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In Vitro Antibacterial Activity of Selected Medicinal Plants Traditionally Used in Iran Against Plant and Human Pathogenic Bacteria

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Abstract

Medicinal plants are widely used for the treatment of different plant diseases and hospital infections caused by bacteria. The present study aims at determining the in vitro antibacterial activity of the medicinal plants traditionally used in Iran against the bacterial species associated with plant diseases and hospital infections. The antibacterial usefulness of methanol extracts of five medicinal plants is tested against seven Gram-negative bacteria and three Gram-positive bacteria using the agar disc diffusion method. Some of the plant extracts showed antibacterial activity against Gram-positive bacteria and Gram-negative bacteria. The high antibacterial activity against the Gram-positive species and Gram-negative was in the extract of *Geum urbanum* against *Bacillus subtilis* (+++) and *Pseudomonas aeruginosa* (+++). Consequently, plant *Geum urbanum* was subjected to gas chromatography-mass spectrometry (GC-MS) analysis. Results show that its antibacterial activity may be due to the presence of eugenol, phenolic acid and tannin. The present study finds clear evidence supporting the traditional use of the plants in treating plant diseases and hospital infections related to bacteria. These plant species showed a moderate to a high antibacterial activity against the bacteria tested.

Keywords: Medicinal plants; Antibacterial activity; Agar disc diffusion method; Inhibition zone; GC-MS analysis.

1. Introduction

Recently, antibiotics have been generated with a target of eliminating the microorganisms which were liable for many diseases as well as the emergence of antibiotic resistance and the failure of chemotherapy are increasing (Fankam et al., 2014). Furthermore, effects the extensive use of the synthetic medicines may lead to serious damages to many of human organs (Gupta et al., 2016). One strategy to avoid this is by using alternative therapeutic agents from plants that are effective against antibiotic resistant bacteria and have low cost (Wikaningtyas et al., 2016). Medicinal plants have great importance in the control of human diseases. Plant materials are calling secondary metabolites used as a source of numerous natural products as a crude extract or as purified products are employing in control and treatment of various types of diseases globally (Cowan, 1999). The World Health Organization (WHO) has stated that medicinal plants are the richest and the best source for obtaining a variety of therapeutic agents (Gupta et al., 2016; Alo et al., 2012). Medicinal plants constitute credible sources for a huge number of modern antibiotics, several of which are usually based on their traditional folk medicine.

Khattab et al. (2016) reported that methanolic/chloroform extract of olive showed activity against Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Iqbal et al. (2014) reported that the methanol extract of Taraxacum officinale was found to be effective against the tested bacterial pathogens Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus and Bacillus Subtilis. Trabelsi et al. (2014) reported that Citrus aurantium (blossoms) extract was active only against Staphylococcus aureus. Soni et al. (2012) reported that the methanol extracts of Datura

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stramonium showed activity against Gram-positive bacteria and little or no antimicrobial activity has against Escherichia coli and Pseudomonas aeroginosa. Sudjana et al. (2009) have investigated the activity of a commercial extract derived from the olive leaves of Olea europaea and indicated that Olea europaea extract did not show broad-spectrum activity and has appreciable activity only against Campylobacter jejuni, Helicobacter pylori and Staphylococcus spp.

In the present study the antibacterial activity of seven medicinal plants against ten pathogenic bacteria was evaluated.

2. Materials and Method

Bacteria Isolates

All pathogenic bacteria used in the present study were includ Bacillus subtilis (PTCC 1023), Staphylococcus aureus (PTCC 1431), Rathayibacter toxicus (ICMP 9525), Escherichia coli (PTCC 1330), Pseudomonas aeruginosa (PTCC 1074), Pseudomonas syringae. syringae (ICMP 5089), Pseudomonas viridiflava (ICMP 2848), Xanthomonas campestris. campestris (ICMP13), Acidovorax avenae and Erwinia amylovora. They were obtained from Sari Agricultural Sciences and Natural Resources University (SANRU), the laboratory of microbiology, Sari, Iran. The isolates were subcultured on Nutrient Agar (NA) plates. The incubation condition was 37°C for quality control species and 27ºC for plant bacteria for 24h. For the antibacterial activity test, a loopful of the organisms were inoculated individually into 5.0ml of nutrient broth and incubated at 37°C for quality control strains and 27ºC for plant bacteria for 24h. 0.2ml from the 24 hours culture organism was dispensed into 10ml sterile nutrient broth and incubated for 3-5 hours to standardize the culture to 10° CFU/ml (Abalaka et al., 2012).

Plants Materials

Different plants, such as *Geum urbanum* (leaves and roots), *Citrus aurantium* (blossoms), *Datura stramonium* (leaves and stems), *Olea europaea* (leaves), *Taraxacum officinale* (leaves and roots), were collected from North of Iran, Mazandaran province (Sari and Chalous cities). Table 1 shows the traditional uses and the plants parts used. The fresh samples were collected and washed with distilled water to remove impurities. The plants were shade-dried and pulverized to powder in a mechanical grinder.

Table 1. Traditional uses, parts used traditionally and bioactive or potentially bioactive components

Species (family)	Traditional uses	Parts used traditionally	Bioactive or potentially bioactive components
Citrus aurantium	Antidepressant, treating all types of negative emotional conditions, states of anxiety, menopause, insomnia, improves elasticity and antiseptic effects	Flowers, Peel, Leaves, Fruit	Adenosine, Asparagine, Tyrosine, Valine, Isoleucine, Alanine, limonexic acid, Geraniol, Eugenol, Menthol, Cinnamic aldehyde, Catechin tannins, Gallic, Tannins, Flavonoids, Polyphenols, Sterols, Polyterpenes and Alkaloids
Geum urbanum	Treatment acute diarrhea, astringent agent for inflammations of the mucosa, gums and treatment of hemorrhoids	Roots, Rhizomes, Leaves	Vicianose, Catechin, Gallic acid, Caffeic acid, Chlorogenic acid, Ellagic acid and Phenylpropanoid
Datura stramonium	Treatment of asthma, sinus infections, burns, ulcers, rheumatism, falling hair, hand ruff, madness, epilepsy, depression and relief of headache, asthma and bronchitis	Stem, Leaves , Flowers, Bark	Alkaloids, Atropine, Hyoscyamine, Scopolamine, Ascorbic-acid and Allantoin
Olea europaea	Analgesic and antiasthmatic	Fruit, Oil, Leaves	Alkaloids, Tannins, Carbohydrates and Proteins
Taraxacum officinale	Treat digestive disorders, infections, bile and liver problems	Flowers, Root, Leaves	Flavonoids, Caffeic, Chlorogenic acid, Terpenoids, Triterpenes and Sesquiterpenes

Preparations of Extracts

Methanol was used to extract the plant samples because it is known to be powerful extraction solvent for antibacterial compounds from plants (Vu *et al*., 2016; Parekh *et al*., 2005). The various parts of the plants were dried under shade at room temperature and then cut into small pieces. About 100g of powdered plant material was macerated in methanol (Kamali *et al*., 2015). The extracted suspensions were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated to dryness using a rotary evaporator (Vu *et al*., 2016; Kamali *et al*., 2015; Khaleel *et al*., 2016). Extracts were then collected into a sterile goblet and were stored in the refrigerator at 4°C for future studies (Kamali *et al*., 2015; Nagat *et al*., 2016; Khaleel *et al*., 2016). Subsequently, all the plant extracts were screened for their antimicrobial activity.

Antibacterial Activity of Extracts

The effects of extracts on bacterial growth were measured *in vitro* by agar disc diffusion method (Essawi and Srour, 2000). 150µl of standardized bacterial suspension was spread over 20mm thick appropriate media with a swab. 20µl of the extracts (with different concentrations of 500, 250, 120, 60, 30 and 10 mg/ml DMSO) and 20µl of the DMSO were put on sterile paper disc and placed on the nutrient agar plates. Plates were incubated at 37°C for quality control strains and 27ºC for plant bacteria for 24h. The assays were performed in four repeats (Kamali *et al*., 2015).

Disc containing DMSO was used as control, which did not effect on the bacterial growth. The measures were done with a ruler in millimeter (mm) and the zones around the discs recorded as inhibition zone for extracts (Kamali *et al*., 2015; Khaleel *et al*., 2016), according to the previously described method of Kamali *et al*. (2015) with some modifications.

Chromatographic Analysis by GC-MS

The essential oil of *Geum urbanum* was analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) for identify and quantify the products composing it (Figure 1). The GC-MS analysis performed on an Agilent 7890A GC apparatus with a 5975 C mass spectrometer detector.

AHP5ms capillary column (30m* 4.6mm (id)* film thickness 0.25 µm) was used. The operating conditions were as follows: Carrier gas: helium, at a flow rate of 1ml/min; split ratio 1:10; injector temperatures: 250ºC; detector temperatures: 290ºC; sample size: 1µL of *Geum urbanum* essential oil, manual injection; oven temperature program: 50ºC as an initial temperature, 5min isothermal raised to 280ºC at a rate of 5ºC/min, then isothermal at 280ºC for 10min; ion source temperature: 230ºC; energy ionization: 70eV; electron ionization spectra with a mass scan range of 50e550. The compounds of *Geum urbanum* essential oil were identified by comparing their Retention Indices (RI) mass spectra with National Institute of Standards and Technology (NIST 08) library data compounds were expressed as percentages of the peak area to the total oil peak area.

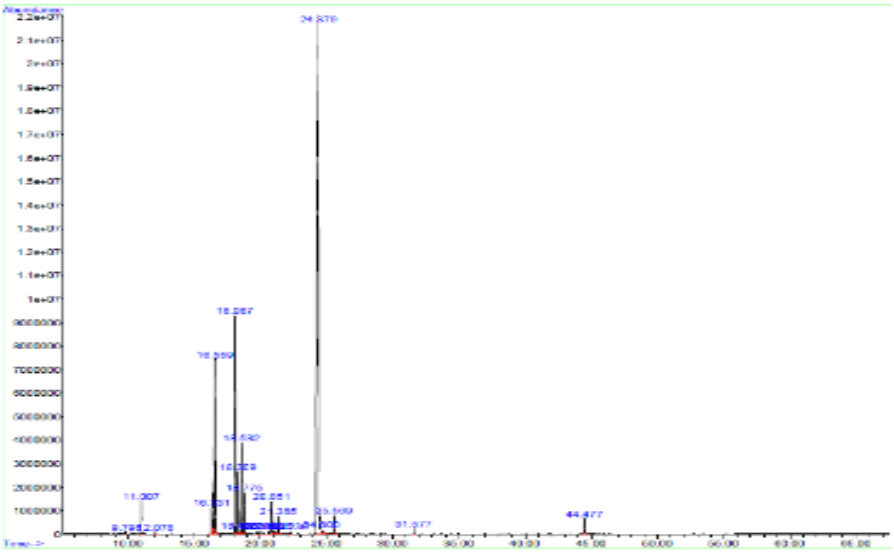

Figure 1: GC – MS chromatograph *Geum urbanum* roots

3. Results

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.

The methanol extract of 5 plants belonging to 5 families were tested against 3 Gram-positive and 7 Gram-negative bacteria using agar disc diffusion method.

Antibacterial Activity

In the present study, zones of growth inhibition around the discs measured with agar disc diffusion method. The antibacterial activity against each bacterium was observed to be varied.

The results revealed that *Geum urbanum* leaves methanolic extracts showed the maximum activity against all bacteria, including *Pseudomonas aeruginosa* (+++), followed by *Pseudomonas viridiflava*, *Bacillus subtilis*, *Rathayibacter toxicus*, *Xanthomonas campestris*, *Acidovorax avenae*, *Staphylococcus aureus*, *Pseudomonas syringae. syringae*, *Erwinia amylovora* and *Escherichia coli*, respectively. The maximum antibacterial activity of *Geum urbanum* root extract was observed against: *Pseudomonas aeruginosa* (+++), followed by *Escherichia coli*, *Pseudomonas viridiflava*, *Rathayibacter toxicus*, *Pseudomonas syringae. syringae*, *Bacillus subtilis*, *Acidovorax avenae*, *Xanthomonas campestris*, *Staphylococcus aureus* and *Erwinia amylovora.* The antibacterial activity of *Datura stramonium* plant leave extract showed the activity against *Pseudomonas viridiflava*, followed by *Rathayibacter toxicus* (+++), *Bacillus subtilis* (+++) and did not show any inhibitory activity against the other test pathogens. Stem extract of *Datura stramonium* showed activity against *Pseudomonas aeruginosa* (+++), *Rathayibacter toxicus* (+++). The *Citrus aurantium aurantium* (blossoms) extract showed the activity against *Pseudomonas viridiflava*, *Xanthomonas campestris* and *Staphylococcus aureus*. The *Olea europaea* (Leaf) showed moderate growth inhibition effects against *Staphylococcus aureus.* Leaves and roots extract of *Taraxacum officinale* did not show any antibacterial activity against the tested pathogens.

The highest antibacterial activity against the Gram-positive species was in the extract of *Geum urbanum* leave against *Bacillus subtilis* (+++). The most active extract against Gram-negative bacteria was extract of *Geum urbanum* for *Pseudomonas aeruginosa* (+++).

GC-MS analysis plays a key role in the analysis of components of plant origin. Among the five plants, *Geum urbanum* showed the significant antibacterial activity. Consequently, root (Figure 1) and leave (Figure 2) of *Geum urbanum* were subjected to GC-MS analysis.

The results of the analysis by GC-MS of the chemical composition of the *Geum urbanum* are presented in Table 2. The major constituents of the oil were Phytol (20.6%), Decane (11.1%), Limonene (10.5%), Beta-bisabolene (10%), Dodecane (9.9%), Bicyclo [3.1.1] hept-2-ene (4.9%) and Tetradecane (CAS) (4.6%). The root major phytoconstituents were Eugenol (57.5%), Benzeneacetonitrile (CAS) (13.1%), 6, 6-dimethyl (11.5%), Myrtenal (4.8%) and Benzeneacetonitrile (CAS) (2.6%).

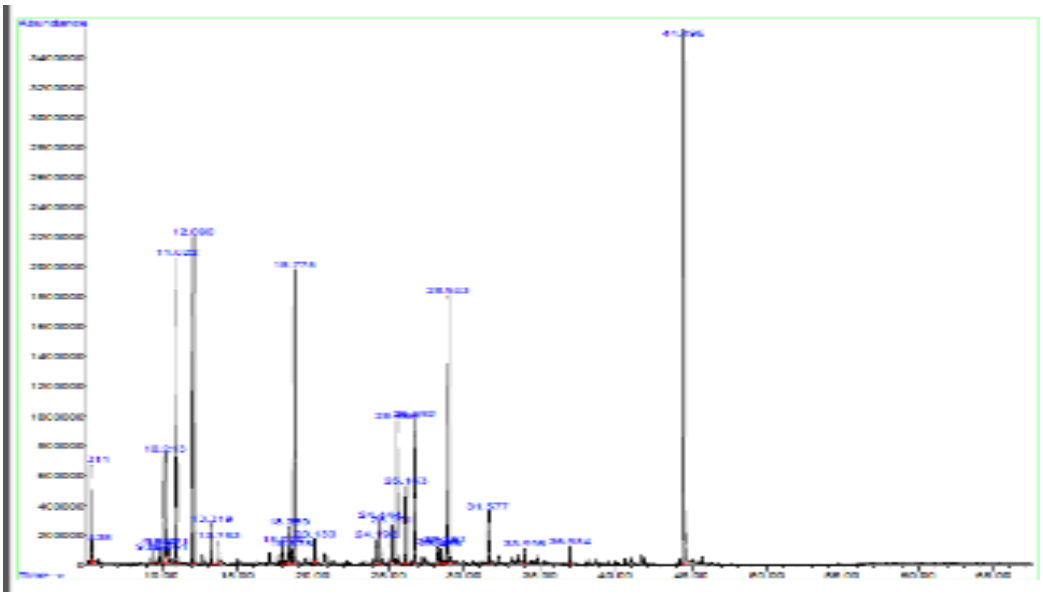

Figure 2. GC – MS chromatograph of *Geum urbanum* leaves

Table 2. The quantitation of antimicrobial activity for plant extracts measured by the agar disc diffusion method. The effectiveness of extracts is demonstrated by the size of the microorganism growth inhibition zone around the filter paper disc

Plant	**Microorganism**									
	B. subtilis	*S. aureus*	*R. toxicus*	*E. coli*	*P. Syringae syringae*	*P. aeruginosa*	*P. viridiflava*	*E. amylovora*	*A. avenae*	*X. campestris*
***Geum urbanum* (leave)**	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Geum urbanum (root)	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
***Citrus aurantium* (*blossoms*)**	-	+++	+	+	+	++	+++	-	+	+++
Datura stramonium (leave)	+++	-	+++	-	+	+	+++	+	++	++
Datura stramonium (stem)	-	-	+++	-	-	+++	-	+	-	-
***Olea europaea* (leave)**	-	+	-	-	-	-	-	+	+	-
***Taraxacum officinale* (leave and root)**	-	-	-	-	-	-	-	-	-	-

a) Diameter of the inhibition zone: no inhibition (-), 8-9.5 mm (+), 10-12 mm (++), > 12 mm (+++).

4. Discussion

Medicinal plants are widely used for the treatment of different plant diseases and hospital infections caused by bacteria. In this paper, the antibacterial activities of extracts from plants used by tribals in Iranian folklore medicine were reported. The results revealed that *Geum urbanum* methanolic extracts showed the maximum activity against the tested bacterial pathogens that the antibacterial activity of *Geum urbanum* may be due to presence of eugenol, phenolic acids and tannins (Kuczerenko *et al*., 2011).

The *Datura stramonium* plant leave extract showed the antibacterial activity against gram positive bacteria *Bacillus subtilis*, *Rathayibacter toxicus* and Gram-negative bacteria *Pseudomonas viridiflava* and little or no antimicrobial activity has against *Escherichia coli* and *Pseudomonas aeroginosa*, our findings are in agreement with the study of Sony *et al*. (2012).

The *Citrus aurantium* (*blossoms*) plant extract showed the activity only against *Pseudomonas aeruginosa.* our findings are in according with Ammar *et al*. (2012). The *Olea europaea* (Leaf) showed moderate growth inhibition effects (9.8–10.4 mm) against *Staphylococcus aureus.* It is in accordance with Sudjana *et al*. (2009). *Taraxacum officinale* (leaves and roots) extracts did not show any antibacterial activity against the tested pathogens but Iqbal *et al*. (2014) reported that the extract of *Taraxacum officinale* was found to be effective against the tested bacterial pathogens *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus, Bacillus Subtilis.* In the present study, we reported a high resolution GC-MS method for the evaluation of the chemical constituents *Geum urbanum* plant. The major bioactive compounds in *Geum urbanum* are eugenol, phenolic acids and tannins as mentioned by Kuczerenko *et al*. (2011).

The results obtained here in further encourage the use of *Geum urbanum* extract in antibacterial formulations due to the fact that *Geum urbanum* extract effectively kills pathogenic bacteria related to plant diseases and hospital infections (Vu *et al*., 2016).

5. Conclusions

All the plant species evaluated in the present study are currently used traditionally for the treatment of various diseases (Table 1) and showed moderate to high antibacterial activity against the bacteria tested. The antibacterial activity of *Geum urbanum* was highly significant for *Pseudomonas aeruginosa* and *Bacillus subtilis*. Finally, the results of the present study clearly elucidate the antibacterial activities of these plants and provide an evidence to support their use in folk medicine.

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Authors' Contributions

Gh. K, T. T and S. Gh designed the experiment and revised the manuscript with co-author. A. G. conducted the experimental work, wrote the article and corrected it.

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