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Original Article

Collection and Identification of Some Selected Medicinal Plants with Antimicrobial Properties from Takhte – Sartashtak Region, Kerman, Iran

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ABSTRACT

Methanolic extracts of twenty selected medicinal plant species collected from the Takhte-e-Sartashtak region, that utilize to prevent and cure different kinds of diseases in traditional medicine, have been examined for antimicrobial activity against some pathogenic microorganisms. These twenty plant species belong to eleven families including, Apiaceae, Asteraceae, Berberidaceae, Cupressaceae, Datiscaceae, Euphorbiaceae, Lamiaceae, Papaveraceae, Rhamnaceae, Rosaceae, and Thymelaceae. They were tested against five microorganisms including two gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* two gram-negative bacteria, *Escherichia coli*, and *Pseudomonas aeruginosa* and one fungus, *Candida albicans* with the method of disk diffusion and determination of growth inhibition zone were tested. Most of the plants showed antimicrobial activity. Results observed methanolic extracts of all of these plant species (except one) were active against at least one or more microorganisms. Only one plant species (*Physospermum cornubiense* (L.) DC., (Apiaceae)) was not active against none of the microorganisms. *Sanguisorba minor* Scop. (Rosaceae) was active against all five microorganisms. Most antimicrobial activities belong to *Ferulago angulata* (Schltdi.) Boiss. (Apiaceae) against *Candida albicans*, also *Glaucium grandiflorum* Boiss. & Huet (Papaveraceae) and *Datisca cannabina* L. (Datiscaceae) against *S. aureus*. Only one taxon, *Sanguisorba minor* Scop. (Rosaceae), was the only active species against *Escherchia coli*. The MIC, MBC, and MFC of plant extracts were approximately different.

INTRODUCTION

In the past, plants have always been important. One of these cases is the importance of medicinal plants as an important natural source for the production of different kinds of drugs against many pathogenic agents for the treatment of various diseases [1]. Humans have been using plants as medicine for thousands of years. Plants' usage to treat and recover diseases has traditionally been common. The use of plants as harmless and effective drugs was common for many years ago in the world and the tendency to use these herbs to fight microbial diseases is increasing [2,3]. Traditional medicine has a special place nowadays in several sections of the world. On the other hand, many microbes are resistant to existing chemical drugs. Microbial resistance to antibiotics today is a medical science problem [4], consequently, there is always a need to develop new and effective drugs [3,5]. Due to the multiple side effects of chemical drugs and the resistance of microbes to these drugs, many biological scientists are now thinking of extracting active pharmaceutical compounds from plants [6]. There is a continuous attempt to discover a method to make new and effective drugs against various pathogens by scientists. The wide range of plants is one of the natural, inexpensive, and clean sources of drugs. About 250,000 high plants are discovered in the world [7], and most of them are unknown for their eventual remedial properties [1,8]. A large number of plants have not been studied for this purpose [9]. Due to a variety of parameters such as different parts of the plant, seasons, variety, weather conditions, and different geographical regions affect the phytochemical compounds of plants and also their medicinal effects, it is recommended to study

the chemical composition and therapeutic properties of similar plants in different geographical areas [10, 11]. Iran has rich and diverse vegetation [12] mostly unknown for the discovery of their bioactivity. However, valuable ethnobotanical studies have been done on the local and usual plant utilization in Iran [13]. In this investigation, a great number of wild plants of the mountainous region of Takht-e-Sartashtak have been collected and identified. The antimicrobial activity of twenty selected medicinal species of them has been examined. The twenty plants were selected with regard to the medicinal use of them that was mentioned in different sources and also based on ethnobotanical knowledge of the native people of the region gathered from different places, about the traditional medicinal use of them. In fact, we tested traditional medicinal plants with different kinds of medical properties, to determine their antimicrobial activity against some pathogenic microorganisms. Methanolic extract of twenty selected plants belonging to eleven families against five microorganisms has been investigated .

MATERIALS AND METHODS

Plant Collection

Fresh plants in spring and summer in 2017 and 2018 from the mountainous region of Takht-e- sartashtak and surrounding areas, with an average of altitude 3000m above sea level, a longitude of 57 ° 6 ´– 57 ° 19 ´ and latitude of 29 ° 28 ´ – 29 ° 17 ´, in Kerman province, southeast of Iran, were collected, dried and identified (using Flora Iranica and Flora of Iran) in the botanical laboratory of the University of Kerman. A voucher number was made for each plant and archived at the Herbarium of Tehran (T) at the Kharazmi University of Tehran. The scientific name of plants was checked on the website of "The Plant List". Dried plant species from whole parts of them (except roots) were powdered and used for extraction. Twenty plants have been selected based on the ethnobotanical knowledge of the native people of the region gathered from different places containing Takht-e-Sartashtak, Hanza, Bondar-e-Hanza, Sarmeshk, Khanehrooghan, and Baghooieh, about the medicinal use of them and also with regard to the medicinal use of them that mentioned in various sources. Some plants (*Physospermum cornubiense* (L.) DC., *Varthemia persica* DC., and *Datisca cannabina* L.) were selected only based on a mention in sources.

Preparation of Methanolic Extracts

Dried and powdered plants were used to prepare the extraction. On 10 g of powdered plant, 50 ml of methanol was poured then, a suspension of plant powder and methanol was placed on the shaker for three days to remove the solvent compounds in the methanol from the plant. After filtering the suspension with filter paper, the derived extract was dried and the powder was prepared for antimicrobial work .

Microorganisms

The identified and standard microbial species were obtained from the Knowledge Base company of Dana Gene Researchers, Kerman, Iran. The bacterial species tested were two gram-positive bacteria, *Bacillus cereus* (PTCC:1015) and *Staphylococcus aureus* (PTCC:1112) two gram-negative bacteria, *Escherichia coli* (PTCC:1330) *Pseudomonas aeruginosa* (PTCC:1074) and one fungus, *Candida albicans* (PTCC:5027).

Antimicrobial Activity Assay

Method of disk diffusion was used [14]. At first, different dilutions of plant extract (mg/ml) were prepared. Thus, prime 0.05 g of dried extract was weighted with an analytical scale and dissolved the extract in 1 ml of the DMSO (Dimethyl sulfoxide) solvent, in which case the dilution of the extract is 50 mg/ml. Similarly, the next dilution was prepared. Each dilution is dispensed with 15 µl of sterilized blank disks and the time is given to dry the extract on the disk. To investigate the effect of plant extract on pathogenic microorganisms, the Muller Hinton Agar culture medium, made in the Merck factory in Germany, was used. After making the culture medium, at first 0.5 McFarland of microorganisms were prepared in sterile physiological serum and 0.2 µl of suspension in aseptic conditions was poured on mentioned culture medium. Then, with a glass spreader, the microbe was cultured on a culture medium evenly. In the next step, on each culture medium, the dilutions of the plant extracts that were previously prepared on the blank disks were placed at regular intervals from each other and also at a distance from the edge of the plate. Then, the plates were incubated for 24 hours at 30 ˚C and examined for antimicrobial activity and observation of zone of growth inhibition around the disks.

Table 1 Family, scientific name, life form, voucher number, Persian or local name, and medicinal use of selected plants in the studied area.

Family, scientific name, life forms, (Voucher number), and Persian or local name	Known and main chemical compounds	Traditional medicinal use(s)[b]	Other medicinal use(s)
Apiaceae, *Ferulag angulata* (Schltdi.) Boiss., G, (T18500), Garchi	(Z)-β- ocimene, terpinolene, α-phellandrene [17]	carminative, anti-distension, (1, 2)	lowering serum lipids and lipid peroxidation, antimicrobial property [18], anticancer effects [19], antioxidant activity [20]
Apiaceae, *Levisticum officinale* W.D.J. Koch, Hc, (T18501), Karafs-e-koohi	α-terpinyl acetate, β-phellandrene [21]	reducing fat and blood sugar, sedative, (1-3)	gastrointestinal disorders caused by cold temper, gout, anemia in young girls [22], antimicrobial activity [15], antiproliferative [21]
Apiaceae, *Physospermum cornubiense* (L.) DC., Hc, (T18502) Shokaran-e-baghi	caryophyllene oxide, limonene, β-caryophyllene [23]		antioxidant and antihemolitic [24]
Asteraceae, *Hertia intermedia* (Boiss.) Kuntze, C, (T18503), Karghich or Karkich	herticin A & B (sesquiterpenes) [25]	insect bite treatment, antiseptic, (1-6)	insect bite treatment, toothache [22], dermal inflation, insect bite treatment [26], pain killer [27], fever, headache, the pain of stomach, and problems of the menstrual cycle [28]
Asteraceae, *Tanacetum parthenium* (L.) Sch. Bip., C, (T18504), Babooneh gavi	camphor, chrysanthenyl acetate, and camphene [11]	anti-inflammatory, anti-spasm, analgesic, anti-headache, anti-migraine, anti-fever, and treatment of menstrual pains, (1-6)	antimicrobial activity [29], fever, sedative, gastric disorders, nerve system relaxant [26], antileishmanial activity [30], anti-inflammatory properties [31]
Asteraceae, *Varthemia persica* DC., C, (T18505), Atr-e-sang	menthol, α- thujene, α- pinene [32]		antimicrobial activity [32], have protective effect against organophosphates toxicity [33]
Berberidaceae, *Berberis integerrima* Bunge, Ph, (T18506), Zereshk	1-methyl malate [34], delphinidin-3-glucoside [35]	blood diluent, treatment of anorexia, reduction of puke and nausea, reduce of gum bleeding, strengthening of the stomach, treatment of constipation, treatment of liver inflammation, treatment of mouth and throat inflammation, (1-6)	blood sugar and depurative [26], antidiabetic, anticancer and antimicrobial activities [36], bone fractures, back pain, wounds, ulcers, infections, blood fat, diabetes, cancer, jaundice, hypertension, blood purification, bone pain [37]
Cupressaceae, *Juniperus excelsa* M. Bieb., Ph, (T18507), Ors or Sarv-e-koohi	cedrol, α- pinene, limonene [38]	wound healing, (5)	antimicrobial activity [39], antifungal activity [13], diuretic, rheumatism, antiseptic, dermal inflations, boils [26]
Datiscaceae, *Datisca cannabina* L., Hc, (T18508), Shebhe shahdaneh	datiscetin 3-rutinoside, galangin 3-rutinoside [40]		antifungal activity [13], diuretic, expectorant, mild laxative [41], purgative [42]
Euphorbiaceae, *Euphorbia gedrosiaca* Rech.f., Aellen & Esfand, Hc, (T18509), Farfiyoon		treatment of corns and warts, (1-6)	treatment of wounds, warts [37]
Lamiaceae, *Mentha longifolia* (L.) L., Hc, (T18510), Pooneh or	Pulegone, iso menthone, 1,8-cineole, borneol, and piperitenone oxide [43]	expectorant, anticonvulsant, relief of nerves, anti-diarrhea, anti-distension, treatment of	antimicrobial activity, treatment of minor sore throat and minor mouth or throat irritation [44], antifungal and

Poodaneh		cold, throat ache relief, menstrual pain relief, treatment of gastrointestinal disorders and stomachache, treatment of insomnia, (1-6)	antioxidant activity [45], antibacterial activity [46], antimicrobial activity [47], antimicrobial and antioxidant activity [43], stomach diseases, stimulant, dysentery, diarrhea [42]
Lamiaceae, *Nepeta glomerulosa* Boiss., Hc, (T18511), Poonehsay	α-pinene, 1,8-cineole, limonene, linalool, *trans*-β-ocimene, humulene epoxide, *trans*-α-bergamotene, α-humulene, camphene [48]	sedative, treatment of joints pains, (1-6)	antifungal activity [13], dermal inflation, pneumonia [26], antibacterial activity [49]
Lamiaceae, *Salvia rhytidea* Benth., Hc, (T18512), Maryamgoli	p-cymene-8-ol, spathulenol, pulegone, sabinene, terpinene-4-ol, α-copaene [50]	antimicrobial effects, (1)	antifungal activity [51], for treatment of cancer, malaria and microbial strains [52], anticancer [53]
Lamiaceae, *Scutellaria multicaulis* Boiss., C, (T18513), Boshghabi		analgesic, sedative, treatment of nervous pains, tonic for the nervous system, (4-6)	antioxidative activity [54]
Lamiaceae, *Stachys acerosa* Boiss., C, (T18514), Sonboleii	cis-chrysanthenyl acetate, 1,8-cineole, α-pinene, linalool, limonene [55]	antimicrobial effects, (1-6)	antibacterial and antioxidant activities [55], antibacterial activity [56], antifungal activity [57]
Lamiaceae, *Thymus carmanicus* Jalas, Hc, (T18515), Avishshan-e-kermani	carvacrol, *p*-cymene, γ-terpinene, thymol, and borneol [58]	expectorant, antitussive, treatment of cold and bronchitis, (1)	cold, sedative, asthma, diarrhea [26], to cure rheumatic and skin disease [59], antioxidant [60], antifungal activity [61], antinociceptive and anti-inflammatory effects [62]
Papaveraceae, *Glaucium grandiflorum* Boiss. & Huet, Hc, (T18516), Shaghayegh	norchelidonine, dihydro chelerythrine, 8-acetonyl dihydro chelerythrine, protopine, allo cryptopine, tetrahydro jattorrhizine, tetra hydro palmatine [63]	analgesic, (5,6)	analgesic activity [64], treatment of pertussis [65]
Rosaceae, *Sanguisorba minor* Scop., Hc, (T18517), Gheytaran or Tootroobah	farnesyl acetate, nonadecane, docosane [66]	lowering blood sugar, treatment of nervous disorders, treatment of bronchitis, removing kidney stones, astringent, stopping bleeding, anti-hemorrhoid, (2-6)	antifungal activity [13], toothache [26, 67]
Rhamnaceae, *Rhamnus persica* Boiss., Ph, (T18518), Siyahtangers or Annab		purgative, treatment of constipation, treatment of some skin diseases, treatment of pain and dental abscess, (2-6)	antileishmanial and toxicity activity [68], toothache [67], allergy and itching in children, wound [37], antioxidant [69]
Thymelaceae, *Daphne oleoides* Schreb., Ph, (T18519), Mazariyoon or Dafneh	daphnetin 8-*O*-β-D-glucopyranoside, 4-ethoxy benzoic acid, 4-hydroxybenzoic acid, grantioidin [70]	laxative, treatment of constipation, diuretic, sudoriferous, wound healing, blain opener, intestine cleaner, (2-6)	purgative, laxative [26], skin diseases [71], antibacterial activity [72]

[a] G: Geophytes, Hc: Hemicryptophytes, C: Chamaephytes, Ph: Phanerophytes.

[b] Ethnobotanical knowledge of the native people of the region gathered from different places contain Takht-e-Sartashtak (1), Hanza (2), Bondar-e-Hanza (3), Sarmeshk (4), Khanehrooghan (5), and Baghooieh (6)

Results observed methanolic extracts of all of these plants (except one) were active against at least one or more microorganisms. The results of the antimicrobial activity of twenty plants against five microorganisms are shown in Table 2.

Table 2 Antimicrobial activity of twenty plants against five microorganisms.

Plants Name	Zone of growth inhibition (mm)				
	E. coli	*S. aureus*	*B. cereus*	*P. aeruginosa*	*C. albicans*
F. angulata (Schltdi.) Boiss.	-	16	11	-	24
L. officinale W.D.J. Koch	-	15	-	11	19
Ph. Cornubiense (L.) DC.	-	-	-	-	-
H. intermedia (Boiss.) Kuntze	-	15	-	-	11
T. parthenium (L.) Sch. Bip.	-	16	-	11	12
V. persica DC.	-	14	-	-	-
B. integerrima Bunge	-	11	10	-	-
J. excelsa M. Bieb.	-	14	-	-	12
Datisca cannabina L.	-	22	10	-	-
E. gedrosiaca Rech.f., Aellen & Esfand	-	12	11	10	-
M. longifolia (L.) L.	-	15	-	-	-
N. glomerulosa Boiss.	-	11	10	13	10
S. rhytidea Benth.	-	13	-	10	11
S. multicaulis Boiss.	-	11	12	12	-
S. acerosa Boiss.	-	-	-	-	12
T. carmanicus Jalas	-	16	12	12	-
G. grandiflorum Boiss. & Huet	-	23	12	10	16
S. minor Scop.	11	13	13	16	15
R. persica Boiss.	-	12	10	11	12
D. oleoides Schreb.	-	-	-	11	-

The microorganisms contain two gram-positive bacteria, *B. cereus* (PTTC:1015) and *S. aureus* (PTTC:1112) two gram-negative bacteria, *E. coli* (PTCC:1330), and *P. aeruginosa* (PTTC:1074) and one fungus, *C. albicans* (PTTC:5027).

Table 3 The MIC, MBC, and MFC of twenty plant species against five microorganisms.

Plants Name	MIC, MBC & MFC of plants extracts against 5 microorganisms (mg/mL)									
	E. coli		*S. aureus*		*B. cereus*		*P. aeruginosa*		*C. albicans*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
F. angulata (Schltdi.) Boiss.	-	-	50	100	50	100	-	-	6.25	50
L. officinale W.D.J. Koch	-	-	50	100	-	-	50	50	12.5	50
Ph. cornubiense (L.) DC.	-	-	-	-	-	-	-	-	-	-
H. intermedia (Boiss.) Kuntze	-	-	50	100	-	-	-	-	50	100
T. parthenium (L.) Sch. Bip.	-	-	50	50	-	-	50	100	25	50
V. persica DC.	-	-	50	100	-	-	-	-	-	-
B. integerrima Bunge	-	-	50	100	100	100	-	-	-	-
J. excelsa M. Bieb.	-	-	50	50	-	-	-	-	50	50
D. cannabina L.	-	-	12.5	50	100	100	-	-	-	-
E. gedrosiaca Rech.f., Aellen & Esfand	-	-	50	50	100	100	50	100	-	-
M. longifolia (L.) L.	-	-	50	100	-	-	-	-	-	-
N. glomerulosa Boiss.	-	-	50	100	100	100	50	50	50	100
S. rhytidea Benth.	-	-	50	100	-	-	50	100	50	50
S. multicaulis Boiss.	-	-	50	100	100	50	50	100	-	-
S. acerosa Boiss.	-	-	-	-	-	-	-	-	50	50
T. carmanicus Jalas	-	-	12.5	50	50	100	50	100	-	-
G. grandiflorum Boiss. & Huet	-	-	50	50	50	100	50	100	12.5	50
S. minor Scop.	12.5	50	50	50	50	100	50	100	12.5	50
R. persica Boiss.	-	-	50	100	100	100	50	100	50	50
D. oleoides Schreb.	-	-	-	-	-	-	50	100	-	-

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, and MFC: Minimum Fungicidal Concentration.

It should be noted that a negative control disk containing a DMSO solvent was used and a commercially available antibiotic disk was used as a positive control .

Determination of MIC, MBC, and MFC

The MIC (Minimum Inhibitory Concentration) refers to the concentration of an extract that can inhibit bacterial growth in vitro, and MBC

(Minimum Bactericidal Concentration) means the least concentration of extract that eliminates the bacteria. The basis for the determination of MIC and MBC is based on the dilution method in Broth Dilution [14]. MFC is a Minimum Fungicidal Concentration. Initially to prepare the standard opacity to add 0.5 mL of 1% of dehydrated barium chloride solution in a 100 mL dish and increase the volume to 100 mL with 1% sulfuric acid. The resulting barium sulfate is called the 0.5 MacFarland standard opacity solution. Now pour 50 mg of herbal extracts powder into 1 mL of solvent to obtain a dilution of 50 mg / mL of the extract. Then in each of the 5 test tubes, we pour 1 ml of the extract solution and add 1 ml of the standard opacity solution to each tube. Next, incubate the tubes for 18 hours and check the results. The last tube that did not show opacity or growth of microorganisms is MIC and the pre-tube is often MBC (for bacteria) or MFC (for fungi). The contents of MBC and MFC tubes should not show any growth on the culture medium.

RESULTS

Results of previous experiments [15,16] demonstrated that water is not a very good solvent for the extraction of chemical compounds from plants, and organic solvents like methanol are better for this work. Methanolic extracts from 20 plants belonging to 11 families were examined for their antimicrobial activity against two gram-positive, two gram-negative, and one fungus, using Muller Hinton Agar culture medium and method of disk diffusion. Scientific name and their plant families, their life form, voucher number, Persian or local name, and also their medicinal uses are shown in Table 1.

Only one plant species (*P. cornubiense* (L.) DC. (Apiaceae)) was not active against none of the microorganisms. Just *Sanguisorba minor* Scop. (Rosaceae) was active against all five microorganisms. Most antimicrobial activity belongs to *F. angulata* (Schltdi.) Boiss. (Apiaceae) against *Candida albicans*, also *Glaucium grandiflorum* Boiss. & Huet (Papaveraceae) and *Datisca cannabina* L. (Datiscaceae) against *Staphylococcus aureus*. Only one taxon, *Sanguisorba minor* Scop. (Rosaceae), were active against *E. coli*. Appear gram-positive bacteria are more sensitive to the extracts than gram-negative. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than against Gram-negative bacteria [73, 74, 16]. Value of diameter of growth inhibition zone of *F. angualta* (Schltdi.) Boiss. (Apiaceae) against *C. albicans* was the maximum that showed the most antimicrobial activity of these plants. The Minimum Inhibitory Concentrations (MIC) of plant extracts were different (Tab.3). Total values of MIC, MBC, and MFC of plants extracts are shown in Table 3. The lowest MIC is associated with *F. angulata* (Schltdi.) Boiss. (Apiaceae) against *C. albicans*.

DISCUSSION

Many previous studies have been conducted to investigate the medicinal and antimicrobial properties of different plant species in the world, and most of these studies have shown positive effects on the treatment of various diseases as well as their antimicrobial activity. The results of this research, like many other studies, indicate that many herbs can be used to cure infections, for example, there is a report of this use in Yemen, and most of the studied plants were positive for antimicrobial activity [75]. Root extracts of *Acacia nilotica* (L.) Delile has antimicrobial activity [76-78]. *Aerva lanata* (L.) Juss. has antimicrobial properties and cytotoxicity [79]. The antimicrobial activity of six terpenoids from Gossypium sp. has been shown [80]. The antimicrobial activities of *Salvadora persica* L. were assayed [81]. The antimicrobial activity of *Tamarindus indica* L. has been shown [82]. The tendency to use natural drugs instead of chemicals, due to their side effects, is increasing day by day. Pharmacologists can obtain new compounds and natural substitutes for chemical drugs by isolating and identifying various extracts of medicinal plants. Extract of some plants has compounds that have antimicrobial properties and can be applied to the treatment of infections [16]. But we must always take into account that the plants are used correctly so that they do not endanger extinction. We should grow plants for different uses so that direct harvesting of nature does not damage plants and natural ecosystems. For example, the populations of the *Levisticum officinale* W.D.J.Koch (Apiaceae) have declined recently because of over-harvesting in the studied area.

CONCLUSION

The results of this research showed that most of the plants had antimicrobial activity but their activities were different. *Sanguisorba minor* Scop., *Glaucium grandiflorum* Boiss. & Huet, *Rhamnus persica* Boiss. and *Nepeta glomerulosa* Boiss. showed the most antimicrobial activity.

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