.5 Well4=0.24 Well5=0.12 Well6=0.06 Well7=0.03 Well8=0.02 Well9=0.008 Well10=0.004

Stock solutions were prepared with dilution factor of 10 as follows:

Antibiotics	Preparation of stock solutions
Azithromycine	4.545mg/mL was prepared by two dilutions to have growth of bacteria and MIC was detected from 45.45µg/mL concentration.
Clarithromycin	4.255mg/mL was prepared by three dilutions to have growth of bacteria and MIC was detected from 4.255µg/mL concentration.
Levofloxacine	3.2786mg/mL was prepared by four dilutions to have growth of bacteria and MIC was detected from 0.3278µg/mL concentration.
Doxycycline	6.203mg/mL was prepared by four dilutions to have growth of bacteria and MIC was detected from 0.6203µg/mL concentration.
Cefuroxime	3.778mg/mL was prepared by two dilutions to have growth of bacteria and MIC was detected from 37.7µg/mL concentration.
Ciprofloxacin	3.840mg/mL was prepared by three dilutions to have growth of bacteria and MIC was detected from 3.84µg/mL concentration.

The solutions were prepared as a 1:10 dilution (1mL antibiotic solution plus 9 mL of solvent).

2.8.2 Antifungal test

Antifungal activity was examined against two pathogenic fungal references available from the microbiological labs at An-Najah National University. The fungi were *Epidermophyton floccosum* and *Candida albicans*. Potato Dextrose Agar (PDA) was used to culture these fungi, recommended as a relatively rich medium for growing a wide range of fungi and prepared according to the manufacturer's instructions.

Plant extract activity 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[35]. The *Candida* concentration in McFarland was $1\times10^{6}$to 5×10^{6}CFU\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^{3}CFU/mL. Aliquots (100 µl) were added to each well, except well number 11.The concentration in first well was 333.33 to 1666.66 CFU/mL.

Antimicrobial activity against *Epidermophyton floccosum* was determined using the agar dilution method[36]. In this method, 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). This was then was applied to the SDA containing different concentrations of plant extract.

MIC was the lowest concentration of plant extract that caused visible inhibition of fungal growth.

In this study, the following two methods were used to determine the effect of the plant extract on two types of fungi:

i. *Candida albicans*: SDA media was used to culture this fungus. Agar (19.5g) was dissolved in 300 mL distilled water (65gin 1000mL), autoclaved and then poured into large plates ready for culturing the fungus on the following day. MIC was performed using MHB and RPMI media. RPMI was prepared by dissolving 1.04 g RPMI in 90mL distilled water. MOPS(3.453 g) was added to this and the pH adjusted to 7 at 25 °C by adding sodium hydroxide (1 mole/mL). Finally, the solution was filter-sterilized using a syringe filter. The same steps and serial dilutions of MIC in bacteria were also used here.

ii. *Epidermophyton floccosum*: SDA was used in agar dilution method. SDA was prepared in test tubes, each tube containing 1 mL. Test tubes were autoclaved and the placed in a water bath at 40 °C. Plant extract (1 mL) was then added with serial dilution. The tubes were left to the following day to allow slants to form.

The fungus was prepared by removing a sample and then adding this to a small plate with Tween and NaCl media. Later, 20µL of the fungal solution was added to each tube and left for 14 days. The results were then recorded.

In addition, two types of antifungal drugs were used to determine the resistance of fungi, mixed in suitable solvents according to solubility.

The concentrations and suitable solvents used are shown in Table 5.

Table 5: Concentrations of antifungal drugs tested and suitable solvents

Antifungal drug and solvent	Concentration in each well (µg/ml)
Terbenafin: Distilled water and methanol.	Well1=250 Well2=125 Well3=62.5 Well4=31.25 Well5=15.6 Well6=7.8 Well7=3.9 Well8=1.1 Well9=0.1 Well10=0.49
Tinidazole: Methanol	Well1=250 Well2=125 Well3=62.5 Well4=31.25 Well5=15.6 Well6=7.8 Well7=3.9 Well8=1.1 Well9=0.1 Well10=0.49

Stock solutions were prepared with dilution factor equal 10.

Antifungal	Preparation of stock solutions
Terbenafin	50mg/mL was prepared by two dilutions to begin MIC from 500µg/mL concentration
Tinidazole	50mg/mL was prepared by two dilutions to begin MIC from 500µg/mL concentration

Drugs were diluted in a 1:10 dilution (1 mL of solution plus 9 mL of solvent).

Chapter Three

Results

3.1 Results

3.1.1 Phytochemical screening tests

Many tests were conducted to determine the presence of organic materials. The results are shown in Table 6 and Table 7.

Table 6:Phytochemical screening test results for *Taraxacum syriacum*

Plant name and extract solvent	Test name and result
***T.syriacum*:** Methanol Hexane Acetone Water	**Protein test (Millon's test)\(Ninhydrine):** negative negative negative negative
 Methanol Hexane Acetone Water	**Carbohydrate tests(Fehling's)\(Benedict's)\(Iodine):** negative negative negative negative
 Methanol Hexane Acetone Water	**Phenol and tannin tests($FeCl_3$):** positive negative positive positive
 Methanol Hexane Acetone Water	**Flavonoid test(Shinoda)/(Alkaline):** positive negative positive positive
 Methanol Hexane Acetone Water	**Saponins test:** negative negative negative negative
 Methanol Hexane	**Glycosides test(Liebermann's)/(Salkowski's):** positive positive

Acetone	positive
Water	negative
	Cardiac steroidal glycoside test: (Killer killiani)
Methanol	negative
Hexane	positive
Acetone	negative
Water	positive
	Steroid test:
Methanol	positive
Hexane	negative
Acetone	positive
Water	negative
	Terpenoids test:
Methanol	negative
Hexane	negative
Acetone	negative
Water	negative

Table 7:Phytochemical screening test results for *A.arvensis*

Plant name and extract solvent	Test name and result
***A.arvensis*:** Methanol Hexane Acetone Water	**Protein test (Millon's test)/(Ninhydrine):** negative negative negative negative
 Methanol Hexane Acetone Water	**Carbohydrate test(Fehling's)/(Benedict's)/(Iodine):** negative negative negative negative
 Methanol Hexane Acetone Water	**Phenol and tannin test($FeCl_3$):** positive negative negative negative
 Methanol Hexane Acetone Water	**Flavonoid test(Shinoda)/(Alkaline):** negative negative negative positive
 Methanol Hexane Acetone Water	**Saponins test:** negative negative negative negative
 Methanol Hexane Acetone Water	**Glycoside test(Liebermann's)/(Salkowski's):** positive positive positive negative
 Methanol Hexane Acetone Water	**Cardiac steroidal glycoside test: (Killer killiani)** positive positive positive negative
 Methanol Hexane Acetone Water	**Steroid test:** positive negative positive negative
 Methanol Hexane Acetone Water	**Terpenoids test:** negative negative negative negative

3.1.2Total phenols and flavonoids results:

In this study, the total phenol and flavonoid contents were calculated in 1g of plant extract and the results are shown in Table 8 and 9:

Table 8:Total phenol content results

Plant and extract solvent	Total phenol content (mg gallic acid/g of plant extract)
T.syriacum:	
Methanol	120.43
Acetone	271.95
Water	143.74
A.arvensis:	
Methanol	151.51

This Table shows that more TPC present in acetone extract of *T.syriacum,* and only in methanol extract of *A.arvensis.*

The calibration curve for gallic acid is shown in Figure 4.

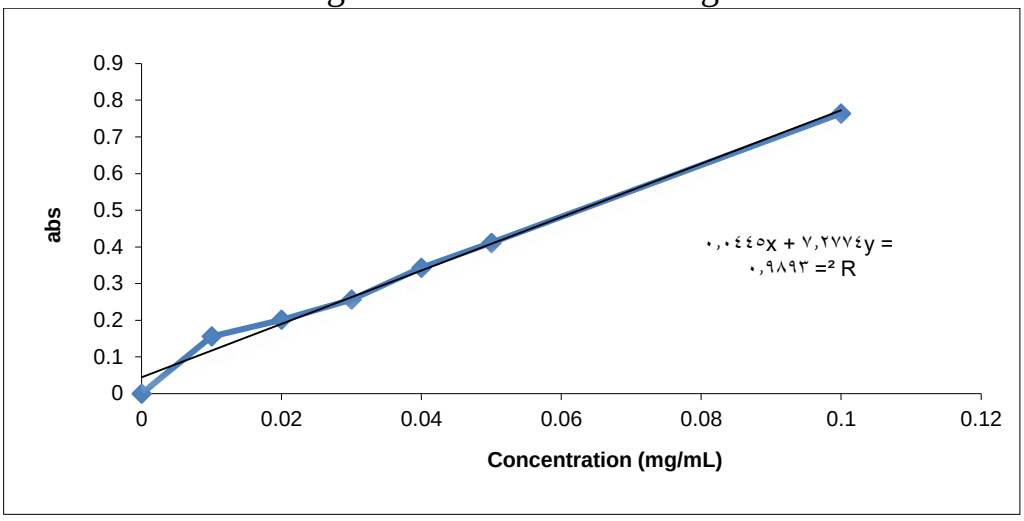

Figure 4: *Calibration curve for gallic acid*

Table 9: Total flavonoid content results

Plant and extract solvent	Total flavonoid content (mg rutin/g plant extract)
T.syriacum:	
Methanol	8.42
Acetone	17.78
Water	27.13
A.arvensis:	
Water	83.31

This Table shows that more TFC present in water extract of T.syriacum, and only present in water extract of A.arvensis

The calibration curve for rutin is shown in Figure 5.

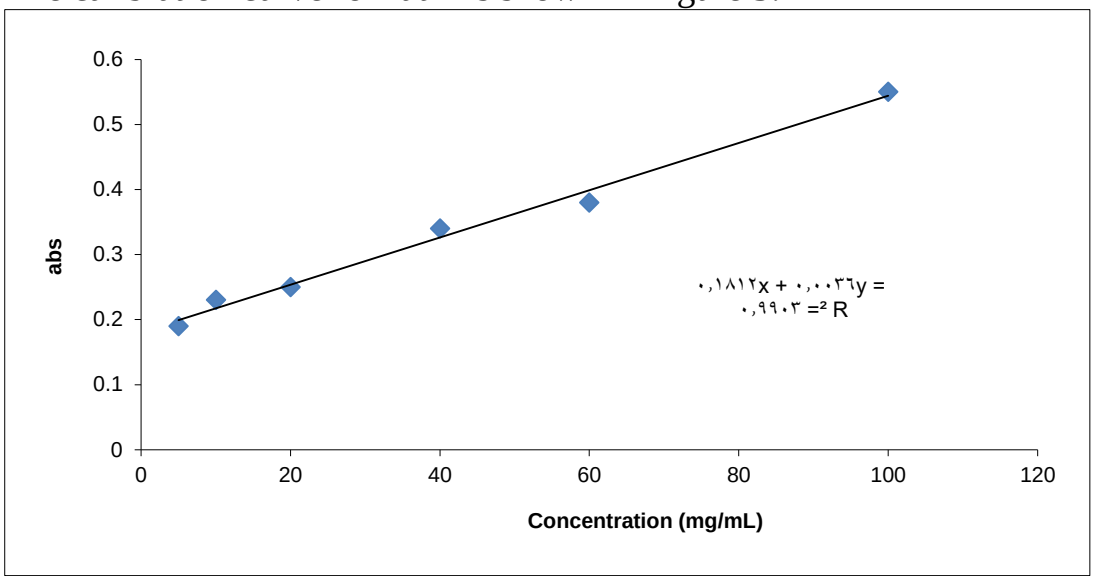

Figure 5: *Calibration curve for rutin*

3.1.3Antioxidant results

The IC_{50} was calculated for each plant after percentage inhibition for DPPH was determined and Trolox was used as reference substance. The results are shown in Table 10 and Table 11.

This Table shows that more TFC present in water extract of T.syriacum, and only present in water extract of A.arvensis.

Table 10: IC_{50}values for antioxidant activity of *T.syriacum and A.arvensis*

Plant and extract solvent	IC_{50} (µg/mL)
***T.syriacum*:** Hexane Acetone Methanol Water	 177.8 707.94578 281.83 95.49
***A.arvensis*:** Hexane Acetone Methanol Water	 11.22 4.86 97.72 724.43
Trolox reference	2.19

This Table shows that the most potent antioxidant activity extract of T.syriacum is water and acetone in A.arvensis.

Antioxidant curves for *A.arvensis* and *T.syriacum* are shown in Figure 6 and 7, respectively.

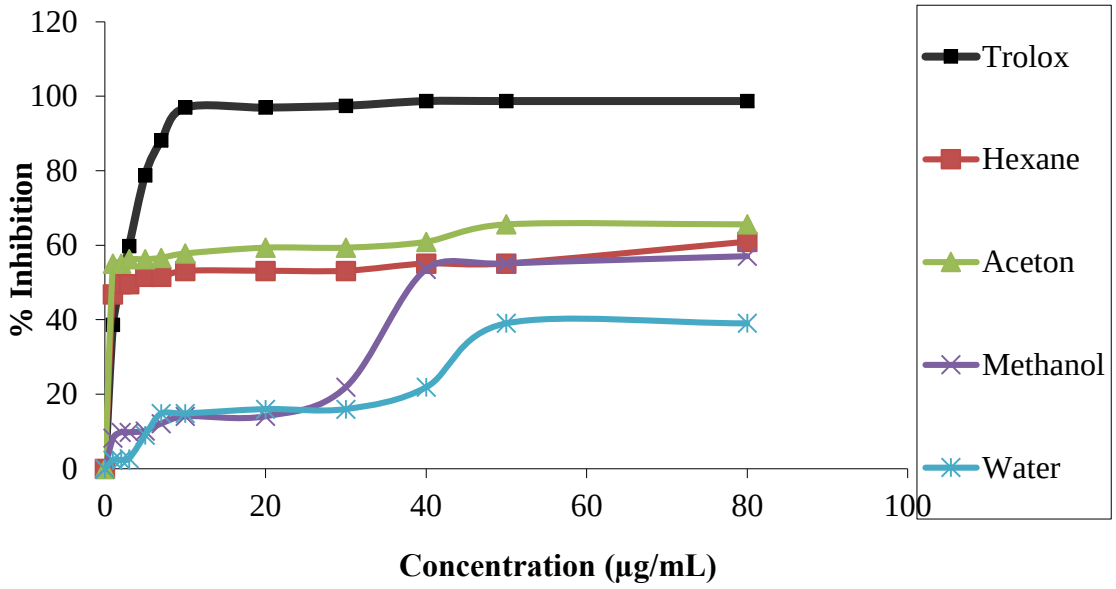

Figure 6: *Antioxidant curve for A.arvensis*

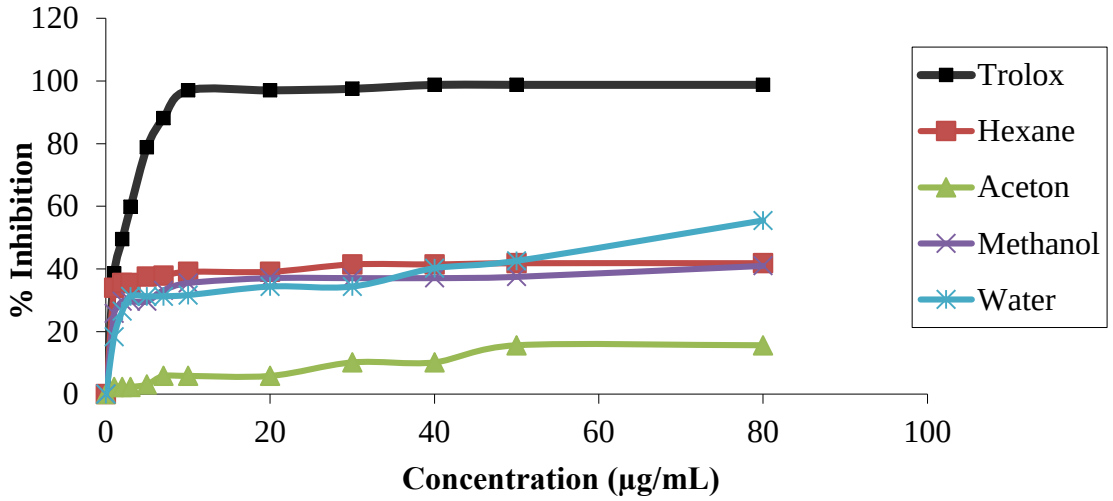

Figure 7: *Antioxidant curve for T.syriacum*

3.1.4. Antilipase tests

The activity of these plants in treating obesity was determined. Antilipase activity was detected using the reference Orlistat. The results are shown in Table 11.

This Table shows that the most potent antioxidant activity extract of T.syriacum is water and acetone in A.arvensis.

Table 11: IC_{50} values for antilipase activity of *T.syriacum and A.arvensis*

Plant and extract solvent	IC_{50} (µg/mL)
***T.syriacum*:**	
Hexane	218.77
Acetone	977.23
Methanol	3311.31
Water	154.88
***A.arvensis*:**	
Hexane	72.44
Acetone	45.70
Methanol	30.90
Water	21.37
Orlistat reference	20.41

This Table shows that the most potent antilipase activity extract of *T.syriacum* is water, and also water in *A.arvensis.*

Antilipase curves for *A.arvensis* and *T.syriacum* are shown in Figure 8 and 9, respectively.

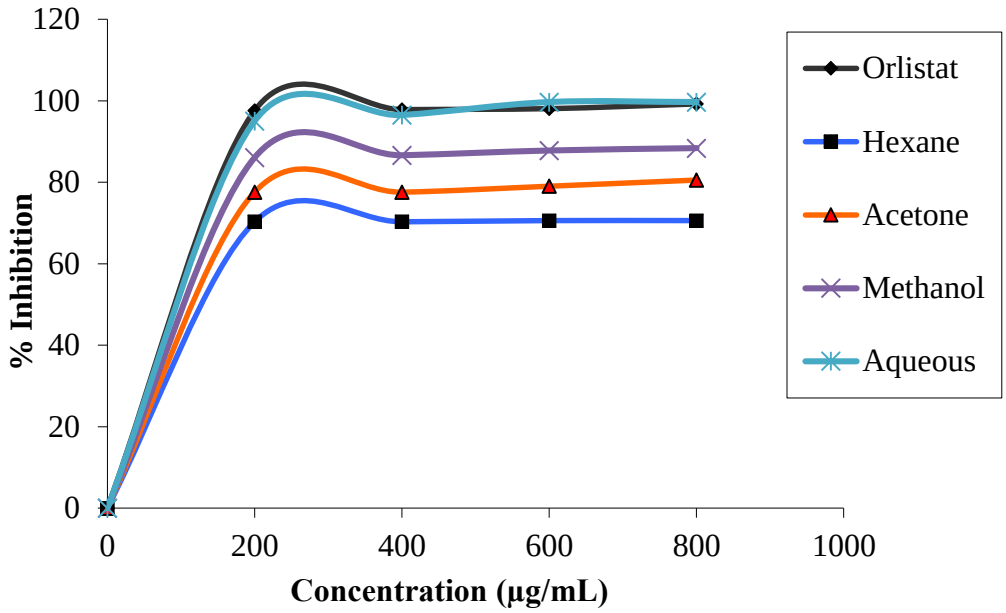

Figure 8: *Antilipase curve for A.arvensis*

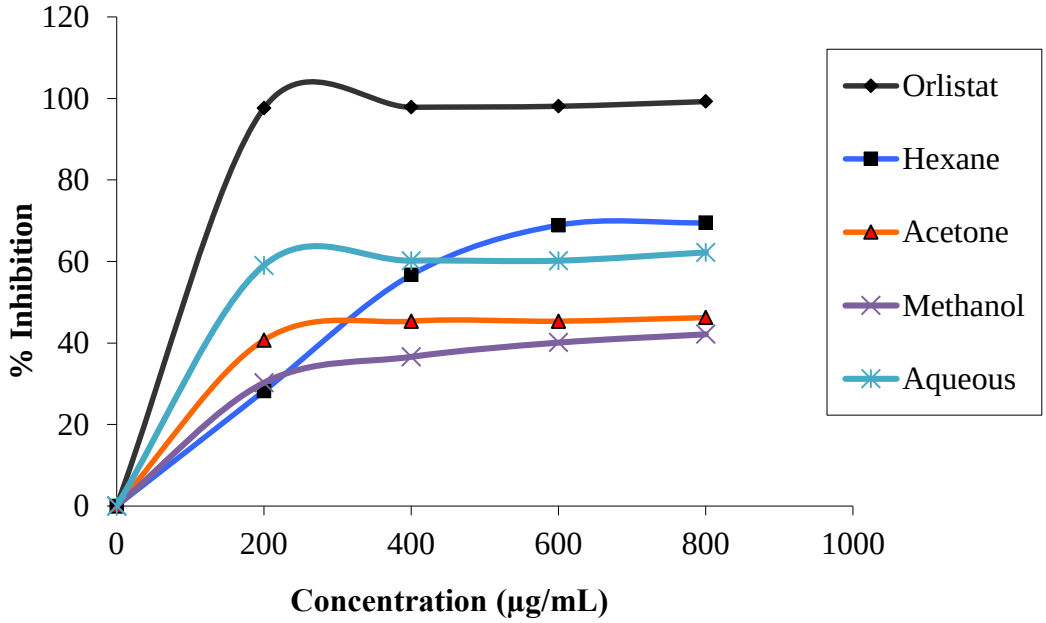

Figure 9: *Antilipase curve for T.syriacum*

3.1.5 Antimicrobial tests:

3.1.5.1 Antibacterial tests

The MIC values were determined for each plant extract with different type of bacteria, as shown in Table 12.

Table 12:MIC values for *T.syriacum* and *A.arvensis* with bacteria

A) *T.syriacum*

Plant extract solvent	Water	Acetone	Methanol	Hexane	DMSO
Bacterial Name	MIC values(mg/mL)				
***Staphylococcus aureus* ATCC 25923**	6.25	12.5	12.5	No inhibition	6.25
***Pseudomonas aeruginosa* ATCC 27853**	25	12.5	6.25	No inhibition	6.25
***Escherichia coli* ATCC 25922**	25	12.5	6.25	No inhibition	6.25
***Shigella sonnie* ATCC 25931**	25	12.5	12.5	No inhibition	6.25

B) A.arvensis

Plant extract solvent	Water	Acetone	Methanol	Hexane	DMSO
Bacteria name			MIC values	(mg/mL)	
***Staphylococcus aureus* ATCC 25923**	25	25	3.125	No inhibition	6.25
***Pseudomonas aeruginosa* ATCC 27853**	12.5	No inhibition	3.125	No inhibition	6.25
***Escherichia coli* ATCC 25922**	12.5	No inhibition	3.125	No inhibition	6.25
***Shigella sonnie* ATCC 25931**	12.5	25	3.125	12.5	6.25

The MIC results with bacteria are shown in Figure 10.

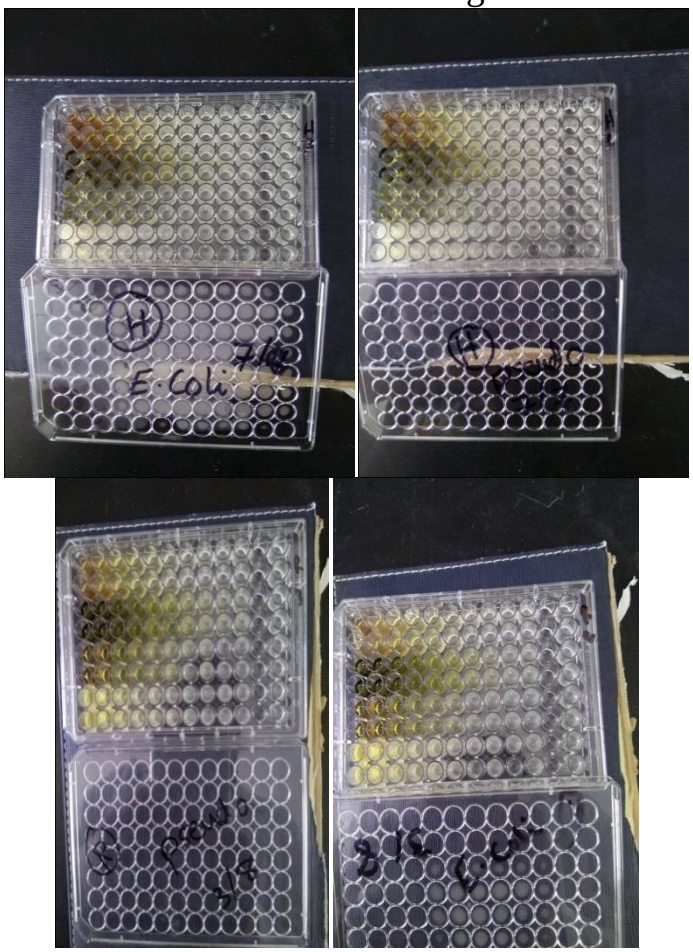

Figure 10: *Some of MIC results with bacteria*

In another way, simple agar diffusion was used, by doing wells and plant extract was added in each well. The results are shown in Table 13.

Table 13:Results of simple agar diffusion

A)T.syriacum

Plant extract solvent	Water	Acetone	Methanol	Hexane
Bacteria name	Diameter			
Escherichia coli	No	No	No	No
Pseudomonas aeruginosa	NO	1 cm	No	No
Staphylococcus aureus	No	No	No	1 cm
Shigella sonnie	No	No	No	No

B) A.arvensis

Plant extract solvent	Water	Acetone	Methanol	Hexane
Bacteria name	Diameter			
Escherichia coli	2 cm	1 cm	1 cm	No
Pseudomonas aeruginosa	No	No	No	No
Staphylococcus aureus	1.5 cm	1 cm	1 cm	No
Shigella sonnie	No	2 cm	1.5 cm	1 cm

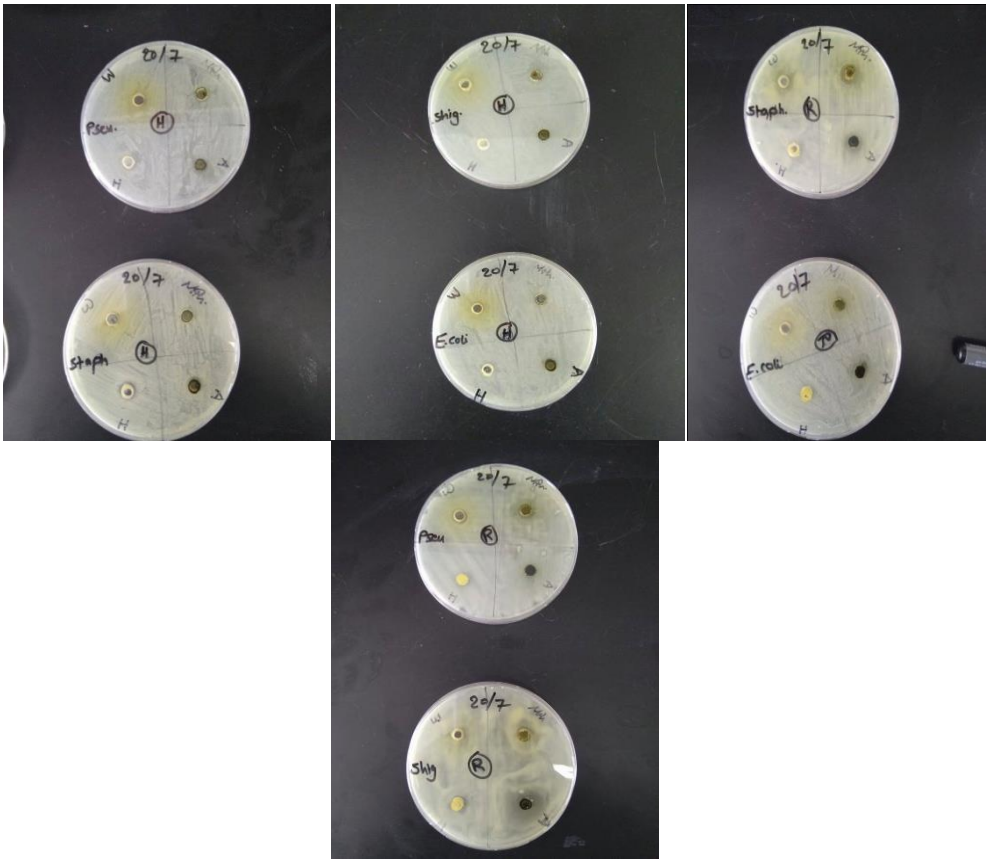

Figure 11: *Photographs of simple agar diffusion*

Also drug resistance for these four bacteria were determined with different type of antibiotics and different dilutions. Results are shown in Table 14.

This Table shows that the most potent antilipase activity extract of T.syriacum is water, and also water in A.arvensis.

Table 14:MIC values for antibiotic resistance of different bacteria

Bacteria	Antibiotic	MIC value (µg/mL)
Staphylococcus aureus **ATCC 25923**	Azithromycine 250mg Clarithromycin 500mg Levofloxacin 500mg Doxycycline 100mg Cefuroxime 250mg Ciprofloxacin 250mg	0.355 0.0083 0.0051 0.077 2.35 0.0075
Pseudomonas aeruginosa **ATCC 27853**	Azithromycine 250mg Clarithromycin 500mg Levofloxacin 500mg Doxycycline 100mg Cefuroxime 250mg Ciprofloxacin 250mg	0.71 0.531 0.00125 0.038 2.35 No growth
Escherichia coli **ATCC 25922**	Azithromycine 250mg Clarithromycin 500mg Levofloxacin 500mg Doxycycline 100mg Cefuroxime 250mg Ciprofloxacin 250mg	0.71 0.132 0.00125 No growth 2.35 0.015
Shigella sonnie **ATCC 25931**	Azithromycine 250mg Clarithromycin 500mg Levofloxacin 500mg Doxycycline 100mg Cefuroxime 250mg Ciprofloxacin 250mg	0.71 1.063 No growth 0.038 2.35 No growth

This Table shows the sensitivity of bacteria to different types of antibiotics

The following MIC results of antibiotics resistance were shown in Figure 12.

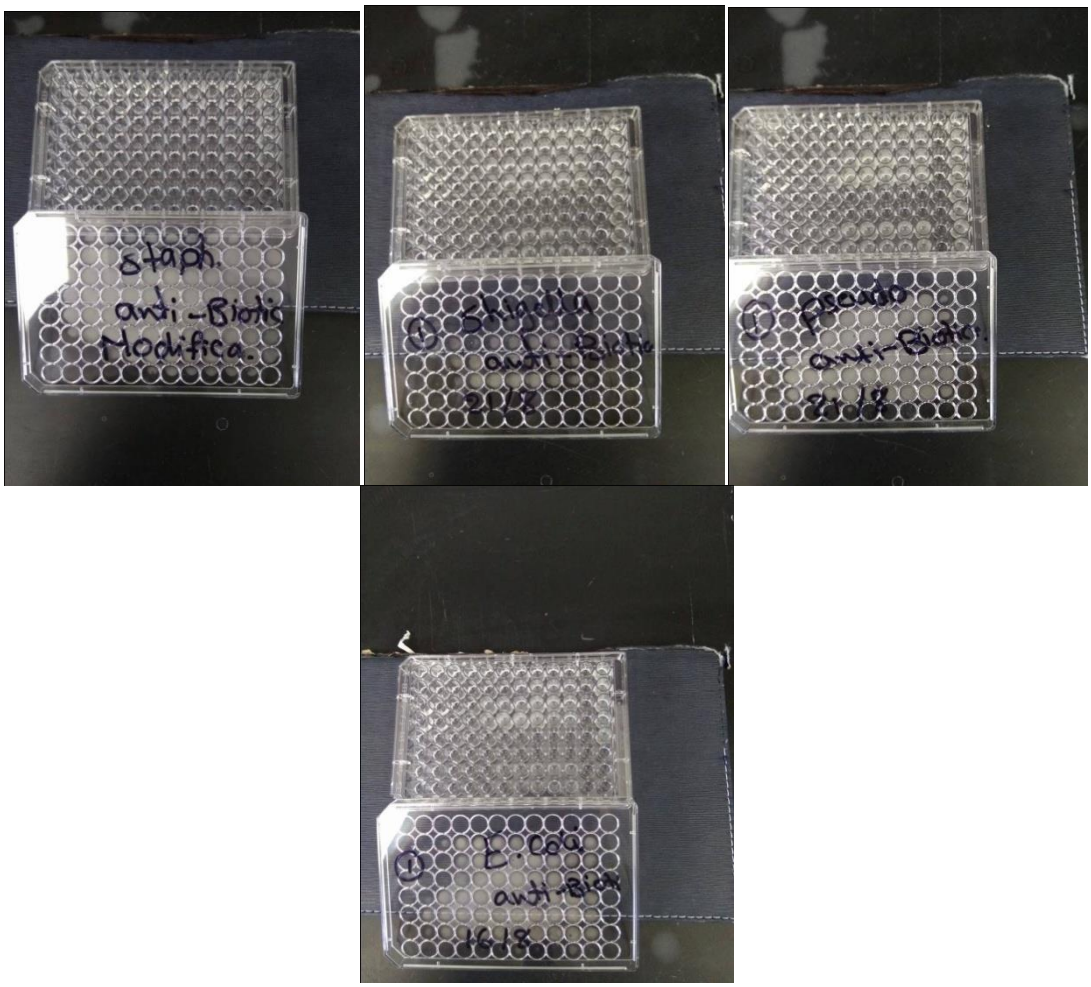

Figure 12: *Some MIC results of antibiotics resistance*

3.1.5.2Antifungal test:

The MIC values for different plant extracts with two types of fungi were determined and are shown in Table 15.

Table 15: MIC values for *Taraxacum syriacum*, and *A.arvensis* with fungi

T.syriacum

Plant extract solvent	Water	Acetone	Methanol	Hexane	DMSO
Fungus	MIC value (mg/mL)				
***Candida albicans* ATCC 90028**	25	3.125	6.25	6.25	3.70%
***Epidermophyton floccosum*ATCC52066**	1.56	0.78	0.78	0.78	6.25%

A.arvensis

Plant extract solvent	Water	Acetone	Methanol	Hexane	DMSO
Fungus name	MIC value (mg/mL)				
***Candida albicans* ATCC 90028**	12.5	6.25	6.25	12.5	3.70%
***Epidermophyton floccosum* ATCC 52066**	0.78	0.78	0.78	0.78	6.25%

The following MIC results with fungi were shown in Figure 13.

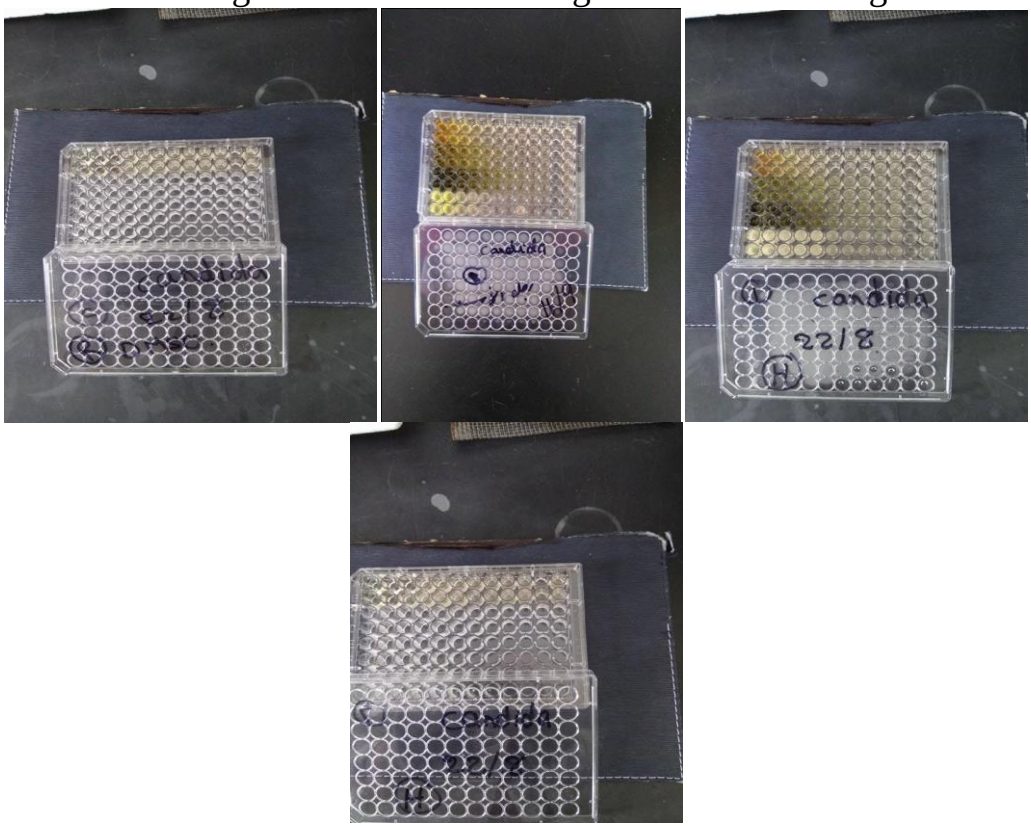

Figure 13: *Some of MIC results with fungi*

The following results of *Epidermophyton floccosum* with plant extracts that were determined are shown in Figure 13.

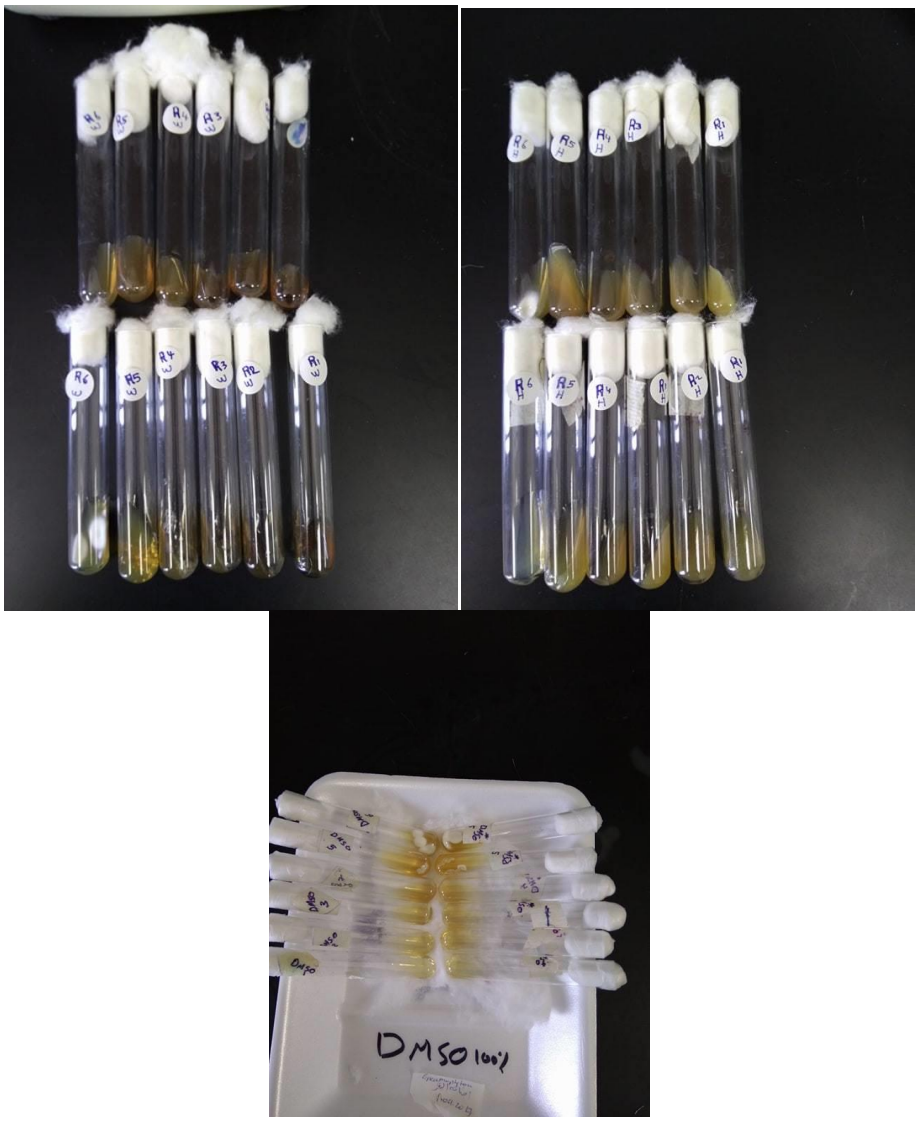

Figure 14:*Photographs of plants with Epidermophyton floccosum fungus*

The MIC values for fungal resistance to the antifungal drugs are shown in Table 16.

Table 16:MIC values for anti-fungal resistance with different fungi

Fungus	Anti-fungal drug	MIC value (µg\mL)
***Candida albicans* ATCC 90028**	Tinidazole 500mg Terbinafine 250mg	No inhibition 18.5185

This Table shows the sensitivity of fungi to different types of antifungal drugs.

Chapter four

Discussion

4.1 Discussion

4.1.1Flavonoids:

Flavonoids are polyphenolic molecules containing 15 carbon atoms and are soluble in water, [37].They are also plant pigments that are synthesized from phenylalanine, associated with the display of colors from flower petals. They regulate plant growth through inhibition of the exocytosis of acetic acid and they influence other biological cells in numerous ways. Flavonoids kill many bacteria and inhibit important viral enzymes, such as reverse transcriptase. They are major functional components of many herbal and insect preparations for medical use. Also flavonoids are found in fruits and vegetables, and they have many beneficial effects, such as antiviral, anticancer, anti-inflammatory and anti-allergic effects[38].

In our current study an aqueous extracts of *T.syriacum* contained high quantity of flavonoid; 1 g of plant extract contained 27.13mg flavonoid. To the best of our knowledge, no previous studies have been conducted with *T.syriacum* plants. But in a study by Liu et al. (2008) 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[39]. Also there is another study Williams et al. (1996)reported three flavonoid glycosides[40], were isolated from *Taraxacum officinale.*

In another side an aqueous extract of *A.arvensis* contained a high quantity of flavonoid; in 1 g of plant extract the quantity was 83.31mg. There is a study on *Alchemilla vulgaris* by, Neagu et al. (2015) reported flavone

content between 360 and 862 µg/mL (for water and ethanol extract)[41]. Also in another study of *Alchemilla mollis* by Nedyalkov et al. (2015) shown that flavonoids concentration varied between 0.966 ± 0.023 and 1.666 ± 0.017 µg/ml ethanol extract [42].

4.1.2 Phenols

Phenolic compounds are reactive metabolites in a wide range of plant-derived foods and are mainly divided into four groups, phenolic acids, flavonoids, stilbenes and tannins[43]. There are many beneficial properties of phenolic components, such as anti-oxidant effects, anti-mutagenic activity and prevention of cardiovascular heart disease. Also, when these components were taken in large doses, they could cause genotoxicity or thyroid toxicity, and interact with other pharmaceuticals and have estrogen activity.

In our current study of *A.arvensis*, a high total phenol content in methanol extract was detected (151.51mg in 1g of plant extract). But in a study conducted by Kiselovaet al.(2006), showed that TPC determined in the tested compounds was between 88.00 and 112.33µg/mL[44]. Another study by Neagu et al. (2015) of *Alchemilla vulgaris*[41] reported total phenolic and condensed tannin content in the mature leaves of 30 *Alchemilla* species. The total phenolic content was found to be different in leaf extracts of these species.

In another side, *Taraxacum syriacum,* the highest content of phenol was detected in acetone extract, equal to 271.95 mg in 1 g of plant extract. To

the best of our knowledge, no previous studies have been conducted with *Taraxacum syriacum*. But in one study of *Taraxacum mongolicum* conducted by Li et al. (2008), showed that total phenolic content of methanol and water extracts from the upper part of the plant were 51.95±0.18 mg/g and 48.16±0.89 mg/g, respectively[45]. Also in another study was published on *Taraxacum officinale* by Amin Miret al. (2012)that investigated the qualitative and quantitative analysis of the major bioactive constituents of *Taraxacum officinale* aqueous and methanol extracts [46].

4.1.3 Antioxidant effect:

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation process is a chemical reaction that can produce free radicals, which lead to chain reactions that cause damage of cells. Thiols or ascorbic acid (vitamin C) terminate these chain reactions so they are anti-oxidant materials[47].

The term antioxidant applies to two different groups of substances, as follows:

i. Industrial chemicals which are added to products to prevent oxidation, such as preservatives in food, cosmetics and fuels.
ii. Natural chemicals which are present in food (fruit and vegetables) and protect the body from oxidative reactions with lipids, proteins or enzymes, by trapping free radicals inside the body.

In our current study, *A.arvensis* had the best antioxidant effect when present in methanol, hexane, and acetone extracts, as shown previously,

with IC_{50}values of 97.72,11.22 and 4.86µg/mL, respectively. But In a study of many plants plus *A.arvensis* for antioxidant effect by Trouillas et al. (2003) it was shown that this plant had a good antioxidant effect and was used in herbal medicines[48]. In another study of *Aphanes arvensis* extracts was conducted by Hamad et al. (2010) which showed that free radical scavenging activity was determined by DPPH method[49]. The methanolic extract showed a scavenging activity nearly equivalent to Trolox and vitamin C and has an IC_{50} value of 4.54µg/mL.

So the current study of *A.arvensis* classifies as good anti-oxidant effect nearly to the previous studies [50]. In another study of *Alchemilla mollis* was conducted by Nedyalkov et al. (2015) that investigated antioxidant capacity of *Alchemilla mollis* in aqueous and ethanol extracts [42].

In *Taraxacum syriacum*, the best antioxidant effect was detected in water extract that IC_{50} was equal to 95.49µg/mL. In a study of *T.syriacum* by Nazari et al. (2015), shown that ethanol extract of *T.syriacum* was more potent[10] [51].

The current study of *T.syriacum* has perfect antioxidant effect in water extract. There was a study of *Taraxacum officinale* was conducted by Park et al. (2011) reported that *Taraxacum officinale* has been widely used in medicine to treat spleen and liver disorders[52].

In another study of *Taraxacum officinale*, conducted by Ivanov (2014), reported that Taraxacum officinale was as potential application as a radical scavenger [53].

4.1.4Antilipase effect

The substances that are used to reduce the activity of lipases found in the intestine are called lipase inhibitors. They bind to lipase enzymes (secreted from the pancreas, are related to dietary triglyceride absorption and catalyze the digestion of dietary triglycerides) in the intestine. Therefore lipase inhibitors prevent the hydrolysis of dietary triglycerides to monoglycerides and fatty acids, so no absorption takes place in the intestine and fat is excreted in the feces rather than being absorbed for use as a source of caloric energy. This mechanism could be used for the treatment of obesity[54]. An example of a lipase inhibitor is Orlistat, which was used in our current study as reference substance, and tends to block absorption of 30% of the total fat intake from a meal. Lipase inhibitors have many side-effects like, oily spotting, in addition to abdominal cramps and hypertension. These side- effects could be controlled by reducing the consumption of dietary fats.

In our study of *A.arvensis*, the best antilipase effect was shown in water, methanol, acetone and hexane extract, with IC_{50}values of 21.37, 30.90, 45.70 and72.44µg/mL, respectively. To the best of our knowledge, no previous studies have been conducted on *A.arvensis*. But in a study of *Alchemilla vulgaris* conducted by Slanc et al. (2006), reported to show inhibitory activity of pancreatic lipase[48]. Another study using *Alchemilla mollis* by Akkol et al. (2015) showed that *Alchemilla mollis vulgaris* is also reported to show inhibitory activity of pancreatic lipase [55].

In *Taraxacum syriacum*, the best antilipase effect was shown in water and hexane extract, with IC_{50}values of 154.88 and 218.77µg\ml, respectively. To the best of our knowledge, no previous studies have been conducted with *Taraxacum syriacum*. In one study using *Taraxacum officinale* by Zhang et al. (2008), it was reported that obesity had become a worldwide health problem[56].

In another study of *Taraxacum officinale* conducted by González-Castejón and García-Carrasco (2014) showed that ability of *Taraxacum officinale* to inhibit adipocyte differentiation and lipogenesis [57].

4.1.5 Antimicrobial resistance

Antimicrobials are medicinal products that kill or inhibit the growth of living microorganisms, usually called antibiotics because they act against bacterial infections[58]. 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. This resistance can be natural or acquired. This resistance may delay and hinder treatment, resulting in complications or even death, so a patient may need more care, as well as the use of alternative and more expensive antibiotics, which have more severe side-effects.

In our study of *A.arvensis*, had antimicrobial effects for bacteria and fungi in different extracts, and MIC values for different type of bacteria and fungi were shown previously. To the best of our knowledge no previous studies

were conducted on *A.arvensis*. But In a study on *Alchemilla vulgaris* by Hamid and Azman(2017), results showed that these plants could act as antioxidants and antimicrobials in food[59]. In Another study using *Alchemilla vulgaris* and *Alchemilla mollis* was conducted by Duckstein et al.(2012) shown that chemical composition has antibacterial activity[60].

In *Taraxacum syriacum*, had antimicrobial effect for bacteria and fungi in different extracts, and had different MIC values were shown previously.

To the best of our knowledge no previous studies were conducted on *T.syriacum*. But In one study on *Taraxacum mongolicum* by Han et al.(2005), it was shown that extractings with various solvents with different polarities have gastric mucosa inflammations[61]. In another study on *Taraxacum mongolicum* was by Zhang et al.(2013), a fungus was isolated from the leaves of *Taraxacum mongolicum* [62]. The culture filtrate displayed antagonism against some pathogenic bacteria owing to the existence of antibacterial compounds. But in a study on *Taraxacum officinale* conducted by Ghaima et al.(2013), the antibacterial and antioxidant activities of *Taraxacum officinale* were studied. Another study of *Taraxacum officinale* by Mir et al.(2013)reported that the value of plants lies in some chemical substances that have a definite physiological action and antibiotic properties[63].

Chapter five

Conclusion and Future Work

Conclusions and future work

Total phenol and flavonoid content in the studied plants showed that *T.syriacum* had a high phenol content in the acetone extract, while *A.arvensis* had a high phenol content in methanol extract. With regard to total flavonoid content, *T.syriacum* had a high content in the aqueous extract and *A.arvensis* also had a high content in the aqueous extract. The antioxidant tests show that *T.syriacum* has more potential effect in water extract, but *A.arvensis* has more antioxidant effect in acetone and hexane extracts. Meanwhile, antilipase activity of *Taraxacum* showed more potential effect in water and hexane extracts, but *Alchemilla* showed more potential effect in water, methanol and acetone extracts. With regard to antimicrobial activity against bacteria and fungi, *T.syriacum* showed more potential against *Staphylococcus aureus*, *Escherichia coli*, *Pseudo*monas *aeruginosa* and *Shigella sonnie* bacteria in water and acetone extracts, and greater potential against *Candida albicans* and *Epidermophyton floccosum* fungi in all extracts, but in *A.arvensis* showed more potent against *Staph*y*lococcus aureus*, *Pseudo*monas a*eruginosa*, *Shigella sonnie*, and *Escherichia coli* bacteria, in water and methanol extracts, and more potent against *Epidermophyton floccosum* fungus only in all extracts. Also some drugs showed effect on bacteria and fungi, briefly, they showed that all types of bacteria were more sensitive to Levofloxacine, ciprofloxacin, and Doxycycline. However, *Candida albicans was* more sensitive to Tinidazole drug. Therefore, our results demonstrate the promising potential of these plants for use in the pharmaceutical industry for the treatment of cancer,

obesity and infectious diseases. However, additional studies are needed to identify the exact bioactive constituents that produce this efficacy and investigate their toxicity and side-effects.

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كلية الدراسات العليا

المسح الكيميائي النباتي والنشاط الدوائي لنباتين طبيين

(*Alchemilla arvensis* and *Taraxacum syriacum*)

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.

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المسح الكيميائي النباتي والنشاط الدوائي لنباتين طبيين
(*Alchemilla arvensis* and *Taraxacum syriacum*)

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الملخص

الإجهاد التأكسدي ومشاكل السمنة والكائنات الدقيقة المقاومة للأدوية المتعددة تمثل تحديات كبيرة للصناعات الدوائية. وقد دفعت هذه المشاكل العلماء إلى فحص للمواد البديلة التي تعمل كمضادات أكسدة قوية ومضاد للسمنة وعوامل مضادة للميكروبات مع أقصى قدر من الفعالية وبعض الآثار الجانبية. منذ بداية التاريخ البشري، أصبحت العلاجات العشبية المختلفة وغيرها من المنتجات الطبيعية مهمة كمصادر بيولوجية لمضادات الأكسدة، علاج للسمنة وعوامل مضادة للميكروبات. لذلك، كانت أهداف هذه الدراسة للتحقيق في مضادات الأكسدة وعلاج السمنة ومضادات للميكروبات من نوعين من النباتات *Alchemilla arvensis* and *Taraxacum syriacum* وبالإضافة إلى ذلك، تم فحص النباتات للمواد الكيميائية النباتية، وتحديدا المحتوى الكلي لPhenols and Flavonoids.

تم فحص النشاط المضاد للأكسدة من خلال إعداد محلول مخزون من المستخلصات النباتية في الميثانول. كما استخدم دواء Trolox كمادة مرجعية في البحث.

تم أيضا تحديد نشاط Lipase البنكرياسي من خلال قياس التحلل المائي لمركب نيتروفينولات وهنا استخدمنا دواء Orlistat كمرجع للقياس وهو مصمم لعلاج السمنة.

من جانب أخر تم فحص الفعاليات البيولوجية المضادة للبكتيريا لهذه المستخلصات النباتية باستخدام عزلات بكتيرية سريرية مقاومة متعددة للأدوية وعدد من السلالات البكتيرية المرجعية التي تم الحصول عليها من مجموعة الأنواع المستنبتة الأمريكية ATCC . والسلالات التي تم استخدامها في الفحص شملت *Pseudomonas aeruginosa* (ATCC 27853),

Escherichia coli (ATCC 25922), *Shigella* sonnie(ATCC 25931), *Staphylococcus aureus* (ATCC 25923).

علاوة على ذلك ، فان الفعالية المضادة للفطريات تم فحصها ضد نوعين من السلالات الفطرية المرجعية(ATCC 52066) *Epidermophyton floccosum.* (ATCC 90028), *Candida albicans*

جرى تقييم الفعاليات المضادة للبكتيريا والفطريات لجميع المستخلصات المائية والعضوية لهذه النباتات باستخدام طرق فحص مختلفة شملت (Micro-broth dilution, Agar diffusion, Agar dilution method).

أظهرت نتائج الفحص أن المستخلص المائي من *Taraxacum syriacum* كان أكثر فعالية مضادات الأكسدة (95.49 / µgمل) من ثم مستخلص الميثانول (281.83 / µgمل) ومستخلص الأسيتون من *Alchemilla arvensis* أكثر فعالية مضادات الأكسدة (4.86 / µg مل) من ثم مستخلص الهكسان (11.22 / µgمل).

وبالنسبة لكونها مضاد للسمنة أظهرت النتائج أن المستخلص المائي من *Taraxacum syriacum* أكثر قوة (154.88 / µgمل) من ثم مستخلص الهكسان (218.77 / µgمل) وكان المستخلص المائي من *Alchemilla arvensis* أكثر فعالية (21.37 / µgمل) من ثم مستخلص الميثانول

(30.90 / µgمل).

وفيما يتعلق بنشاط مضاد للميكروبات ضد البكتيريا والفطريات، تم تقييم *Taraxacum syriacum* أن لها فعالية ضد البكتيريا المختارة في البحث في مستخلص الماء والاستيون وأيضا ضد الفطريات التي تم اختيارها في البحث، وتقييم *Alchemilla arvensis* أن لها فعالية ضد البكتيريا المختارة للبحث في المستخلص المائي و الميثانول ولها تأثير فقط على فطر *Epidermophyton floccosum* .

في النهاية لقد قمنا في البحث باختبار مقتطفات من *Alchemilla arvensis* and *Taraxacum syriacum* لمضادات الأكسدة، والسمنة و مضادات الميكروبات. وأوصت هذه الدراسة أن تستخدم هذه النباتات في أدوية علاج السرطان، وأيضا للسمنة، بالإضافة إلى استخدامها كأدوية مضادة للجراثيم ومضاد للفطريات لذلك إن تم استخدام هذه النباتات بالطريقة الصحيحة والجرعات المناسبة فمن الممكن الاعتماد عليها وحدها كعلاج دون أي تدخل للصناعة الدوائية.