

## Original Article

## Evaluation of *in vitro* Antimicrobial, Antidiabetic and Antioxidant Potential of *Alyssum homalocarpum* and Green Synthesis of the Silver Nanoparticles

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### Abstract

In the present work synthesis of silver nanoparticles using *Alyssum homalocarpum* (Fisch. & C.A.Mey.) Boiss. extract has been considered. The methanolic extract of *A. homalocarpum* was concentrated and analyzed using Gas Chromatogra. Also silver nanoparticles were synthesized by the bio-reduction of silver nitrate solution (1 mm) using the methanol extract. Scanning electron microscopy (SEM), and Fourier transforms infrared spectroscopy (FT-IR), have been used to determine physicochemical properties of silver nanoparticles. - Glucosidase inhibition assay, -amylase inhibition activity, and IC<sub>50</sub> test have been performed and the results reported. Folin- Ciocalteu reagent and aluminium chloride colorimetric methods have been used to estimate total phenolic and flavonoid content of the extract. Six bacteria and four fungi were used to measure antimicrobial of extract. 9,12,15-Octadecatrien-1-ol, n-Hexadecanoic acid, 2-Pyrazoline, 2,4-Decadienal, and 9,12-Octadecadienoic acid as most important compounds have been determined. The extract showed strong - glucosidase inhibitory activity (18.01 µg/mL) and also DPPH radical scavenging (IC<sub>50</sub>: 64 µg/mL). The maximum antibacterial activity was investigated against *Salmonella typhi* (30.9 mm).

**Keywords:** Antibacterial, Enzyme, Nanoparticles, Synthesis

### Introduction

Phenolic compounds in *plants* are an essential part of the human diet, and are of considerable interest due to their *antioxidant* properties [1]. In recent years antioxidant and phenolic content of a wide variety of medicinal plants, including grains [2], *Thymus vulgaris* L [3], *Helichrysum monizii* Lowe [4], Turkey herbal plants [5], *Moringa oleifera* leaves [6], *Melilotus albus* and *Dorycnium herbaceum* [7], and *Salvia* species [8] have been evaluated and reported. The *Alyssum homalocarpum*. is one of the biggest genera, which has been known with up to 230 species in the world with main distribution in Asia and Eastern Europe. The first main goal of the present work is the green synthesis of silver nanoparticles using *Alyssum* L. aqueous extract. Existence of stabilizer components

in *A. homalocarpum* extract is one of the most important factors in green synthesis of silver nanoparticles. Green synthesis of silver nanoparticles using aqueous plant extract due to the simplicity and eco-friendly in many numerous studies has been performed [9-11].

Diabetes mellitus (DM) is a disease characterized by hyperglycemia due to absolute (type I) or relative (type II) deficiency of insulin. Inhibition of carbohydrate hydrolyzing enzymes (such as - amylase and -glucosidase) can be an important strategy in the management of blood glucose. These enzymes are the potential targets in the development of lead compounds for the treatment of diabetes. Herbal medicines with antidiabetic potential are gaining popularity in the treatment of diabetes mellitus because of their efficacy, low incidence of side effects, and low cost [12,13].

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Also in light of the evidence of the rapid global spread of resistant clinical isolates, the need to search for plants containing new antimicrobial substances is of paramount importance [14,15]. In the present research *in vitro* enzyme inhibitory and antimicrobial activities of the seed extract of *A. homalocarpum* have been examined.

## Material and Methods

All chemicals used in this study, such as Na<sub>2</sub>CO<sub>3</sub>, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin's reagent, Gallic acid, methanol and ethanol purchased from Merck (Darmstadt, Germany). Required stock solutions were prepared suitably using deionized water, and diluted to the required initial concentration. The absorption studies were carried out using Jusco (Japan) UV-Visible spectrophotometer V-570 model. The shape and surface morphology of samples were investigated by scanning electron microscope (SEM, VEGA model, TESCAN Company, Czech) under an acceleration voltage of 20 kV. Fourier transform infrared (FTIR) spectra of samples were obtained using Bruker-Germany VBRTEX70 spectrophotometer.

### GC-MS Analysis

GC-MS analysis was carried out on a Perkin Elmer Turbo Mass Spectrophotometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The GC/MS apparatus was Agilent technology 5975 system, capillary column of HP-5MS (30 m×0.25 mm×0.25 μm). The oven temperature program was initiated at 70 °C, held for 2 min, and then raised up to 300 °C at a rate of 10 °C/min (held for 10 min). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injector temperature was 280 °C. The spectrums of the components were compared with the database of NIST (National Institute of Standards and Technology) and Wiley 7n.1 mass computer library.

### Preparation of Seed Extracts

*A. homalocarpum* seeds purchased from the Shefa company (Sannandaj, Iran). The seeds were then washed with double-distilled water and dried at oven- at 30 °C for 1 hour. The dried *A. homalocarpum* seed was crushed in a laboratory mill and sieved until it attained a small particle size. Dry seeds (10 g) were extracted using

methanol (250 mL) in a soxhlet extractor (model 6718, Ace Glass Inc., Vineland, NJ.). The extracts were filtered and then concentrated under reduced pressure in a rotary evaporator (Model RE 200). The resulted extracts were stored at -18 °C in the dark until analysis.

### Determination of Antioxidant Activity

The DPPH method [16] was used to determine antioxidant activity of *A. homalocarpum* seeds extract. 200 μL of the methanolic solutions (20 mg/mL) were added to 1 mL of a 0.09 mM DPPH solution. The decrease in absorbance at 517 nm was measured every minute during 60 min, in the dark. The DPPH radical scavenging effect was expressed, based on the Trolox calibration curve as μmol Trolox equivalent per 100 g of the dried plant (μmol equiv. Trolox/100 g dried plant). The scavenging percentage was calculated according to: Scavenging effect (%) = [(control absorbance - sample absorbance)/(control absorbance)] × 100 where control absorbance is the absorbance at 517 nm of 100 μM DPPH solution without addition of the extract/fractions, sample absorbance is the absorbance at 517 nm of 100 μM DPPH with 5–100 μg/mL of sample. Trolox was used as a reference standard. BHT and Oligopin® were also used for comparison. EC50 value (concentration of extract necessary to reduce by 50% the initial quantity of DPPH) was determined by a graph plotting percentage inhibition against concentration.

### Determination of Total Phenolic Content

The total phenolic content (TPC) was determined based on the Folin-Ciocalteu method [17]. 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. The TPC was expressed as gallic acid equivalents in mg/100 mL of *A. homalocarpum* seeds extract. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 0.2 to 4 mg/L.

### Green Synthesis of Silver Nanoparticles

For the synthesis of silver nanoparticles, the plant extract (20 mL) was added to different concentrations (1 mM, 2 mM, 3 mM) of AgNO<sub>3</sub>

solution. The reaction was carried out at 25 °C for 24 h. During the experiment, pH of solutions was checked and fixed at 3 [18]. The samples were analyzed every 24 h by visual inspection and UV/VIS spectroscopy.

#### Determination of Antimicrobial Activity

The *A. homalocarpum* extract was tested against pathogenic organisms *Bacillus cereus* (PTCC 1015), *B. subtilis* (ATCC 1399), *Staphylococcus aureus* (ATCC 25213), *Escherichia coli* (PTCC 2405), *Salmonella typhi* (PTCC 1609), *Aspergillus niger* (PTCC 5012), *Candida albicans* (PTCC 5027), *Dreschlera turcica* and *Fusarium verticillioides*. All the used microbial strains were obtained from the Pasteur Institute of Iran. The different levels of zone of inhibition were measured as described method previously [19]. A series of dilutions with concentrations ranging from 50 to 100 µL for nanoparticles were used in the experiment against every microorganism tested. Gentamicin, streptomycin, clindamycin and streptomycin and nystatin were used as positive control in antibacterial and antifungal assays, respectively. A DMSO solution was used as a negative control for the solvents influence. The antimicrobial activity was calculated as a mean of the three replicates.

#### -Glucosidase Inhibitory Activity (AGI)

AGI analysis has been performed based on the previously described method with slight modifications [12,13]. In a 96-well plate reader, a reaction mixture containing 112 µL of potassium phosphate buffer (0.1 M, pH 6.8), 20 µL of alpha-glucosidase (1 unit/mL) and 8 µL of sample extract (dissolved in DMSO) was pre-incubated for 15 min at 37 °C, and then 20 µL of pNPG (2.5 mmol/L) was added to the mixture as a substrate. After further incubation at 37 °C for 15 min, the reaction was stopped by adding 80 µL of Na<sub>2</sub>CO<sub>3</sub> solution (0.2 mol/L). All the enzyme, inhibitor and substrate solutions were made using the same buffer. Acarbose was used as a positive control and water as negative control. P-nitrophenol's release kinetics were measured spectrophotometrically with a microplate spectrophotometric reader Multiskan MS™ (Labsystems, Minneapolis, USA) for 5 min with intervals of 30 seconds at 405 nm. The -glucosidase inhibitory activity was expressed as the IC<sub>50</sub> according to the percentage inhibition and calculated by the following equation:

$$\% \text{ inhibition} = [1 - (OD_{\text{sample}} - OD_{\text{sampleblank}}) / (OD_{\text{control}} - OD_{\text{blank}})] \times 100$$

where OD<sub>sample</sub> is the absorbance of PBS+enzyme + sample+pNPG; OD<sub>sampleblank</sub> is the absorbance of PBS+sample+pNPG; OD<sub>control</sub> is the absorbance of PBS+enzyme+pNPG; and OD<sub>blank</sub> is the absorbance of PBS+pNPG.

IC<sub>50</sub> value was defined as the concentration of extract inhibiting 50% of -glucosidase activity under the assay conditions. The result of three replicate experiments were stated as Mean± Standard Deviation.

#### -Amylase Inhibitory Activity (AAI)

AAI technique was used according to Yu *et al.* method[20]. 1mL of the *A. homalocarpum* extract was pre-incubated with -amylase 1 unit/mL for 30 min and then 1 mL (1% w/v) starch solution was added. The mixture was further incubated at 37 °C for 10 min. Then the reaction was stopped by adding 1 mL dinitrosalicylic acid reagent (12.0 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaOH and 96 mM 3, 5- dinitrosalicylic acid solution) and the contents were heated in a boiling water bath for 16 minutes. A blank was prepared without *A. homalocarpum* extract and another without the amylase enzyme, replaced by equal quantities of buffer (20 mM Sodium phosphate buffer with 6.7 mM Sodium chloride, pH 6.9 at 20° C). The absorbance was measured at 540 nm. The reducing sugar released from starch was estimated as maltose equivalent from a standard graph. Acarbose was used as positive control. The -amylase inhibitory activity was calculated by using the equations:

$$\% \text{ reaction} = (\text{maltose})_{\text{test}} / (\text{maltose})_{\text{control}} \times 100$$

$$\% \text{ inhibition} = 100\% - \% \text{ reaction}$$

The results were expressed as the mean±SD of three replicates.

## Results

#### GC-MS Analysis

The GC-MS profile of identifying compounds is given in Fig. 1. The chromatogram peaks were integrated and compared with the database in the GC-MS library. GC-MS analysis of major compound in Table 1 revealed the presence of different alcoholic compounds, etc.

**Table 1** Major components identified in the methanol extracts of the studied *Alyssum homalocarpum* (Fisch. & C.A.Mey.) Boiss.

RT	Compound	Area%	Molecular formula
17.054	2-Acetyl-1,4,5,6-tetrahydropyridin	4.82	C <sub>7</sub> H <sub>11</sub> NO
17.438	2-Decenal	1.89	C <sub>10</sub> H <sub>18</sub> O
18.954	2,4-Decadienal	3.67	C <sub>10</sub> H <sub>16</sub> O
20.075	Heptane, 2,2,3,3,5,6,6-heptamethyl	5.03	C <sub>14</sub> H <sub>30</sub>
29.396	2-Pyrazoline	3.23	C <sub>3</sub> H <sub>6</sub> N <sub>2</sub>
33.694	n-Hexadecanoic acid	5.51	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
36.875	9,12-Octadecadienoic acid	1.59	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>
37.012	9,12,15-Octadecatrien-1-ol	15.79	C <sub>18</sub> H <sub>32</sub> O
37.367	9,12,15-Octadecatrienoic acid	3.85	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>
42.477	1-Naphthalenamine	1.3	C <sub>10</sub> H <sub>9</sub> N
54.041	gamma.-Sitosterol	3.86	C <sub>29</sub> H <sub>50</sub> O

**Table 2** Antimicrobial activity of the *Alyssum homalocarpum* (Fisch. & C.A.Mey.) Boiss. extract against various pathogenic microbial strains.

	Mean (average) inhibition zone [mm] <sup>A</sup>								
	<i>Bacillus cereus</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhi</i>	<i>Aspergillus niger</i>	<i>Candida albicans</i>	<i>Dreschler a turcica</i>	<i>Fusarium verticillioides</i>
50*	12±0.40	9±0.01	10±0.21	10±0.31	14± 0.4	11±0.21	6± 0	13±0.10	13±0.61
75	14±0.01	15±0.10	12±0.49	16±0.14	18.1±0.4	13±0.51	6± 0	16±0.07	14±0.12
100	18.1±0.28	22±0.01	19±0.9	21±0.03	30.9±0.4	20±0.18	6± 0	23±0.01	18±0.02
Standard <sup>B</sup>	Gentamicin	Streptomycin	Clindamycin	Gentamicin	Streptomycin	Nystatin	Nystatin	Nystatin	Nystatin
-	25.33± 0.57	25.00±00	9.66± 0.12	19.66± 0.43	8.66±15	15±0.12	15.5±0.26	22±0.57	16±0.33
DMSO	-	-	-	-	-	-	-	-	-

Note: Each value is the mean±standard deviation of three replicates. <sup>A</sup>Includes diameter of disc (6 mm). <sup>B</sup>tested at a concentration of 30 mL/disk. (-) No inhibition detected. \*: concentration of the nanoparticle (µL).

### Characterization of Silver Nanoparticles

In the present work silver nanoparticles have been prepared using the *A. homalocarpum* extracts as reducing agent. The visual appearance of all the AgNO<sub>3</sub> solution, changed shortly after the addition of the plant extract, indicating that a reduction reaction took place. UV–VIS spectral in Fig. 2 confirms stability of synthesized silver nanoparticles in plant extract.

### FTIR Analysis

FT-IR spectrum analysis was used to investigate variations in the functional groups of the silver nanoparticles. FT-IR spectra for the silver nanoparticles is presented in Fig. 3. As can be observed, the silver nanoparticles spectrum shows absorption bands, mainly at 1025, 1384, 1458, 1641, 2362, 2925, 2855 and 3461 cm<sup>-1</sup>. Absorption peaks at about 1025, 1458 and 1641 cm<sup>-1</sup> represent amide linkage groups. Furthermore, the peaks near

3461 and 2925 cm<sup>-1</sup> were assigned to OH stretching.

The representative microscopy image of the silver nanoparticles is given in Fig. 4. The SEM image showd the homogeneous and relatively smooth surface of the silver nanoparticles. Most of the nanoparticles were enlarged to the range of 27–42 nm.

### Antioxidant Activity

The inhibitory effects of methanolic extract on the linoleic acid peroxidation, expressed as IC<sub>50</sub>, is equal to 64±0.09 µg/mL. The potential of antioxidant activity showed moderate antioxidant activity. The phenolic content calculated as Gallic acid equivalent, and is 154.68±3.22 g of the dry weight of seed.

### Enzyme Inhibitory Activity

#### -Glucosidase inhibition

The results showed that the extract was contained of high -glucosidase inhibitory activity (18.01

$\mu\text{g/mL}$  in 100  $\text{mg/mL}$  concentration) which is comparable with standard inhibitor Aacarbose (16.9  $\mu\text{g/mL}$ ).

#### -amylase Inhibition

In the present research, the inhibitory activity of the extract on -amylase was weak (221.87  $\mu\text{g/mL}$  in 100  $\text{mg/mL}$  concentration).

#### Antimicrobial Activity

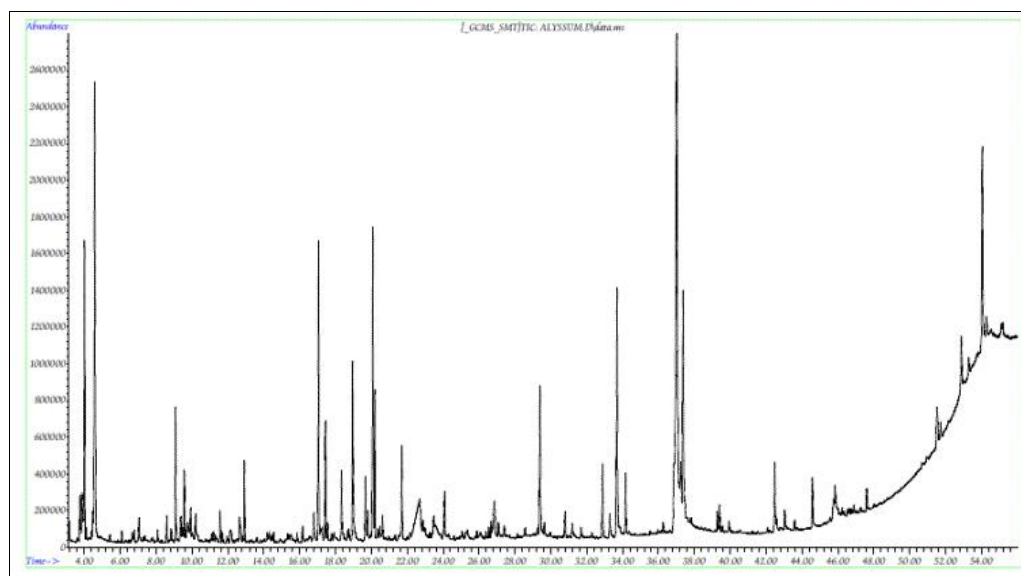
The result of antibacterial activity of *A. homalocarpum* extract was summarized in Table 2. Among all the tested bacteria minimum activity was observed against *Bacillus cereus* (18.1 mm) and maximum activity was demonstrated against *Salmonella typhi* (30.9 mm) in 100 concentration.

## Discussion

Silver nanoparticle solution shows dark yellowish – brown color in aqueous solution due to the surface Plasmon resonance phenomenon [10,21]. Characterization of silver nanoparticles is very important due to using Ag-Nps for different application. The surface structure of silver nanoparticles has been characterized by physical and chemical properties. The FT-IR bands (absorption bands, mainly at 1025, 1384, 1458, 1641, 2362, 2925, 2855 and 3461  $\text{cm}^{-1}$ ) denote

stretching vibrational bands responsible for compounds exist in plant extract or may be held responsible for efficient capping and stabilization of obtained AgNPs [22] (Fig. 3). It is known that antioxidants cause chemical changes in the precursor facilitating formation of silver nanoparticles at room temperatures. Natural antioxidants used as chemical agents for dehydration of lingo-cellulosic materials at low temperatures (Fig. 4). The seed extract showed high phenol content ( $154.68 \pm 3.22$ ) and DPPH activities ( $64 \pm 0.09$ ). 9,12-Octadecatrien-1-ol, n-Hexadecanoic acid, 2-Pyrazoline, 2,4-Decadienal, and 9,12-Octadecadienoic acid are the major compounds found in the extract. These chemicals could be responsible for biological activity of extract. Generally The identified compounds possess biological properties in many researches [23, 24, 25]. For instance, 2-2-Pyrazoline [26], 2,4-Decadienal [27], and 9,12-Octadecadienoic acid (Linoleic acid) show the antioxidant effect or antimicrobial effect. Generally significant antibacterial and antifungal effect were observed against *Salmonella typhi* (30.9 mm) and *Dreschler aturcica* respectively.

The antimicrobial activities can be ascribed to the presence of 2-Pyrazoline.



**Fig. 1** Total Ion Chromatogram (TIC) of ethanol extract of seed of *Alyssum homalocarpum* (Fisch. & C.A.Mey.) Boiss.

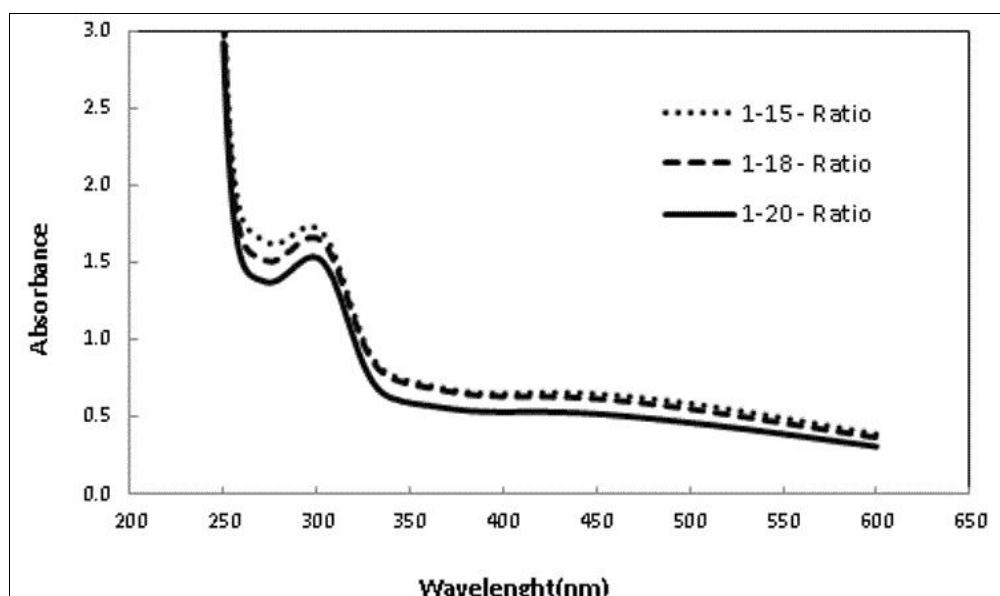


Fig. 2 UV-visible spectra of silver nanoparticles at different ratio of  $\text{AgNO}_3$ -plant extract.

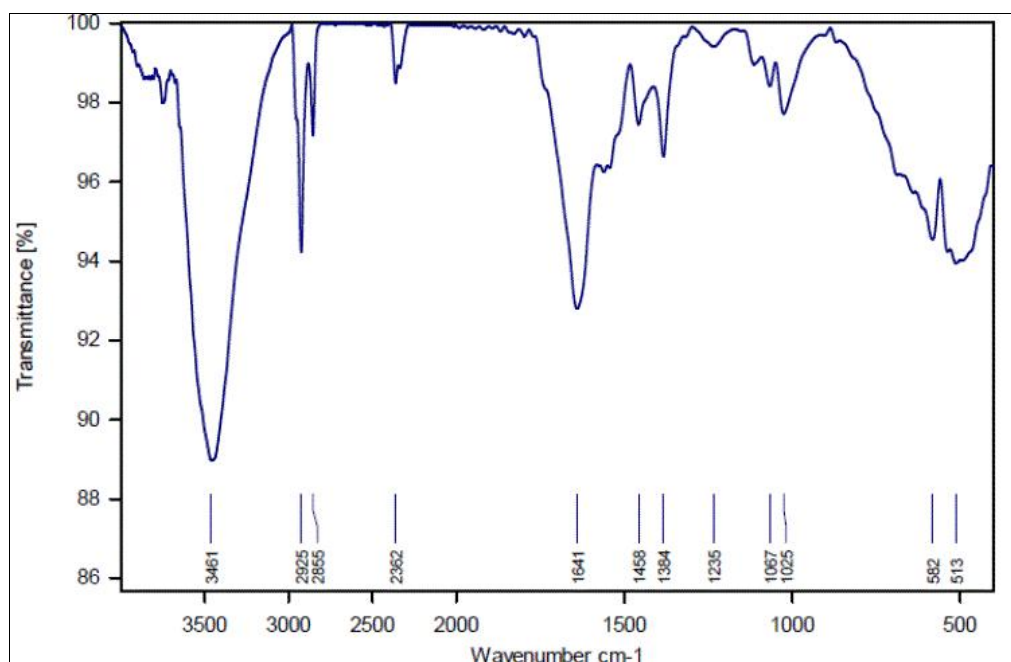
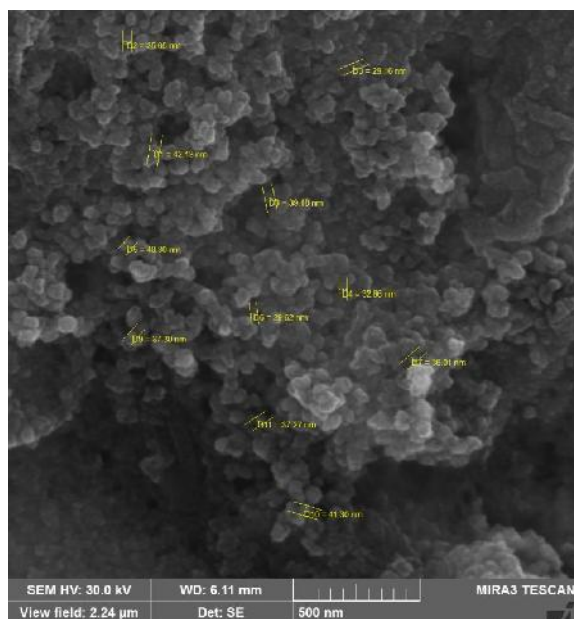


Fig. 3 FT-IR spectra for the silver nanoparticles solution.

Also in the case of antidiabetic activity of the extract, it exerted the significant  $\alpha$ -glucosidase inhibitory activity ( $\text{IC}_{50}$ =18.01  $\mu\text{g}/\text{mL}$ ) in comparing to the standard drug quercetin ( $\text{IC}_{50}$ =16.9  $\mu\text{g}/\text{mL}$ ). the present method predicts the high phenol content and DPPH activities of seed extract, whereas Souri *et al.* [28] investigated activity ( $\text{IC}_{50}$ ) against peroxidation of linoleic acid (2 mg/ml) and phenolic content of *Allysum* against breast ductal carcinoma cell line. Also Zay and Mammadov the phenolic composition, and antioxidant, antibacterial and cytotoxic activities of the methanolic extracts obtained from three

*Alyssum* L. taxa investigated. They reported  $\text{IC}_{50}$  of three *Alyssum* L. taxa between  $0.117 \pm 0.006$  -  $0.102 \pm 0.001$  mg/mL [29]. *homalocarpum*  $94.25 \pm 4.01$  ( $\mu\text{g}/\text{ml}$ ) and  $165.68 \pm 3.22$  mg/100g dry). Kashani *et al.*[30], respectively, ethanolic extracts of selected medicinal herbs from Iranian flora including *A. homalocarpom* Fisch evaluated for their cytotoxic effect on different cell lines and Cytotoxic activity recorded in the mentioned study revealed good potential antiproliferative activity of *Allysum homalocarpum* extract with  $\text{IC}_{50}$  value of  $285.63 \pm 44.42$   $\mu\text{g}/\text{ml}$



**Fig. 4** SEM image of silver nanoparticles formed by *Alyssum homalocarpum* (Fisch. & C.A.Mey.) Boiss. extract.

All findings suggest that the seed extract of *A. homalocarpum* could be a good source of natural antioxidant, antidiabetic and antimicrobial compounds that can be used as therapeutic agents in related disease.

## Conclusion

In the present work antimicrobial, antidiabetic, antioxidant, and phenolic content of methanol extract of *A. homalocarpum* has been determined. The results show the potential antioxidant activity is moderate. This work also focused on synthesizing of silver nanoparticles from silver nitrate solutions using *A. homalocarpum* extract. These antimicrobial activities can be ascribed to the presence of 2-Pyrazoline. Also in the case of the antidiabetic activity of the extract, it exerted the significant  $\alpha$ -glucosidase inhibitory activity ( $IC_{50}$ =18.01  $\mu$ g/mL) in comparing to the standard drug quercetin ( $IC_{50}$ =16.9  $\mu$ g/mL). All findings suggest that the seed extract of *A. homalocarpum* could be a good source of natural antioxidant, antidiabetic and antimicrobial compounds and can be used as therapeutic agents in related disease.

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