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Original Article

Evaluation of Antibacterial Activities of Some Medicinal Plants, Traditionally Used in Iran

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Abstract

The aim of this study was to assess the antibacterial activities of some medicinal plants extracts traditionally used in Iran. Hydroalcoholic extracts obtained from different parts of five plants including *Rosmarinus officinalis* L. (rosemary), *Syzygium aromaticum* L. (Clove)., *Arctium lappa* L. (Burdock) , *Coriandrum sativum*, *Myrtus communis* with traditional medicinal use were examined for their antibacterial activities against some gram-negative strains including *Pseudomonas aeruginosa*, *Salmonella typhi*, Proteus mirabilis, Klebsiella oxytoca and Shigella dysentriae. The disc diffusion method was applied to screen the antibacterial efficacy of the extracts. Gentamicin was used as control. This study showed that the extracts obtained from *Syzygium aromaticum* L., *Arctium lappa* L. and *Myrtus communis* had antibacterial activity against Proteus mirabilis. In addition, only the *Myrtus communis* extract had some inhibiting effect on the growth of *Pseudomonas aeruginosa*. The result of the current study revealed that some of the studied plants could be considered as potential source of antimicrobial agents and supports the traditional applications of a number of the tested plants as antibacterial reagents.

Keywords: Antibacterial agent; *Arctium lappa* L.; *Coriandrum sativum*; *Myrtus communis*; *Rosmarinus officinalis* L.; *Syzygium aromaticum* L..
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1. Introduction

Even today, herbal remedies are widely practiced in Iran and many developing countries [1]. Using herbal remedies for treatment of diseases goes back to thousands of years in Iran, and there are several scientific documents in this era. For example, Rhazes and Avicenna were using herbal medicines for treatment of patients and wrote many books on this topic [2, 3]. However, treatment of infections has been remarkably effective since the discovery of antibacterial medicines, appearance of resistant pathogens as well as adverse side effects of certain antibiotics [4-6] have led to the search for new antibacterial agents, specially from medicinal plants [7-

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Table 1. Name (scientific and local), part used and traditional applications of the tested medicinal plants in Iran

Scientific name	Local name	Part used	Traditional applications
Rosmarinus officinalis L.	Rosmary	Aerial part with flowers	Diuretic, Anti-inflammatory, topical analgesic, Digestive, Anti-infection
Syzygium aromaticum L.	Mikhak	Flowers	Digestive, Sedative, Anti-nausea, Digestive, mouth wash, Anti-infection
Arctium lappa L.	Baba adam	Roots	Anti-inflammatory , topical analgesic, Anti-infection
Coriandrum sativum L.	Geshniz	Seeds	Digestive, Anti-diarrhea, mouth wash, Anti-infection
Myrtus communis L.	Moord or Moort	Leaves	Anti-diarrhea, Hair-tonic

10]. This study was performed to screen the antibacterial activities of some medicinal plants, traditionally used in Iran. *Rosmarinus officinalis* L. (Laminaceae), *Syzygium aromaticum* L. (Myrtaceae), *Arctium lappa* L. (Asteraceae), *Coriandrum sativum* L. (Apiaceae), *Myrtus communis* L. (Myrtaceae) were tested against common Gram-negative bacteria. The scientific and local names of the tested plants, used parts and their traditional indications in Iran are shown in Table 1.

2. Methods and materials

2.1. Plant materials

A. lappa roots were collected from Hamedan Medicinal plant garden and dried at room temperature. For the rest of tested medicinal plants (Table 1), dried samples were purchased from a local store in Hamadan.

2.2. Preparation of the extracts

The dried and grounded plant parts, as mentioned in Table 1, were extracted with ethanol-water 80% (v/v), by maceration for 4 h at room temperature. The ratio of the solvent to dried samples was 10:1 [11]. The extracts were filtered and concentrated to 5% of volume at 40 °C using a rotary evaporator. The residues were transferred to small vials and kept at 4 °C prior to use.

2.3. Bacterial cultures

Bacterial cultures of gram-negative species *Pseudomonas aeruginosa*, *Salmonella typhi*, Proteus mirabilis, Klebsiella oxytoca and Shigella dysentriae, were used to evaluate the antimicrobial properties of the selected extracts. The bacterial strains were obtained from Hamedan University of Medical Sciences and sub-cultured in nutrient agar. The plates were incubated for 24 h at 37 °C. A single colony from the plates was transferred into 4 ml fluid of Luria Bertuni medium and incubated overnight at 37 °C and 200 rpm in a shaking incubator. The cells were harvested by centrifugation at 4 °C and washed twice and resuspended in Ringer solution to provide the turbidity of the 0.5 McFarland standards for disc diffusion method [12].

2.4. Antibacterial assays

The antimicrobial activity of the tested extracts was monitored using paper disc diffusion method that is a highly recommended for routine assessment of preliminary antimicrobial screening. This was performed by standard NCCLS methodology, using nutrient agar plates, inoculated with a 0.5 McFarland standard of the selected bacteria [12]. The filter paper discs of about 6 mm in diameter were cut by punching machine from Whatman No. 1 filter paper. The discs were autoclaved at 121 °C and impregnated by 3, 6, 9, 12 and 15 µl of the extracts, respectively, and placed on the nutrient agar plates. After 24 h incubation at 37 °C, inhibition zone diameters were read

Table 2. Antibacterial activity screening of the tested medicinal plants extracts, traditionally used in Iran as inhibition zone diameter (mm) and standard deviation.

Scientific name	Volume of the extracts impregnated to discs	Mean diameter of inhibition zone (mm) *Pseudomonas aeruginosa*	*Salmonella typhi*	Proteus mirabilis	Klebsiella oxytoca	Shigella dysentriae
Gentamicin sulfate	(10 µg/disc)	18±1	15±0.2	12.7±0.6	10±0.2	15±0.7
Rosmarinus officinalis L.	3 µl	0	0	0	0	0
	6 µl	0	0	0	0	0
	9 µl	0	0	0	0	0
	12 µl	0	0	0	0	0
	15 µl	0	0	0	0	0
Syzygium L. *aromaticum*	3 µl	0	0	11±0.1	0	0
	6 µl	0	0	16.7±0.5	0	0
	9 µl	0	0	17.3±0.4	0	0
	12 µl	0	0	18.3±0.4	0	0
	15 µl	0	0	19±1	0	0
Arctium lappa L.	3 µl	0	0	10±0.5	0	0
	6 µl	0	0	13±1	0	0
	9 µl	0	0	13±0.4	0	0
	12 µl	0	0	13±0	0	0
	15 µl	0	0	15±0.8	0	0
Coriandrum sativum L.	3 µl	0	0	0	0	0
	6 µl	0	0	0	0	0
	9 µl	0	0	0	0	0
	12 µl	0	0	0	0	0
	15 µl	0	0	0	0	0
Myrtus communis L.	3 µl	0	0	14.3±0.1	0	0
	6 µl	0	0	16±0	0	0
	9 µl	0	0	17.7±0.2	0	0
	12 µl	5.3±0.4	0	19.3±0.1	0	0
	15 µl	8±0.1	0	20±0.5	0	0

with callipers and the bacteriostatic properties of the active extracts against the bacteria was evaluated. Gentamicin sulphate (Alborz Daru, Iran), 10 µg/disc, and ethanol (80%) were used as positive and negative controls, respectively. The test was repeated three times for each extract.

3. Results

The results for antibacterial activity screening of the selected plant extracts are listed in Table 2. Among five plants examined, *A. lappa*, *M. communis* and *S. aromaticum* showed antibacterial activity against *P. mirabilis* and almost no effect on the other selected bacteria. At the highest concentration examined, *M. communis* extract revealed some antibacterial activity against *P. aeruginosa*. In the present study, *R. officinalis* and *C. sativum* did not show any antibacterial activity against the selected strains. The negative control, ethanol 80%, did not show any inhibition effects and its mean diameter was considered zero (not shown in the Table 2).

4. Discussion

The present study was carried on to determine the *in vitro* antimicrobial activity of some medicinal plants used by Iranian people to evaluate the scientific base of their application. Although the plants differed slightly in their activity against tested micro-organisms, three evaluated extracts; *S. aromaticum*, *A. lappa* and *M. communis* were

active against *P. mirabilis*. This was in good agreement with results obtained by Saeed and Tariq (2008) for clove [13]. They showed the aqueous infusion and decoction and also essential oil of *S. aromaticum* were active against *P. mirabilis* and had no effect on *S. typhi*. However, they reported antibacterial effect against *P. aeruginosa* for the all three extracts of clove. It could be due to susceptibility of the bacterial strains or different extraction method they applied.

Although some antibacterial activities have been reported for the extracts obtained from burdock [14, 15], to the best of our knowledge this is the first report describing the antibacterial effect of burdock root extract on *P. mirabilis*. It has been reported that, the crude preparation of *M. communis* had antibacterial effect on *P. mirabilis* [16].

P. mirabilis causes serious diseases in human including nosocomial infections. In addition, *P. mirabilis* is the most frequent cause of infection-related kidney stones [17]. It is shown that Rheumatoid arthritis is linked to *P. mirabilis* infection [18]. Due to the importance of *P. mirabilis* in such diseases, plants like *S. aromaticum*, *A. lappa* and *M. communis*, which show high activity against *P. mirabilis*, are of great importance.

In this study, *P. aeruginosa* was only susceptible to *M. communis* extract. Similarly, Hashemi *et al*. (2011) revealed that the methanolic extract of *M. communis* was active against *P. aeruginosa* [19]. In another study conducted by Rasooli *et al*. (2002), the essential oil of *M. communis* did not show any inhibiting effect on the growth of *P. aeruginosa* [20]. It could be concluded that, the active ingredients in the ethanolic extract of *M. communis* (and not in the essential oils) are responsible for the anti *P. aeruginosa* effect. However, in another study conducted by Owlia *et al*. (2009) [21], the essential oil of *M. communis* was significantly active against *P. aeruginosa*. In addition, it has been reported that, the existence of α-pinene in the essential oil of *M. communis* is responsible for the anti *P. aeruginosa* activity of this medicinal plant [21].

Although in the current study burdock extract showed no anti *P. aeruginosa* effects, Holetz *et al*. (2002) reported some degree of activity against the bacterium [15]. The difference could be due to the different maceration processes used. They employed 90% ethanol for 48 h, while in the current research 80% ethanol was utilized for only 4 h. It could be concluded that, more maceration time with higher concentration of ethanol would extract more anti *P. aeruginosa* chemical constituents from the burdock.

Infection with *P. aeruginosa* is a serious problem in hospitalized patients with burns, cancer and cystic fibrosis. Due to multiple antibiotic resistances, antibiotic susceptibility testing of clinical isolates is mandatory [17, 22]. Since *P. aeruginosa* infection is one of the most life threatening conditions, there is a need to identify novel substances active towards this pathogen. This means that the current study together with previous investigations support the antibacterial properties of *M. communis* against *P. aeruginosa*.

Although the ethanolic extract of *R. officinalis* did not show any antibacterial activity in the current study, its essential oil showed some anti *S. typhi* activity in a research conducted by Bozin *et al*. (2007) [23]. In addition, the essential oils obtained from rosemary had anti *P. aeruginosa* effects [24, 25].

Although this study did not show any antibacterial effects for the ethanolic extract of *C. sativum* on the tested bacteria, the essential oil, aqueous infusions and aqueous decoctions obtained from *C. sativum*, had some antibacterial activities [26, 27].

5. Conclusion

The preliminary study on the selected medicinal plants supports some of the

traditional claims of effective anti-infective and could initiate further study that may eventually facilitate the use of these medicinal plants as antimicrobial agents in developing countries. However, additional studies will also be needed for further pharmacological, toxicological and clinical evaluation of these traditional medicinal plants.

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