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Evaluation of Antibacterial Activities of Some Medicinal Plants from North-West Iran

[1, 2, 3]Farzaneh Lotfipour, [1, 2] Hossein Nazemiyeh, [1] Fatemeh Fathi-Azad, [1]Neyereh Garaei, [1]Sanam Arami, [1]Sima Talat, [1]Fatemeh Sadegpour, [1]Rana Hasanpour

Abstract

Objective(s)
Aim of the present study was to screen the antibacterial activities of some medicinal plants extracts traditionally used in Azarbaijan area (Iran).
Materials and Methods
Thirty-six extracts obtained from different parts of ten plants including *Tanacetum balsamita* L. (Copmositae), *Muscari caucasicum* Baker (Hyacinthaceae), *Equisetum arvense* L. (Equisetaceae), *Achillea millefollum* L. (Copmositae), *Stachys fruticulosa* M. Bieb. (Labiatae), *Stachys schtschegleevii* Sons. ex, Grossh. (Labiatae), *Salvia sahendica* Boiss & Buhse (Labiatae), *Phlomis caucasica* Rech. f. (Labiatae), *Etchium italicum* L. (Boraginaceae) and *Thalictrum minus* L. (Ranunculaceae) from north-west Iran with traditional medicinal use were examined for their antibacterial activities against some Gram-negative strains such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella paratyphi* and *Serratia marcescens*, also, Gram-positive strains of *Staphylococcus aureus, Micrococcus luteus, Staph. epidermidis, Streptococcus pneumoniae* and *Bacillus cereus*. The filter paper disc diffusion method as well as broth serial dilution technique were applied to screen the antibacterial efficacy of the extracts and determination of minimum inhibitory values.
Results
Results indicated that the majority of tested plant extracts had antibacterial activity at least against one of the selected bacteria, with the exception of *Muscari caucasicum*. Methanol extract of the aerial part of *Thalictrum minus* L. (Ranunculaceae) showed the most potent antibacterial activity against *Staph. aureus* with MIC value of 0.3125 mg/ml.
Conclusion
The results of this study show that most of the studied plants are potentially a good source of antimicrobial agents and support the traditional applications of some of the tested plants.

Keywords: Antibacterial activity, Disc diffusion, Iranian medicinal plants

1- Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
*Corresponding author: Tel: +98 -411- 3392580; Fax: +98 -411- 3344798; email: farzaneh.lotfipour@gmail.com
2- Research Center for Pharmaceutical Nanotechnology, Tabriz University of Medical Sciences, Tabriz, Iran
3- Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Introduction

Evidence of use of herbal remedies in Iran goes back to the history itself; also, there are lots of scientific documents in this area. For example Rhazes (860-930), a Persian physician, who adopted treatment based on herbs and foods, and avoided synthesized medicine, except for necessity. Ibn Sina (Avicenna, 980–1037) wrote many books on a wide range of topics but he is perhaps most famous for his 'Laws of Medicine' which contains sections on the formulation of medicines, general medicine and others that discuss the herbal medicines in details (1-3).

Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (4). However, treatment of infections has been remarkably effective since the discovery of antibacterial drugs, appearance of some resistant pathogens as well as undesirable side effects of certain antibiotics (5-9) have led to the search for new antibacterial agents, in particular from medicinal plants (10-13). The present study was to screen the antibacterial activities of some local medicinal plants extracts traditionally used in Azarbaijan area (Iran); *Tanacetum balsamita* L. (Copmositae), *Muscari caucasicum* Baker (Hyacinthaceae), *Equisetum arvense* L. (Equisetaceae), *Achillea millefollum* L. (Copmositae), *Stachys fruticulosa* M. Bieb. (Labiatae), *Stachys schtschegleevii* Sons. ex Grossh (Labiatae), *Salvia sahendica* Boiss & Buhse (Labiatae), *Phlomis caucasica* Rech. (Labiatae), *Etchium italicum* L. (Boraginaceae) and *Thalictrum minus* L. (Ranunculaceae) against common Gram-negative and positive bacteria. The scientific and local names of the tested plants, parts used and their traditional applications are listed in Table 1.

Table1. Name (scientific and local), family, part used and traditional indications of some medicinal plants from North-west of Iran.

Family	Scientific name	Local name	Part used	Traditional indications
Copmositae	*Tanacetum balsamita* L.	Gia sevze	Leaves	Digestive, diuresis, antitusive, analgesic/anti-inflammatory
Equisetaceae	*Equisetum arvense* L.	Dom asb (Horse tail)	Whole plant	Diuresis, osteoporosis
Compositae	*Achillea millefollum* L.	Bomadaran	Flowers	Anti-inflammatory, anti-infection
Labiatae	*Stachys fruticulosa* M. Bieb.	Sonbole gachdust	Aerial parts	Anti-inflammatory
Labiatae	*Stachys schtschegleevii* Sons. ex Grossh.	Pulk	Aerial parts	Anti-inflammatory
Labiatae	*Salvia sahendica* Boiss. & Buhse	Maryam goli sahandi	Aerial parts	Anti-inflammatory, anti-infection
Labiatae	*Phlomis caucasica* Rech.f.	Gush barreh gafgazi	Aerial parts	Analgesic, anti-infection, digestive
Boraginaceae	*Echium italicum* L.	Have chuve	Root and aerial parts	Anti-infection
Ranunculaceae	*Thalictrum minus*. L.	Ghare-gheytarma	Root and aerial parts	Anti-infection
Hyacinthaceae	*Muscari caucasicum* Baker	Khazih	Aerial parts	Anti cough

Materials and Methods

Plant materials

The different parts of plant samples (Table 1) collected and dried at room temperature. The specimens were identified by Dr. H. Nazemiyeh, Mr. Ghahreman and Mrs Eslampanah (Department of Pharmacognosy, Faculty of Pharmacy, Tabriz University of Medical Sciences) and a voucher specimen was kept at the Herbarium of Faculty of Pharmacy, Tabriz University of Medical Sciences.

Preparation of the extracts

The dried and grounded plant parts were extracted with different solvents (*n*-hexane, dichloromethane, methanol, chloroform) by maceration for 3 days at room temperature. The extracts combined, filtered and concentrated under reduced pressure at 45 °C till dryness. The residues transferred to small vial and kept at 4 °C before use.

Bacterial cultures

Bacterial cultures of Gram-negative species *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Salmonella paratyphi* (ATCC 4420) and *Serratia marcescens* (ATCC 33077) as well as Gram-positive strains namely *Staphylococcus aureus* (ATCC 6538), *Micrococcus luteus* (ATCC 10240), *Staphylococcus epidermidis* (ATCC 12228), *Streptococcus pneumoniae* (ATCC 12401) *and Bacillus cereus* (ATCC 9372) were used to evaluate the antimicrobial properties of the selected extracts. The bacterial strains obtained in lyophilized form (purchased from Institute of pasture, Iran) which were cultured in Luria Bertuni agar medium (Scharlau Spain) after suspending them in sterile distilled water. The plates incubated for 24 hrs at 37 °C. Single colony from the plates was transferred into 4 ml fluid of LB medium and incubated over night at 37 °C and 200 rpm in a shaking incubator. The cells harvested by centrifugation at 3000 rpm (Behdad, Iran) for 15 min and at 4 ˚C. Subsequently, they were washed twice and re-suspended in Ringer solution to provide the turbidity of the 0.5 McFarland standards for disc diffusion method or the concentration range of 10^5-10^6 CFU/ml for broth dilution method (14).

Antibacterial assays

The antimicrobial activity of the tested extracts was monitored using paper disc diffusion method that is a highly recommended method for routine assessment of preliminary antimicrobial screening. This was performed by standard NCCLS methodology, using Mueller- Hinton plates, inoculated with a 0.5 McFarland standard of the selected bacteria. The filter paper discs were impregnated by the extracts (10 µl) and placed on the agar (14). After 48 hrs incubation at 37 °C, inhibition zone diameters read with calipers. The bacteriostatic properties of the active extracts against the most susceptible bacteria namely *Serra. marcescens, Staph. aureus, M. luteus, Staph. epidermidis* and *B. cereus* determined by an evaluation of the Minimum Inhibitory Concentration (MIC). The extracts mixed with Fluid Casein Digest Soya Lecithin Medium (twin pack, Himedia, India) in decreasing concentrations; the tubes inoculated with a 1 ml of inocolum of the tested bacteria (final concentration of 10^5-10^6 CFU/ml). After 24 hrs of incubation at 37 °C, the tubes were screened for any evidence of bacterial growth. MIC was defined as the lowest concentration of plant extract that completely suppressed the bacterial growth (15). Tubes of DMSO (10%), as solvent for preliminary dissolving or at least homogenization of the extracts and gentamycin sulfate, as positive control, also included.

Results

The results for antibacterial activity screening of the selected extracts are shown in Table 2. Among ten plants examined, all of them showed antibacterial effect at least against one of the selected bacterial strains, except *Muscari caucasicum* that showed no activity against any of the tested strains. *Staph. aureus* with 45.5%, *Staph. epidermidis* with 36.5% and *M. luteus* with 33.5% susceptibility were the top three susceptible strains. The most potent effect, related to methanol extract of *Thalictrum minus* that inhibits *Staph. aureus* with inhibition zone diameter of 18.5 mm. MIC values for the active extracts are indicated in Table 3. As shown in Table 3, methanol extract of *Thalictrum minus* with the MIC value of 0.3125 mg/ml against *Staph. aureus* was the most active extracts and the most susceptible bacterium respectively, which confirmed the results of disc diffusion method.

Table 2. Antibacterial activity screening of the selected extracts of the tested medicinal plants from North-west of Iran as inhibition zone diameter (mm).

Scientific name	Part of plant	Extracts	Inhibition zone diameter (mm)								
			A	B	C	D	E	F	G	H	I
Tanacetum balsamita L.	Leaves	Cyclohexane	-	-	-	-	-	10	12	-	14
		Dichloromethane	-	-	-	13	9	11	-	-	-
		Methanol	-	-	-	12	-	-	11	-	-
Muscari caucasicum Baker	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	-	-	-	-	-	-
Equisetum arvense L.	Whole plant	Aqueous	-	-	-	-	-	-	-	-	-
		Ethyl acetate	-	-	-	11	9	-	10	-	-
		Chloroform	-	-	-	-	9	-	-	-	9
Achillea millefollum L.	Flower	Aqueous	-	-	-	-	-	-	-	-	-
		Ethyl acetate	-	-	-	-	9	10	12	-	-
		Chloroform	-	-	-	9	15	-	11	-	-
Stachys fruticulosa M. Bieb.	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	-	12	-	-	-	9
Stachys schtschegleevii Sons. ex Grossh.	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	8	13	11	9	-	9
Salvia sahendica Boiss. & Buhse	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	12	14	10	11	-	10
Phlomis caucasica Rech. f.	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	10	13	-	-	-	9
Etchium italicum L.	Root	Cyclohexane	-	-	12	11	11	14	12.5	-	11
		Dichloromethane	-	-	-	-	8	7	9	-	9
		Methanol	-	-	-	-	-	-	7	-	7
	Aerial parts	Cyclohexane	-	-	-	-	-	-	-	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	7	-	-	7	-	10
	Root	Cyclohexane	-	-	-	-	-	-	15.5	-	-
		Dichloromethane	-	-	-	-	-	-	-	-	-
		Methanol	-	-	-	-	-	-	9.5	-	-
Talictrum minus L.	Aerial parts	Cyclohexane	-	-	-	-	-	-	16.5	-	-
		Dichloromethane	-	-	-	-	-	17.5	-	-	-
		Methanol	-	-	10.5	-	18.5	-	7	-	9

A: *E. coli,* B: *P. aeruginosa,* C: *Sal. paratyphi,* D: *Serra. marcescens*, E: *Staph. aureus,* F: *M. luteus,* G: *Staph. epidermidis,* H: *Strep. pneumonia,* I: *B. cereus.*

Table 3. Minimum inhibitory concentration (MIC) of the most active plants extracts and antibiotic (Gentamicin sulfate) against some of the bacteria.

Scientific name	Extract	MIC value (mg/ml) against:				
		Serra. marcescens	*Staph. aureus*	*M. luteus*	*Staph. epidermidis*	*B. cereus*
Tanacetum balsamita L.	Cyclohexane	-	-	2.5	1.25	1.25
	Dichloromethane	1.25	2.5	-	-	-
	Methanol	1.25	-	2.5	1.25	-
Equisetum arvense L.	Dichloromethane	2.5	2.5	-	2.5	-
	Methanol	-	2.5	-	-	2.5
Achillea millefollum L.	Dichloromethane	-	2.5	2.5	2.5	-
	Methanol	2.5	0.625	-	2.5	-
Stachys fruticulosa M. Bieb.	Methanol	-	2.5	-	-	2.5
Stachys schtschegleevii Sons. ex Grossh.	Methanol	2.5	1.25	2.5	2.5	2.5
Salvia sahendica Boiss. & Buhse	Methanol	2.5	1.25	2.5	2.5	2.5
Phlomis caucasica Rech. f.	Methanol	2.5	1.25	-	-	2.5
Etchium italicum L. (root)	Cyclohexane	2.5	2.5	1.25	1.25	2.5
	Dichloromethane	-	2.5	2.5	2.5	2.5
Thalictrum minus L. (root)	Cyclohexane	-	-	-	0.25	-
Thalictrum minus L. (aerial parts)	Cyclohexane	-	-	-	0.625	-
	Dichloromethane	-	-	0.625	-	-
	Methanol	2.5	0.3125	-	-	2.5
Gentamicin sulfate		0.8	1.2	0.2	1.2	1.2

Discussion

The present study was conducted to investigate the *in vitro* antimicrobial activity of some local medicinal plants used by people of North-west of Iran to evaluate the scientific base of their application. However, the plants differ significantly in their activity against test microorganisms, nearly all of the extracts evaluated, with the exception of *Muscari aucasicum*, were active against at least one of the Gram-positive strains. The most susceptible bacteria group was the Gram positive strains, among them *Staph. aureus* and *Staph. epidermidis* that cause serious infections in human and other animals including superficial skin lesion, localized abscesses, and food poisoning (16) were in the first positions. Due to the importance of *Staph. aureus* in the above mentioned conditions, plants such as *Thalictrum minus* that showed high activity against *Staph. aureus* is of great importance.

Gram-negative strains except *Serra. marcescens* almost showed no susceptibility, this finding is in good agreement with results obtained by several researches. Duarte and co-workers (2005), Skaltsa *et al* (1999, 2003) as well as Khanavi and co-workers that evaluated the antimicrobial effect of methanol and essential oils of different strains of the Stachys genus showed similar results (17-20). This could be due to several possible reasons; one is the presence of a double membrane surrounding each bacterial cells. This outer membrane excludes certain drugs and antibiotics from penetrating the cell, partially accounting for why Gram-negative bacteria are generally more resistant to antibiotics than other Gram-positive bacteria (21-22).

Three of the least susceptible bacteria were *E. coli*, *Strep. pneumonia* and *P. aeruginosa*. The latter is one of the most commonly-isolated nosocomial pathogen accounting for a significant percentage of hospital-acquired infections. Due to multi-resistance feature of *P. aeruginosa*, finding an effective antimicrobial agent against this microorganism is a difficult task, resulting in the increasing trend of nosocomial infections in hospitals and health care centers (23).

The most potent antibacterial effect related to methanol extract of *Thalictrum minus* that inhibit *Staph. aureus* with inhibition zone diameter of 18.5 mm. MIC values which were determined only for the active extracts, are indicated in Table 3. Methanol extract of *Thalictrum minus* with the MIC value of 0.3125 mg/ml against *Staph. aureus* was the most active extract and the most susceptible bacterium respectively, which confirm the results of disc diffusion method.

Although the nature and number of active antibacterial components involved in each extract are not clear, but the broad spectra of activity of several plants extracts such as *Thalictrum minus, Salvia sahendica, Achillea millefollum* and *Etchium italicum,* especially methanol extracts, however, are promising and the isolation of active constituents of each extract can be the subject of next researches.

Conclusion

In conclusion, some of the people from North-west of Iran employ medicinal plants for their health problems like inflammation, infection, and urinary tract disorders. The results of this study have shown that most of the studied plants are potentially a good source of antimicrobial agents and support the traditional medicinal application of some of the tested plants.

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