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Research paper

Phytochemical, antimicrobial and antioxidant preliminary screening of a traditional Palestinian medicinal plant, *Ononis pubescens* L.

Nidal Amin Jaradat[a,*], Motasem Al-Masri[b], Abdel Naser Zaid[a], Fatima Hussein[a], Fuad Al-Rimawi[c], Aseel Abu Mokh[a], Jehan Abo Mokh[a], Sameer Ghonaim[a]

[a] Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, P.O. Box 7, Nablus, Palestine
[b] Department of Biology and Biotechnology, Faculty of Science, An-Najah National University, P.O. Box 7, Nablus, Palestine
[c] Department of Chemistry, Al-Quds University, P. O. Box 51000, Jerusalem, Palestine

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ABSTRACT

Introduction: Antibiotic resistance has become a serious global concern, and the discovery of novel antimicrobial herbal constituents may provide valuable solutions to overcome this problem. It is important to identify new sources of natural antioxidants and antimicrobials. The present study describes for the first time the antioxidant, and antibacterial activities of various fractions of *Ononis pubescens* L., a traditional Palestinian medicinal plant.
Methods: Antimicrobial activity was tested against selected strains from American Type Culture Collection (ATCC) and clinical isolates including *Shigella sonnie, Staphylococcus aureus*, *Entrococuss feacium, Escherichia coli*, *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *Candida albicans* and *Epidermatophyto flacosum* using minimum inhibitory concentration (MIC) assay, while antioxidant activity was analyzed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method.
Results: A mixture of phytochemicals was found in all of the studied fractions of *O. pubescens,* which also showed remarkable potential with antioxidant and antimicrobial activities.
Conclusion: The current study provides initial data that justify the use and importance of Ononis species in Palestinian folkloric medicine. Our results showed that *Ononis pubescens n*-hexane fraction has powerful antibacterial bioactivity against *Staphylococcus aureus,* as well as the acetone, *n*-hexane and methanol fractions which showed excellent potential against *Epidermatophyto flacosum* fungi, while the acetone fraction showed the highest antioxidant activity among other fractions. Further investigations are needed to identify and characterize these constituents.

1. Introduction

Humans have used natural products like plants, microorganisms, animals, and marine organisms as medicines to treat various diseases, even prior to the invention of writing. According to fossil records, the human utilization of plants for treatment of diseases may be traced back to 60,000 years [1,2].

Knowledge about natural products and their benefits were found the hard way, by seeking food from the edible plants and materials which led to various reactions such as diarrhea, vomiting, or other toxic reactions and even death. After, the discovery of fire and technological breakthroughs, the possibility of developing drugs emerged [3]. Plants are rich sources of many bioactive secondary metabolites that have the potential to treat different afflictions, for example, alkaloids, coumarins, flavonoids, polyphenols, unsaturated lactones, thiosides, saponins, cyanosides and essential oils [4,5]. In recent years, phytochemical secondary metabolites have been extensively investigated as a source of therapeutic agents. Thus, it is anticipated that phytochemicals with potential biological properties such as the antibacterial activity will be used for the treatment of various diseases including bacterial infections [6,7]. Antibiotic resistance has become a serious global concern, and the discovery of novel antimicrobial herbal constituents may provide valuable solutions to overcome the problem [8,9]. New resistance mechanisms are emerging and spreading globally, threatening the ability to treat common infectious diseases, resulting in prolonged illness, disability, and death [10,11].

An imbalance between free radicals and antioxidant systems can cause oxidative stress in the human body which may result in several chronic and fatal diseases such as Parkinson's, cardiovascular disease, Alzheimer's, and cancer. In response, humans produce antioxidant compounds and enzymes which neutralize these free radicals, but due to the decreasing levels of these naturally occurring compounds as a

* Corresponding author.
E-mail address: nidaljaradat@najah.edu (N.A. Jaradat).

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result of environmental factors and other reasons, this may cause an increase in oxidative stress which can lead to cell damage and death. Supplementation containing antioxidants is required at this stage, to prevent the damage which are caused by these free radicals from natural or synthetic sources. The investigation of pharmacological active and safe antioxidants are crucial. The DPPH assay is a simple and efficient method which based on the measurement of the scavenging ability of antioxidants towards the stable radical DPPH molecule [12–14]. Therefore, there is a pressing need for searching of new antimicrobial and antioxidant medications with diverse mechanisms of action.

There are various species among the genus *Ononis* (Leguminosae family), which have been shown to have different pharmacological actions such as; antihypertensive [15], antioxidant [16], antibacterial [17], antifungal [18], analgesic [19], anticancer activities [5,20].

In Palestine, there are fifteen widespread species of the genus *Ononis* - *O.alopecuroides*, *O. biflora*, *O. hirta*, *O. mitissima*, *O. mollis*, *O. natrix*, *O. ornithopodioides*, *O. phyllocephala*, *O. pusilla*, *O. serrata*, *O. sicula*, *O. spinosa*, *O. variegate*, *O. viscosa* and *O. pubescens* [21] *Ononis pubescens* L. *(O. pubescens*) is also known as Downy Rest Harrow, and is an annual herbaceous plant growing wildly in the Mediterranean regions especially in Palestine and distributed in the woodlands, shrublands, and semi-steppe shrublands. The leaves are simple, lanceolate, entire margined, and pointed apex. They are also trifoliate in the lower part of the stems, but unifoliada in the high part and covered with yellow, multicellular, glandular, and sticky hairs [21,22].

The *O. pubescens* aerial parts contain alkyl-isocoumarins, alkylbenzoic acid and alkylresorcinols derivatives [23]. Recently, increased the interest rate of alkylresorcinols due to their biological activity, having been described as inhibitors of glycerol-3 phosphate dehydrogenase and as the molecules that mediate DNA strand scission [24,25].

The present investigation was intended to screen and to evaluate the antioxidant and antimicrobial effects of the aqueous, methanol, acetone and *n*-hexane fractions of *O. pubescens*. This plant was chosen because it has been used traditionally from the ancient times as medicines and food for cattle in Palestine [26,27].

To the best of our knowledge, there are no previous studies in the literature about the antimicrobial and antioxidant properties of this traditional plant making this study the first one.

2. Material and methods

2.1. Instrumentation

Shaker incubator apparatus (Memmert shaking incubator, Germany), Rotavap (Heidolph OB2000-VV2000, Germany), grinder (Moulinex 1, China), balance (Rad wag, AS 220/c/2, Poland), freeze-dryer (Mill rock technology-BT85, China), sterile syringe filter (0.45 μm 25 mm) hydrophobic (PTFE, China), stir-mixer (Tuttnauer, Jerusalem),weighing scale (Adam Equipments, USA) Incubator with Co_2 (Tuttnauer, Israel), and multichannel micro-pipet (Eppendorf, Germany)

2.2. Chemicals and reagents

Methanol, NaOH, *n*-hexane and acetone were purchased from Loba Chemie, India and Millon's reagent, Benedict's reagent and Ninhydrin solution were obtained from Alfa Agar, England. In addition, Molish's reagent, H_2SO_4 and iodine solution were purchased from Alfa-Aesar, England. Chloroform and HCl were obtained from Sigma-Aldrich, Germany. Moreover, magnesium ribbon, acetic acid, $FeCl_3$ and DMSO (Dimethyl sulfoxide) were obtained from Riedeldehan, Germany. In addition, the nutrient broth was purchased from Himedia, India. While, BBL Mueller Hinton II broth, Difco Sabouraud Dextrose Agar, and Bacto tryptic soy broth were purchased from Dickinson and company Sparks, USA as well as McConkey agar was obtained from Himedia Laboratories, India.

2.3. Plant material

The entire *O. pubescens* plant (roots, stems, leaves, and flowers) was collected in April 2016 from Bethlehem region of the West Bank/ Palestine. Taxonomical identification was performed at the Department of pharmacy in Pharmacognosy and Herbal Products Laboratory, An-Najah National University, and the voucher specimen code was Pharm-PCT-1680.

Once dried at room temperature (after two weeks), the plant material was ground and stored in cool dry conditions in the absence of light.

2.4. Preparation of plant four fractions

The dried powder of O. *pubescens* was fractionated sequentially by adding solvents of increasing polarity: hexane (non-polar solvent), acetone (polar aprotic solvent), methanol (polar protic solvent) and water (polar protic solvent). About 25 g of the grounded entire plant was soaked in 500 ml of four different solvents (water, methanol, acetone, *n*-hexane) separately and placed each one in a shaker device at 100 rounds per minute for 72 h at room temperature, and then stored in the refrigerator for 4 days. After that, each organic fraction was filtered and concentrated under vacuum on a rotator evaporator. While aqueous fraction was dried using freeze dryer. Finally, all crude fractions were stored at 4 °C in the refrigerator for further use [28].

2.5. Phytoconstituents screening

Qualitative phytoconstituents screening for primary and secondary plant metabolites such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, protein, monosaccharide, reducing sugar, starch, phenols, saponins, steroids, terpenoids, and tannins were carried out according to the standard qualitative analysis of phytogenic compounds as described by Trease and Evans, 1983 [29] and Harborne, 1998 [30].

2.6. Microbial isolates

The examined bacterial and fungal isolates were obtained from American Type Culture Collection. The selected species of microorganisms are frequently isolated at clinical settings in our region and some possess multidrug resistance. The isolates included three Gram-positive strains: *Staphylococcus aureus* (ATCC 25923), *Staphylococcus aureus* (MRSA), and *Enterococcus faecium* (ATCC 700221) and three Gram-negative strains: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Shigella sonnie* (ATCC 25931). Meanwhile, the fungal isolates included *Candida albicans* (ATCC 90028) and *Epidermatophyto flacosum* (ATCC 10231).

2.7. Antimicrobial test

Different fractions were screened for antimicrobial activity by using the well diffusion method. Bacterial suspension was prepared by picking some colony of overnight agar culture of the test organism, and adding it to a test tube containing 5 ml of nutrient broth, then the turbidity was compared with that of McFarland nephelometer tube No. 0.5 ($1.5*10^8$ cfu/ml); then it was diluted by taking 1000 μl of suspension and it was added to 2 ml of nutrient broth ($0.5*10^8$ cfu/ml). The MIC is the lowest concentration of an antimicrobial that inhibits the growth of a microorganism after 18–24 h. Each fraction was subjected to serial broth dilution technique to determine their minimum inhibitory concentration for all tested microorganisms.

2.7.1. Determination of MIC

Microbroth dilution method was used to determine antibacterial

activities of plant fractions. Into each well of micro- titration plate, 100 µl of Mueller-Hinton broth was placed. A volume of 100 µl of plant fraction (50 mg/ml) was pippeted into the first well and mixed using the micropipette. This was followed by transferring 100 µl from the well to the next well. The processes of dilution are repeated up to well number 11 (negative control of bacterial growth) from which 100 µl was discharged after mixing. Well number 12 was free from a plant fraction and was considered the positive control of bacterial growth. Each examined bacterial strain suspension (e.g. *Staphylococcus aureus, Escherichia coli* etc.) was inoculated with 1 µl of a bacterial suspension (from 5×10^7 CFU/ml bacterial suspension) into all wells except well number 11 (negative control). In well number 12 (positive control of bacterial growth), the inoculated bacteria must grow because it's just a broth and if the bacteria did not grow this means there was a problem in preparation of media or bacterial inoculums. The inoculated plate was incubated at 35 °C for 24 h. The lowest concentration of plant fraction that inhibited visible bacterial growth was considered the MIC. Each plant fraction was examined twice in each run [31,32].

2.7.2. Determination of anti-yeast activity of plant fractions

The MIC of the studies plant fractions against *Candida albicans* was determined by broth microdilution similar to [33,34]. In this procedure, RPMI1640 was used instead of MHB.

2.7.3. RPMI1640 preparation

A weight of 1.04 g of RPMI powder was dissolved in 90 ml sterile, distilled water. MOP (3.456 g) was added to the solution. The pH of the solution was adjusted to 7 at 25 °C by using 1 mol/l NaOH. Then, sterile distilled water was added up to 100 ml. The solution was sterilized by filtration through 0.45 µm syringe filter.

2.7.4. Broth microdilution

A volume of 100 µl of RPMI 1640 broth media was placed in each well. Then, 100 µl of plant fraction was placed in first well and mixed. This was followed by 100 µl from the first well to the second one. This step was repeated up to wells number 11 from which 100 µlwas discharged after mixing. Well number 12 contained only RPMI 1640 broth and represents the positive control of yeast growth. The concentrations of plant fraction in the micro-wells were assigned to evaluate their antifungal activities ranged from 0.065 to 55 mg/ml.

2.7.5. Inoculum preparation

Candida albicans was sub-cultured on sabouraud dextrose agar at 37 °C for 24 h. Five colonies were placed in 5 ml 8.5% saline. Then, cell density was adjusted to 0.5 McFarland's standard (0.08 to 0.1 absorbance at 625 nm) to obtain a yeast concentration 1×10^6 to 5×10^6 CFU/ml. This suspension was of *Candida albicans*, was diluted 1:50 and then 1:20 with broth medium (RPMI 1640 broth), which results in 1×10^3 to 5×10^3 CFU/ml, from which 100 µl was pippeted into each well except well number 11, which represented negative control of yeast growth. All of the inoculated plates were incubated at 35 °C. The incubation period was 48 h. The final concentrations of microbial cells were about 0.5–2.5 $\times 10^3$ colony-forming unit (CFU)/ml.

2.7.6. Determination of anti-mould activity

Epidermatophyton floccosum mould inhibition by plant fraction was evaluated by agar dilution method [34,35]. Sabouraud dextrose agar (SDA) was placed in tubes and kept at 40 °C water bath after sterilization by autoclave. Plant fractions were serially diluted with SDA. The prepared concentrations were from 0.78 to 25 mg/ml for aqueous fractions and organic fractions. Then, the prepared tubes were allowed to solidify in slanted position at room temperature. A suspension with turbidity similar to 0.5 McFarland standard which was prepared from a fresh culture of *Epidermatophyton floccosum*. Then 20 µl of the suspension of was added to all tubes. Tubes with SDA only were used as positive control of the mould. Results were taken after 10 days of incubation at 25 °C. Minimum inhibitory concentration was the lowest concentration that completely inhibits the growth of *Epidermatophyton floccosum*.

2.8. Antioxidant DPPH method

A stock solution of a concentration of 1 mg/ml in methanol was firstly prepared for the plant fractions and Trolox. The working solutions of the following concentrations (1, 2, 3, 5, 7, 10, 20, 30, 40, 50, 80, 100 µg/ml) were prepared by serial dilution with methanol from the stock solution. DPPH was freshly prepared at a concentration of 0.002% w/v. The DPPH solution was mixed with methanol and the above-prepared working concentration in a ratio of 1:1:1 respectively. The spectrophotometer was zeroed using methanol 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. The percentage of antioxidant activity of the plants and the Trolox standard were calculated using the following formula:

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

Where:

B = optical density of the blank,

S = optical density of the sample.

The antioxidant half maximal inhibitory concentration (IC_{50}) for the plant samples and the standard were calculated using BioDataFit edition 1.02 (data fit for biologist). Determination of antioxidant activity was carried out in triplicate for each sample. The obtained results were presented as means ± standard deviation (SD) and then were compared using an unpaired *t*-test.

3. Results

The yields of the fractionation for aqueous, acetone, methanol and *n*-hexane solvents which were conducted under the same working conditions were assessed (Table 1) and phytochemical markers detection in each collected fraction was performed (Table 2) during the separation process.

The highest extraction yield for *O. pubescens* was obtained using methanol (23%) and the qualitative phytochemical screening results revealed that cardiac glycosides were found in the aqueous, acetone and *n*-hexane fractions, while phenols were identified in methanol and acetone fractions, as well as tannin, were found in the acetone and aqueous fractions. In addition, steroids were identified in the aqueous, acetone and *n*-hexane fractions, while terpenoids were found in all fractions except methanol fraction. Meanwhile, flavonoid was identified in the methanol and acetone fractions and carbohydrates were found in all the studied plant fractions. The most effective fraction was acetone fraction as its rich in several bioactive phytochemical classes

3.1. Antimicrobial activity

The organic fractions of *O. pubescens* exhibited antimicrobial bioactivity against the growth of the majority of screened pathogens included in this study. While, the aqueous *O. pubescens* fraction did not

Table 1
Fractionations yields of *O. pubescens* aqueous, acetone, methanol and hexane solvents.

Fractions	Dried plant weight (g)	Plant fractions (g)	Yields, %
Hexane	25 g	2.12 g	8.48%
Acetone	25 g	4.15 g	16.6%
Methanol	25 g	5.75 g	23%
Aqueous	25 g	1.73 g	6.92%

Table 2
Phytochemical screening tests for the aqueous, methanol, acetone and *n*-hexane fractions of *O. pubescens.*

Phytochemical Compounds	Aqueous fraction	Methanol fraction	Acetone fraction	n-Hexane fraction
Cardiac glycosides	+	–	+	+
Saponin glycoside	–	–	–	–
Alkaloids	–	–	–	–
Protein	–	–	–	–
Starch	–	–	–	–
Phenols	–	+	+	–
Carbohydrates	+	+	+	+
Tannin	+	–	+	–
Steroids	+	–	+	+
Reducing sugar	–	–	–	–
Monosaccharide	–	–	–	–
Terpenoids	+	–	+	+
Flavonoid	–	+	+	–

Table 3
Antimicrobial activities (MICs) of *O. pubescens* methanol, aqueous, *n*-hexane and acetone fractions.

Microorganisms	MIC, mg/ml acetone fraction	MIC, mg/ml *n*-hexane fraction	MIC, mg/ml methanol fraction	MIC, mg/ml aqueous fraction
Staphylococcus aureus	12.5 mg/ml	1.563 mg/ml	25 mg/ml	NI
MRSA	25 mg/ml	6.25 mg/ml	6.25 mg/ml	NI
Shigella sonnie	12.5 mg/ml	NI	25 mg/ml	NI
Entrococuss feacium	6.25 mg/ml	12.25 mg/ml	12.5 mg/ml	NI
Escherichia coli	12.5 mg/ml	25 mg/ml	25 mg/ml	NI
Pseudomonas arginosa (ATCC 27853)	12.5 mg/ml	12.5 mg/ml	6.25 mg/ml	NI
Candida albicans	NI	12.5 mg/ml	12.5 mg/ml	NI
Epidermatophyto flacosum (ATCC 10231)	3.125 mg/ml	3.125 mg/ml	3.125 mg/ml	NI

NI: No Inhibition.

exhibit any antibacterial or antifungal bioactivity as presented in Table 3.

3.2. Antioxidant properties

DPPH free radical scavenging assay was adopted for evaluation of antioxidant power for each prepared aqueous, acetone, *n*-hexane, and methanol *O. pubescens* fractions. Trolox was used as a reference standard compound in various concentrations, which were ranged from 1 to 100 µg/ml. The zero inhibition concentration was considered for the DPPH and methanol without any plant fraction.

Fig. 1 shows the inhibition of DPPH for each *O. pubescens* fraction that used to make a comparative in-vitro evaluation of antioxidant activities.

The results revealed that an excellent antioxidant activity, with IC_{50} 8.67 µg/ml, was exhibited by *O. pubescens* acetone fraction, followed by *O. pubescens* aqueous fraction with IC_{50} 15.14 µg/ml and followed by *O. pubescens* methanol fraction with IC_{50} 19.41 µg/ml, while the *n*-hexane fraction was the least antioxidant fraction with IC_{50} 34.67 µg/ml. All these results were comparable to a Trolox standard that has IC_{50} 2.11 µg/ml. The detailed results are shown in Table 4 with their percent of inhibition according to Trolox.

4. Discussion

Due to its geographical position, Palestine has interesting potential in the field of medicinal plants and is classified as one of the most

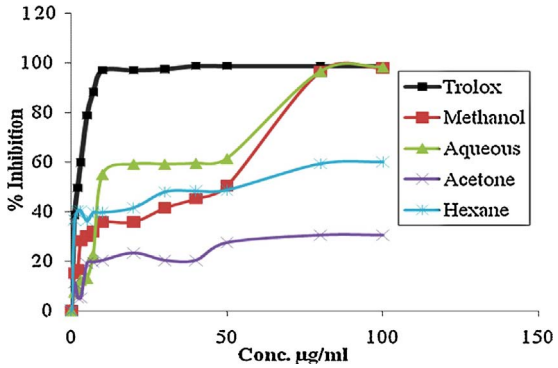

Fig. 1. Inhibition activity of Trolox reference standard compound and four *O. pubescens* fractions.

Table 4
DPPH free radical scavenging activity (IC_{50}) and inhibition percentages according to Trolox of *n*-hexane, acetone, methanol, and aqueous fractions of *O. pubescens.*

Inhibition, % according to Trolox	IC_{50} (µg/ml)	Fraction type
6.09	34.67 ± 0.97**	n-hexane
24.37	8.67 ± 0.77**	Acetone
10.88	19.41 ± 1.1***	Methanol
13.96	15.14 ± 1.09**	Aqueous
100	2.11 ± 0.88***	Trolox

Where: ** indicate a p value of ≤ 0.001 and *** indicate a p value of ≤ 0.0001.

botanically rich countries in the Mediterranean region. In fact, the Palestinian flora is diverse with about 27500 species and subspecies, of which about 300 plants are currently used in folk medicine. This ecological diversity is the natural reservoir of bioactive molecules, which is of great interest to the cosmetic, food and pharmaceutical industries [36,37].

The majority of recent medicines that have been discovered from plants or other natural products and have been investigated due to human experience and subsequently clinical trials. Accordingly, for the treatment of specific diseases, the pharmacologically active compounds have been isolated and analyzed its entity to produce specific medications like penicillin, morphine, pilocarpine and many others [38,39].

In the 20 century, the advanced chemical analytical techniques have emerged to facilitate isolation of the natural active ingredients. In addition, the evolution of drugs receptor theory which suggests a molecular interaction of unique chemical structure compounds to body macromolecules like proteins and nucleic acid which resulting in discovering the required therapeutic agents [40–42].

Recently, investigations of new sources of natural antioxidant agents have been attracting an increasing attention because these compounds are inexpensive and safe since some chemical synthetic antioxidants showed health risk and toxicity [43–46].

The present study evaluated the antioxidant activity of *O. pubescens* four fractions with a wide range of polarity by using free radical scavenging DPPH method. In summary, *O. pubescens* acetone fraction showed an excellent antioxidant activity, with IC_{50} 8.67 µg/ml, followed by *O. pubescens* aqueous fraction with IC_{50} 15.14 µg/ml and followed by *O. pubescens* methanol fraction with IC_{50} 19.41 µg/ml, while the *n*-hexane fraction was the least antioxidant fraction with IC_{50} 34.67 µg/ml. All these results were comparable to Trolox standard that has IC_{50} 2.11 µg/ml.

In a study which was conducted by El Guiche et al. in Morocco which evaluated the antioxidant activity by using DPPH method in *Ononis natrix* plant, found that the methanol fraction has 90.12 µg/ml [47]. Moreover, in another study which was conducted by Valyova et al. found that the antioxidant activity of methanol extract of *Ononis spinosa* by using the same method was 59.1 µg/ml [48]. Therefore, our

studied species have higher antioxidant potential than previously conducted studies on other *Ononis* species.

Throughout the history of humanity, bacterial infections and their related diseases have killed many people around the world. Therefore, during the 20th century with the discovery of antibiotics such as penicillin and others hallmarked the beginning of the antibiotic era, which has saved the lives of people around the globe [49].

On the other hand, worldwide misusage of antibiotics has caused the emergence and spread of antibiotic-resistant strains of bacterial pathogens. The emergence of highly resistant strains of bacterial pathogens such as VRSA and MRSA, Gram-negative bacteria that are resistant to Carbapenems and ESBL, antibiotic resistant tuberculosis, draws the attention of scientists to the threat that is imposed by the spread of antibiotic-resistant bacteria strains. Therefore, microbiologists and pharmacologists are seriously concerned about the possibility of going back to the pre-antibiotic era as more and more antibiotics are becoming useless in treating infections caused by such strains [50,51].

This serious concern about going back to the pre-antibiotic era has triggered massive investigations that aim to discover new drugs as alternatives to antibiotics for treatment of bacterial infections. Most of these investigations have been focusing on folkloric plants which used to treat bacterial infections [52,53] and for many decades enormous investigations have shown that phytogenic products produced various chemicals with antibacterial and antifungal properties [54,55].

Interestingly, in the Palestinian ethnomedicine, numerous plants have been used for the treatment of infectious diseases, O. pubescens being one these plants which has been reported in the folk medicine for treatment of upper respiratory system infections [27].

Antibacterial tests were conducted against six ATCCs and clinical isolates bacterial stains while antifungal tests were established against two fungal pathogens. Table 3 showed that the higher inhibition activity (the lowest MIC) of the acetone fraction was against *Entrococuss feacium* bacteria and against *Epidermatophyto flacosum* fungi with MICs 6.25 mg/ml and 3.125 mg/ml, respectively, furthermore the *n*-hexane fraction showed the highest inhibition activity against *Staphylococcus aureus* with MIC 1.563 mg/ml and the best antifungal inhibition activity was against *Epidermatophyto flacosum* with MIC 3.125 mg/ml. In addition, the best antibacterial activity of the methanol fraction was against *Pseudomonas arginosa* and MRSA with MIC6.25 mg/ml for both of them, while it has the best antifungal activity was against *Epidermatophyto flacosum* with MIC 3.125 mg/ml. But unfortunately, the aqueous fraction was inactive against all the studied bacterial and fungal strains.

That means that the best activity against *Staphylococcus aureus* strain was *O. pubescens n*-hexane fraction with MIC 1.563 mg/ml, and the best activity against MRSA were *O. pubescens n*-hexane and methanol fractions with MIC 6.25 mg/ml for both fractions. Furthermore, the best activity against *Shigella sonnie* was reached with *O. pubescens* acetone fraction with *MIC* 12.5 mg/ml. While, the best antibacterial property against *Entrococuss feacium* was achieved by *O. pubescens* acetone fraction with MIC 6.25 mg/ml. The best antibacterial activity against *Escherichia coli* was also *O. pubescens* acetone fraction with MIC 12.5 mg/ml. While, the best antibacterial bioactivity against *Pseudomonas aeruginosa* was *O. pubescens* methanol fraction with MIC 6.25 mg/ml.

Our results showed that the antifungal bioactivity of the acetone, *n*-hexane and the methanol *O. pubescens* fractions were noticeably high against *Epidermatophyto flacosum* with MIC 3.125 mg/ml for all these fractions. Meanwhile, the aqueous fraction did not show any bioactivity against this fungus. Moreover, the *n*-hexane and methanol *O. pubescens* fractions showed antifungal bioactivity against *Candida albicans* with MIC 12.5 mg/ml, while the aqueous and acetone *O. pubescens* fractions were inactive against this pathogen.

The separation of active compounds of *O. pubescens* plant has not been attempted in this study as separation with column chromatography requires huge amounts of the plant which was not available at this time. In addition, isolation, chemical structure elucidation, and determination of structure activity relationship (SAR) of the most therapeutic active compounds from *O. pubescens* fractions are limitations in this study. However, these experiments will be our future goal.

5. Conclusion

The studied plant four fractions revealed a potential interest in their antioxidant activities, and the majority of the studied organic fractions inhibited relevant microbial strains thereby validating its folk use in the treatment of upper respiratory system infections. Further phytochemical investigations directed at isolation and structural identification are recommended, which may lead to new antimicrobial compounds and in vivo studies that may validate the in vitro findings.

Authors contribution

All research done by the authors.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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None.

Ethical approval

N/A.

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