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Original Article

# Assessment of Heavy Metal Accumulation and Antibacterial Activity of Some Medicinal Herbs Grown in an Industrial Area of Steel Production, Ahvaz

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## Abstract

Unfavorable conditions can have effect on secondary metabolites production in the plants. The aim of present study was to evaluate the effect of heavy metals accumulation on antibacterial potential of *Chenopodium murale* L., *Senecio glaucus* L., *Malva parviflora* L. and *Calendula arvensis* L. grown in an industrial area of steel production in Ahvaz, southwest of Iran. The cadmium (Cd), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb) and zinc (Zn) values were analyzed in different parts of these medicinal herbs. The antibacterial activity of methanol and ethanol extracts of the plants was surveyed in standard disc diffusion method against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*. In addition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of these extracts were determined. High concentration of Cd was found in the roots of *M. parviflora* and *S. glaucus*. The concentration of other metals in these plants was within the allowed limits. Both of ethanolic and metanolic extracts exhibited the highest antibacterial effect against *E. coli* and *P. aeruginosa*. MIC and MBC values of alcoholic extracts were determined against *E. coli* and *P. aeruginosa* as the most sensitive bacterial species. It seems that antibacterial activity of studied plants is not essentially correlated with their heavy metal concentration.

*Key words:* Alcohol extracts, Antibacterial agents, Ethanolic extracts, Medicinal plants, Metals assay, Methanolic extracts

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## 1. Introduction

Heavy metals are elements with a specific weight higher than 5 g/cm$^3$. Some heavy metals such as Cu, Fe, Mg, Zn and Co are essential elements for normal growth, development and metabolism of plant.

However, high concentrations of these elements lead to physiological disorders in plants. Other heavy metals including Pb, Cd, Hg, Cr, *etc*. are not considered as essential and nutritional elements and even in low amounts are highly toxic for plants and animals. Accumulation of heavy metals in the body of organisms can lead to toxicity and harm. Heavy metals are released into the environment as a result of industrial and agricultural activities [1]. Heavy metal accumulation by different plant species has been recorded in the areas with industrial or mining activities in Iran such as Hamadan iron and copper mine [2], Isfahan Mobarakeh steel company [3], Sarcheshmeh copper mine [4] and Ahvaz steel production companies [5]. Heavy metals monitoring in plants grown in industrial areas can provide useful information about the environmental health and safety. Plants are able to uptake and accumulate heavy metals in various organs and tissues. The amount of excess metal ions in food chain results in damage to ecosystems and threatens the human health, if these plants be used as food or pharmaceutical products [6]. Use of plants in treatment of many diseases has long been considered. It is mostly believed that medicinal plants, due to natural origin, do not have side effects. However, cases of toxicity following the use of medicinal plants have been reported due to the presence of heavy metals [7]. Hence, the World Health Organization (WHO) has stressed that the plants with medicinal and curative function, should be assayed for the presence of heavy metals [8]. Analysis of heavy metal content is necessary for final production and processing of herbal products. Some studies illustrated the presence of different amounts of heavy metals in medicinal plants [9, 10, 11, 12, 13]. High concentrations of heavy metals have been found in some medicinal plants grown in areas with industrial activities such as *Punica granatum* [14], *Boviea volubilis*, *Eucomis autumnalis*, *Discorea dregena* [15] and *Withania somnifera* [16]. Heavy metal stress enhances production of reactive oxygen species (ROS) and it then induces oxidative stress in plants. Plants employ various enzymatic and non-enzymatic mechanisms to reduce oxidative stress. Phenolic compounds, carotenoids, flavonoids and other secondary metabolites represent an important role in scavenging ROS caused by heavy metal stress [17]. These metabolites comprise abundant medicinal and therapeutic properties. Medicinal significance of plants is often dependent on the quality and quantity of secondary metabolites [18]. It has been established that some of these compounds, due to antioxidant and antibacterial properties, contain pivotal role in prevention of the growth and proliferation of pathogens [19]. Plant secondary metabolites may be synthesized during normal growth and development stages or in response to environmental stresses such as heavy metals contamination. The amount of heavy metals in medicinal plants is certainly effective on chemical ingredient, efficiency and biological activity of pharmaceutical plant compounds [13]. There is limited information in relation to the effect of heavy metals accumulation on

antibacterial activities of medicinal plants grown in industrial regions of Ahvaz, Iran. Therefore the aim of this study is to evaluate the effect of heavy metal stress on antibacterial potential of *Chenopodium murale* L., *Senecio glaucus* L., *Malva parviflora* L. and *Calendula arvensis* L., as medicinal herbs that have been considerably distributed in areas surrounding steel production industries in Ahvaz, southwest of Iran.

*Chenopodium murale* L. is an annual herb that belongs to Amaranthaceae family. The presence of secondary metabolites such as, flavonols and their glycosides, sterols, alkaloids and terpenes has been revealed in *C. murale* [20]. This species, as a potherb, is used instead of spinach and has laxative and anthelmintic properties. Different species of *Chenopodium* are widely used in traditional medicine. Antiviral, antimicrobial, antifungal, antioxidant, anthelmintic, antineoplastic and immunomodulatory effects have been reported for some species of *Chenopodium* [20].

*Senecio glaucus* L. is an annual herbaceous plant from Asteraceae family. The existence of bioactive compounds such as, phenolics, saponins, flavonoids and tannins and some volatile essential oils in *S. glaucus* has been reported [21]. Members of *Senecio* genus are known to produce pharmacologically active and various phytochemicals for example pyrrolizidine alkaloids, sesquiterpenoids, diterpenoids and treterpenoids. Moreover, antibacterial, antimicrobial, molluscicidal and cytotoxic activities have been identified for these plants. In traditional remedies, different species of *Senecio* are used for wound healing and treatment of coughs, bronchitis, asthma and eczema [22].

*Malva parviflora* L. is an annual or perennial herb from Malvaceae family. *Malva* is used as food; as well as in traditional and modern medicines. Scientific evidence indicates that different parts of this plant, due to therapeutic properties, are effective in the treatment of many gastrointestinal, dermatological, urological, haemorrhoidal, menstrual and vaginal disorders [23]. In addition, antioxidant, anticancer [24] and antibacterial properties [25] have been reported for *Malva*. Pharmaceutical effects of *Malva*, especially in the leaves and flowers, can be attributed to the presence of phenol derivatives, polysaccharides, tannins, anthocyanidines, mucilages, terpenoids, vitamins and sterols [23].

*Calendula arvensis* L., as an annual herb from Asteraceae family [26] *Calendula* is a very important herb for medicinal and industrial uses, as well as food additives. The various types of bioactive phytochemicals such as triterpenoids, sesquiterpenes, flavonoids, essential oils and carotenoids have been reported in *Calendula* [26]. Some parts of the plant, mostly the flowers, have been applied in the treatment of fever, jaundice, menstrual complaints and skin injuries [27]. The flowers in particular have been displayed antibacterial and antioxidant activities [28].

Ahvaz, as capital of Khuzestan Province in Iran, is an industrial city due to the existence of oil and gas resources and steel production companies. Most of the people living in rural areas of Khuzestan often use medicinal herbs

from wild populations grown in environment. Therefore, with regard to the utilization of these plant species mentioned in traditional medicine and home remedy and also concerning the growth location of these plants, where steel production activities release metal particles into the environment, this study was conducted to assess heavy metals concentration in four medicinal herbs and investigate the effect of heavy metals content on antibacterial potential of these plant extracts.

## 2. Materials and Methods

### *2.1. Plant Material and Extracts Preparation*

The whole plants of *C. murale* L., *S. glaucus* L., *M. parviflora* L. and *C. arvensis* L. as well as soil samples were collected from around of steel production industries, located in southeast of Ahvaz in Khuzestan province. The plants were identified based on the herbarium of Biology Department in Shahid Chamran University of Ahvaz and washed with sterilized distilled water. To study the antibacterial activities, the leaves and stems, roots and flowers were separately shade-dried at room temperature for 10 days and ground to fine powder. Then, 10 ml of 80% ethanol or methanol (v/v) was added to 1 g of herbal powder. Following one minute vortex, the samples were centrifuged at 3000 rpm for 15 min. The supernatant was used to survey antibacterial activity [29].

### *2.2. Analysis of Heavy Metals*

The Mn, Zn, Fe, Ni, Cd and Pb concentrations were measured in the plant and soil samples. For this purpose, fresh shoot, root and flower of each plant sample were dried in oven at 72 ºC for 48h, subsequently, plant dry matter were powdered and extracted according to Kovacs *et al*. (1996). Powdered samples were mixed with 10 ml of 65% $HNO_3$ and remained overnight at room temperature. Then, the digests were heated at 85 ºC to evaporate acid. One ml of 30% $H_2O_2$ was added; consequently, the digests were filtered and diluted with deionized water to 50 ml. To evaluate heavy metals total concentration, 1 g of sieved soils was mixed with 15 ml of 65% $HNO_3$ and 10 ml of 37% HCl. After heating and desiccating, 30 ml of 1 N HCl was added. The digests were then filtered and made up to 50 ml volume with deionized water [31]. Heavy metals concentration in plant organs and soil samples was determined using a Flame Atomic Absorption Spectrometer instrument (GBC, SAVANTAA scientific equipment, Australia).

### *2.3. Evaluation of Antibacterial Activity*

The antibacterial effect of alcoholic extracts was surveyed against two gram-positive bacteria *i.e*., *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633), and two gram- negative bacteria *i.e*., *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 9027) using standard disc diffusion method (Kirby-Bauer method), as indicated by Motamedi *et al*. (2014). Briefly the listed bacteria were grown

in Mueller Hinton broth medium (MHB, Merck-Germany) at 37 ºC to achieve a final concentration of cells according to 0.5 McFarland turbidity. Bacterial suspensions were lawn cultured on Mueller Hinton agar (MHA, Merck-Germany) with a sterile cotton swab. After preparing different concentrations of ethanolic and methanolic extracts (50, 100, 200 and 400 mg/ml), sterile blank paper discs (6 mm) were saturated with these extracts so that the effective dose of extracts per disc was obtained as 2, 4, 8 and 16 mg, respectively. To evaporate alcoholic solvents, the discs were left at room temperature for 30 min. Then, the discs were put on lawn bacterial cultures of MHA. Simultaneously, saturated discs with solvents were also prepared and used to investigate the possible antibacterial effect of solvents. Furthermore, standard antibiotic discs were also tested against bacterial species. The plates were incubated at 37 ºC for 24 h. The diameter of the growth inhibition zone around was measured for each disc in millimeters. In order to determine minimum inhibitory concentration (MIC) of plant extracts, macro broth dilution method was used and it was considered for the most sensitive bacterial species verified in the disc diffusion method [29]. A set of diluted solutions of alcoholic extracts including 0.5, 1, 2, 4, 8, 16, 32 and 64 mg $ml^{-1}$ were added to the tubes containing 1 ml of MHB medium and 100 µl bacterial suspensions with 0.5 McFarland turbidity. The tubes were incubated at 37 ºC for 24 h. The MIC was defined as the lowest extract concentration which did not display any visible growth of bacteria after incubation time. To find minimum bactericidal concentration (MBC), a loop full of bacterial suspensions containing the extracts was cultured on MHA medium and incubated at 37 ºC for 24 h. These suspensions exhibited no visible growth in the MIC assay. The MBC was regarded as the highest dilution of extract that did not lead to colony formation on MHA medium.

*2.4. Scanning Electron Microscopy Analysis*

In order to find possible effect of extracts on bacterial cell wall, SEM analysis was planned. A bacterial suspension was prepared from bacterial colonies at the margin of inhibition zone. Prepared smear was coated with gold and studied using SEM (Hitachi Japan S4160) of central laboratory in Shahid Chamran University of Ahvaz.

## 3. Results and Discussion

Table 1 shows heavy metals concentrations in the plant species. Also, the data about antibacterial effect of methanolic and ethanolic extracts of four medicinal species is presented in Table 3 and 4. No Cd content was found in these plants, with the exception of *M. parviflora* and *S. glaucus* roots. The maximum and minimum levels of Fe were determined in the shoots of *M. parviflora* and *C. murale* as 8.80 and 0.123 mg/kg DW, respectively. The highest Mn content was assayed in aboveground organs of *M. parviflora* including shoots and flowers as 7.35 and 7.30 mg/kg DW, respectively. Mn concentration was zero in the shoots of *C. murale*. The highest and the lowest Ni concentration were

**Table 1.** The concentartion of heavy metals in various parts of the plants (mg/kg DW). NF= No flower.

| Plant species | Cd | Fe | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|---|
| *C.murale* root | 0 | 2.64 | 0.334 | 1.30 | 0 | 0.363 |
| Shoot | 0 | 0.123 | 0 | 0.081 | 0 | 0 |
| Flower | NF | NF | NF | NF | NF | NF |
| *S. glaucus* root | 0.685 | 7.87 | 0.680 | 0.325 | 0 | 0.770 |
| Shoot | 0 | 4.32 | 0.272 | 0.081 | 0 | 0 |
| Flower | 0 | 2.14 | 0.450 | 0.081 | 0 | 0.312 |
| *M. parviflora* root | 0.789 | 2.56 | 0.421 | 0.079 | 0 | 0.700 |
| Shoot | 0 | 8.80 | 0.735 | 0.204 | 0 | 2.54 |
| Flower | 0 | 3.46 | 0.730 | 0.325 | 0 | 0.741 |
| *C. arvensis* root | 0 | 2.62 | 0.352 | 0.420 | 0 | 0.107 |
| Shoot | 0 | 4.26 | 0.435 | 0.07 | 0.830 | 1.03 |
| Flower | 0 | 4.36 | 0.475 | 0.330 | 0 | 0.560 |

measured in the roots of *C. murale* (1.3 mg/kg) and the shoots of *C. arvensis* (0.07 mg/kg). Lead accumulation in the shoots of *C. arvensis* was equal to 0.830 mg/kg. On the other hand, it was not determined any content of Pb in other plants. Total concentration of heavy metals in the soil of this area is illustrated in Table 2. The values of heavy metals in the soil were measured as Fe > Mn > Zn > Pb > Ni > Cd.

The data about antibacterial effect of methanolic and ethanolic extracts of four medicinal species is presented in Tables 3 and 4. Maximum antibacterial activity was determined by the methanolic extracts of *S. glaucus* (14 mm, reproductive organ) and *C. murale* (14 mm, root) against *E. coli*, at 4 and 16 mg effective doses per disc, respectively. Methanol extracts of four plants showed an insignificant antibacterial activity against *S. aureus* and *P. aeruginosa* (Table 3). As shown in table 4, maximum inhibition effect of ethanolic extracts on the growth of *P. aeruginosa* was caused by the shoots of *C. murale* (14 mm) and the flowers of *S. glaucus* (15 mm) at 16 mg effective dosage per disc. Ethanolic extracts of various parts of mentioned species inhibited the growth of *S. aureus*. Methanol and ethanol extracts of *M. parviflora* and *C. arvensis* demonstrated antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus* in some cases. With the exception of methanolic extracts of *C. arvensis* roots, no other antibacterial activity was found for alcohol extracts against *B. subtilis*. The results of MIC and MBC assays are showed in table 5. As formerly mentioned, the most sensitive bacteria identified in the disc diffusion method, were selected to verify MIC and MBC. Methanol extract of *M. parviflora* flowers against *P. aeruginosa* had MIC with 1 mg/ml and MBC 32 mg/ml. MIC values for ethanol extracts from shoot of *M. parviflora* and *C. murale* were determined against *P. aeruginosa* as 4 and 2 mg/ml, respectively, whereas no values was found for MBC, *i.e.*, these extracts are bacteriostatic pharmaceuticals. The MIC index of methanolic extract of *S. glaucus* flowers was 64 mg/ml for *E. coli*, while its MBC was

**Table 2.** Total concentration of heavy metals in the soil of area (mg/kg DW).

| Cd | Fe | Mn | Ni | Pb | Zn |
|---|---|---|---|---|---|
| 0.02 | 66.40 | 12.60 | 0.243 | 0.8 | 4.66 |

**Table 3.** Antibacterial activity of methanolic extracts from herbaceous plants. The values are diameter of inhibition zone in mm, NI= No inhibition was observed, NF= No flower.

| | ***E. coli*** | | | | ***P. aeruginosa*** | | | | ***S. aureus*** | | | | ***B. subtilis*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant species** | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | |
| | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** |
| *C.murale* root | 13 | 12 | 12 | 14 | NI | 7 | 7 | 7 | 7 | 7 | 7 | 7 | NI | NI | NI | NI |
| Shoot | NI | NI | NI | NI | 7 | NI | 7 | NI | 7 | NI | 7 | NI | NI | NI | NI | NI |
| Flower | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF |
| *S. glaucus* root | NI | 8 | 8 | 10 | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| Shoot | NI | NI | NI | NI | NI | NI | NI | NI | 7 | 7 | 7 | 7 | NI | NI | NI | NI |
| Flower | 7 | 14 | 13 | 12 | 7 | 7 | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| *M. parviflora* root | NI | NI | NI | 7 | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| Shoot | NI | NI | NI | 7 | NI | 7 | 8 | 9 | NI | NI | 7 | 8 | NI | NI | NI | NI |
| Flower | 7 | 7 | 7 | 7 | 8 | 9 | 9 | 10 | 8 | 8 | 9 | 9 | NI | NI | NI | NI |
| *C. arvensis* root | 7 | 7 | 7 | 7 | NI | NI | NI | NI | 7 | 7 | 7 | 9 | NI | NI | 7 | 8 |
| Shoot | NI | NI | NI | NI | 7 | 7 | 7 | NI | 7 | 7 | 7 | 7 | NI | NI | NI | NI |
| Flower | NI | NI | NI | NI | NI | 7 | 8 | 8 | 7 | NI | 7 | 7 | NI | NI | NI | NI |

greater than 64 mg/ml. At present, the use of plants, as natural resources of pharmaceutical products, is of great concern, especially in developed countries. Two main reasons can be sited in this regard: (1) herbal products compared to synthetic compounds and antibiotics possess fewer side effects, (2) frequent and improper application of antimicrobial agents has been led to the development of antibiotic-resistant strains and treatment failure [12]. Nevertheless, the quality and efficiency of herbal medicinal ingredients are dependent on environmental conditions [18]. For instance, the presence of heavy metals in *Marsilea minuta* resulted in reducing its antimicrobial activity [32]. Okem *et al*. (2014) illustrated that there was no significant correlation between phenolics contents and heavy metals levels in verified medicinal plants. Many medicinal plants growing in the industrial and mining areas are able to uptake and accumulate high amounts of heavy metals. High concentrations of Ni, Cu and Pb have been reported in *Punica granatum* leaves [14]. The study on *Boviea volubilis*, *Eucomis autumnalis* and *Discorea dregena* indicated the presence of elevated levels of Cd and As [15]. High amounts of various heavy metals have been found in *Withania somnifera* [16]. As a consequence, heavy metals concentration of medicinal plants must be controlled to safeguard the public health. In accordance with the recommendations of the WHO (2007), the amounts of elements in

medicinal herbs should be checked to produce final products. Some heavy metals such as Pb and Cd, even at very low concentrations, are highly toxic and dangerous for organisms. The permissible limits of these metals in the plant tissues have been reported 10 and 0.03 mg $kg^{-1}$, respectively [13]. Other metals such as Ni, Zn and Mn are involved in physiological functions of the plants within the permitted limits by 1.5, 50 and 100 mg/kg, respectively [33]. As shown in the table 1, the values of heavy metals in different parts of each plant species are variable. With the exception of Cd in the roots of *S. glaucus* and *M. parviflora*, the amount of the other metals is lower than the allowable limits of WHO (2007). Cadmium is an extremely toxic element. Increased Cd concentration in the human body leads to an increase in urinary beta-microglobulin and kidney disorder [34]. Despite high levels of Cd, in most cases the listed bacteria were resistant to alcoholic extracts of *M. parviflora* root (Tables 3 and 4). Nevertheless, shoot and flower of *M. parviflora* exhibited moderate antibacterial activity against *E. coli*, *P. aeruginosa* and *S. aureus*. The study on methanol, hexane and water extracts of *M. parviflora* showed a strong antibacterial activity against a wide range of gram-positive and gram-negative bacteria [25]. *M. parviflora*, as a medicinal herb, is rich in phenolics, alkaloids, flavonoids and saponins [23]. Based on these results, with the presence of Cd in the root, ethanolic extracts of *S. glaucus* flower illustrated a significant inhibitory effect against *P. aeruginosa* (Table 4). There are different reports about the effect of heavy metal levels on the antibacterial activity of plant extracts. Street *et al*. (2009) expressed that the antimicrobial activity increased in *Merwilla plumbea* treated with high concentration of Cd. Aqueous extracts of *Verbascum speciosum*, collected from heavy metal contaminated areas, showed high antibacterial activity against *Salmonella paratyphi* [36]. The plants exposured to heavy metals stress enhance the activity of antioxidant enzymes and synthesis of secondary metabolites such as phenolics and flavonoids to guarantee their survival [17]. Thus, increased antioxidant activity could be one of the main reasons for the improvement of antibacterial activity under heavy metals stress. As previously mentioned in introduction [20, 21, 23, 26], the plants studied in this work are rich sources of phenolic compounds. Bactericidal or bacteriostatic properties of phenolic compounds are mainly is dependent on the concentration used and also their substituted groups on their phenol nucleus *e.g*., hydroxyl, alkyl, acetate, *etc*. Phenolic compounds can incorporated in lipid bilayer of plasma membrane in bacterial cell and disrupt membrane homeoviscous adaptation mechanism. Hence, the majority of activities related to the membrane in bacterial cell will be failed which can be led to growth inhibition or death of bacterial cell [37]. Elevated levels of phenolic compounds have been reported in some medicinal plants under heavy metal stress [38]. On the other hand, despite the high accumulation of heavy metals, some medicinal plants have represented poor antibacterial

**Table 4.** Antibacterial activity of ethanolic extracts from herbaceous plants. The values are diameter of inhibition zone in mm, NI= No inhibition was observed, NF= No flower.

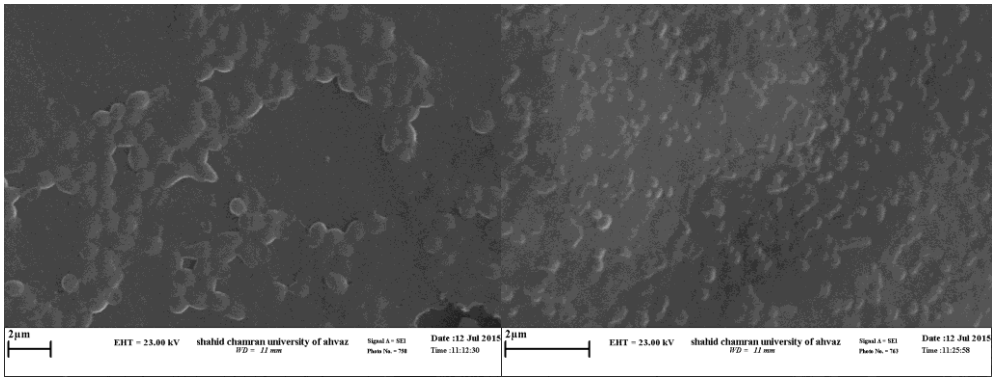
| | ***E. coli*** | | | | ***P. aeruginosa*** | | | | ***S. aureus*** | | | | ***B. subtilis*** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Plant species** | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | | **Effective dose of extract (mg/disc)** | | | |
| | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** | **2** | **4** | **8** | **16** |
| *C.murale* root | 6 | 7 | 8 | 8 | 11 | 7 | 8 | 10 | 13 | 8 | 7 | 6 | NI | NI | NI | NI |
| Shoot | 7 | 9 | 8 | 7 | 9 | 12 | 13 | 14 | 8 | 9 | 11 | 12 | NI | NI | NI | NI |
| Flower | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF | NF |
| *S. glaucus* root | NI | NI | NI | 8 | 7 | 7 | 7 | 9 | 7 | 8 | 8 | 8 | NI | NI | NI | NI |
| Shoot | NI | NI | NI | NI | NI | NI | NI | 11 | 8 | 10 | 10 | 10 | NI | NI | NI | NI |
| Flower | NI | NI | NI | NI | 9 | 11 | 13 | 15 | 7 | 7 | 7 | 9 | NI | NI | NI | NI |
| *M. parviflora* root | NI | NI | NI | 7 | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI | NI |
| Shoot | NI | NI | NI | 7 | NI | 7 | 8 | 9 | NI | NI | NI | NI | NI | NI | NI | NI |
| Flower | 7 | 7 | 7 | 7 | 8 | 9 | 9 | 10 | 8 | 9 | 8 | 9 | NI | NI | NI | NI |
| *C. arvensis* root | 7 | 7 | 7 | 7 | NI | NI | NI | NI | 7 | 7 | NI | 7 | NI | NI | NI | NI |
| Shoot | NI | NI | NI | NI | 7 | 7 | 7 | NI | 8 | 8 | NI | NI | NI | NI | NI | NI |
| Flower | NI | NI | NI | NI | NI | 7 | 8 | 8 | 9 | 8 | 10 | 11 | NI | NI | NI | NI |

properties [13]. This indicates that antibacterial activity is not essentially dependent on heavy metals level in medicinal plants. It has been suggested that decreased synthesis of antimicrobial substances or inactivation of bioactive phytochemicals is resulted in a decrease in antimicrobial properties due to phytochemical-metal complex formation [32]. Moreover, it seems that the plants diminish synthesis of phenolics to prevent from negative effects of phenoxyl radicals created at high concentrations of heavy metals [39].

Regardless of the amount of heavy metals, maximum inhibition effect was observed by both methanol and ethanol extracts against gram-negative bacteria (Tables 3 and 4), so that the root methanolic extract of *C. murale* showed the highest inhibitory effect on the growth of *E. coli*. In addition, the greatest inhibitory activity was found by ethanolic extracts of *S. glaucus* flowers and *C. murale* shoots against *P. aeruginosa*. Ethanolic extracts of *C. murale* contain abundant flavonoids [20]. Some species of *Chenopodium* illustrated bactericidal properties against *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis* [40]. The study on *S.*

**Table 5.** MIC and MBC of ethanolic and methanolic extracts from *C. murale* and *M. parviflora*. The values are in mg/ml.

| | ***P. aeruginosa*** | | ***P. aeruginosa*** | ***E. coli*** |
|---|---|---|---|---|
| | **Ethanolic Extracts** | | **Methanolic Extracts** | |
| | *C. murale* shoot | *M. parviflora* shoot | *M. parviflora* flower | *S. glaucus* flower |
| MIC | 2 | 4 | 1 | 64 |
| MBC | - | - | 32 | > 64 |




**A** **B**

**Figure 1.** Effect of *C. murale* root extract on *S. aureus*. Cell deformation is obviously can be found in treated bacteria (A). Deformed *P. aeruginosa* as a consequent of treatment with *S. glaucus* flower ethanolic extract (B). Cell swelling and deformation from rod to irregular shape is the major change in treated bacteria.

*glaucus* indicated that *E. coli*, *P. aeruginosa* and *S. aureus* were resistant to shoot and root methanoilic extracts of this plant, while root extracts prevented the growth of *B. subtilis* [21]. *S. glaucus* contains saponins, phenolics and flavonoids which protect the living cells against oxidative stress and have anticancer activity as super antioxidants [21]. Thus, it is supposed that antibacterial activities of *C. murale* and *S. glaucus* are largely due to the presence of these phytochemicals.

The results of SEM analysis are presented in figure 1. According to the results of this work, the extracts that showed considerable antibacterial effect in disc diffusion assay probably were able to induce structural changes in target bacteria. It seems that the target site is cell wall and plasma membrane of bacteria and these extracts may be caused deformity in bacterial cell structure. This cell wall damage was more obvious for rod shaped bacteria such as *P. aeruginosa*. The results of SEM analysis showed that the effective extracts are active pharmaceuticals which affect on bacterial cell wall and cause deformation of bacteria. There is no similar structure to bacterial cell wall in eukaryotic cells, therefore the compounds that can affect on cell wall synthesis or polymerization might be safely used to control of bacterial infections without side effects [29]. It is supposed that the studied medicinal plants in this research are good candidates for finding new antibacterial agents which can disrupt bacterial cell wall and cause bacterial death. Since these agents are mainly bactericidal, the risks of bacterial survival and recurrence of infection will be reduced.

## 4. Conclusion

According to the results of this study, the concentration of heavy metals (with exception of Cd) in mentioned plants were below the permissible limits. Nevertheless, the amounts of heavy metals in both the soil and plant are dependent on seasons, soil factors and the activity level of steel production companies. The data indicates that methanolic and ethanolic extracts showed the highest inhibition effect on growth of gram-negative

bacteria including *E. coli* and *P. aeruginosa*. Based on the SEM results, it supposed that the effective extracts of studied plants exert antibacterial abilities through the destruction of cell wall in the bacteria, and these plants might be considered as antibacterial agents to control bacterial infections. Studied herbs widely grow in the region of steel production industries, where density of rural populations is high. Due to the belief in folk medicine, these people consume wild plants for the home remedies. Thus, long-term use of these herbs may increase the level of metals such as Cd in the human body. For a better understanding, it is required that qualitative and quantitative analysis of heavy metals in the medicinal plants of this area is carried out during different seasons. Furthermore, it is suggested that the antimicrobial activity of these plants is verified with different concentrations of Cd under controlled conditions.

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