Journal of Medical Bacteriology

Antimicrobial Activities of some Plant Extracts against Phytopathogenic Fungi and Clinical Isolates in Iran

Mohammad Reza Majid Khoshkholgh Pahlaviani [1], Reza Kazemi Darsanaki [2], Sirus Bidarigh [3]*

[1] *Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran.*

[2] *Young Researchers and Elites Club, Lahijan Branch, Islamic Azad University, Lahijan, Iran.*

[3] *Department of Agriculture, Lahijan branch, Islamic Azad University, Lahijan, Iran.*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 09 Jun 2018
Revised: 12 Jun 2018
Accepted: 13 Jul 2018
Published: 06 Oct 2018

Keywords:
Antimicrobial activity, Oxalis corniculata, Plant extracts, Peganum harmala, Salvia officinalis.

ABSTRACT

Background: Natural products from plants as environmentally safe options have received attention for controlling various phytopathogenic diseases. In this study, the antimicrobial activities of three plant aqueous and alcoholic extracts (*Salvia officinalis*, *Peganum harmala* and *Oxalis corniculata*) against phytopathogenic fungi (10 fungal isolates associated with diseased tomato fruits) and clinical isolates (10 bacterial isolates) and *Candida albicans*, as a pathogenic yeast model, were investigated in the Gilan province of northern Iran.

Methods: After phytochemical screening of plant extracts, antimicrobial activity of the extracts evaluated by standard methods for determination of MIC and MBC. Results of the phytochemical screening of aqueous and alcoholic leaf extracts of the selected three plants revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids.

Results: All three extracts of the plants tested showed varying degrees of antimicrobial activities against both phytopathogenic fungi and bacteria. Generally, the methanol extracts were more active than other extracts for *S. officinalis* and *P. harmala*, whereas for *O. corniculata* water extract showed more antimicrobial activity.

***Conclusion*:** The study has been able to establish and document the important medicinal plants which can be used in the management of phytopathogens and infectious diseases in Iran.

• ***Please cite this paper as:*** Khoshkholgh Pahlaviani MRM, Kazemi Darsanaki R, Bidarigh S. Antimicrobial Activities of some Plant Extracts against Phytopathogenic Fungi and Clinical Isolates in Iran. *J Med Bacteriol.* 2018; **7** (3, 4): pp.5-16.

*Corresponding Author: Mohammad Reza Majid Khoshkholgh Pahlaviani, Department of Microbiology, Faculty of Basic Sciences, Lahijan Branch, Islamic Azad University, Lahijan, Iran.

Tel: +98- 013-42239042., *E-mail:* Khoshkholgh@liau.ac.ir

Introduction

There is an increasing worldwide interest in using plants as natural products in food, pharmaceutical and cosmetic industries. Bioactive compounds in medicinal plants led to wide usage of plant products in these industries as dietary supplements, botanical drugs and functional foods, etc. Plants also have been used as treatments for various diseases such as psoriasis, hypertension, cholesterol, fever, eczema and diarrhea. Thus, today their scientific validation was provided by identification and isolation of bioactive phytochemicals (1). Phytochemicals are the secondary metabolites that have several subgroups possessing various bioactivities such as antioxidant, antimicrobial, antivirus, anticancer, etc., (2).

Infectious diseases are a leading cause of death, worldwide. In the last decades, the clinical efficacy of many synthetic antibiotics is being threaded by the emergence of a serious problem which can be defined as multidrug- resistant pathogens (3). Multidrug-resistant (MDR) in both human and plant has developed due to the indiscriminate usage of commercial antimicrobial drugs that have widely applied in the treatment of infectious diseases. Therefore, scientists have tried to discover new antimicrobial substances from plants sources. It is known that the natural products and their derivatives hold more than 50% of all the drugs in clinical usage with one quarter originating from higher plants (3). Pathogenic fungi are the infectious agents in plants and controlled by synthetic fungicides; however, the use of these is increasingly restricted due to the harmful effects of pesticides on environment and human health (4).

In this study, the purpose was to determine the antimicrobial activities of some plant extracts against 10 phytopathogenic fungi and 11 clinical isolates in Iran.

Materials and Methods

Collection and extraction of plant materials

The plant materials used were *Salvia officinalis*, *P. harmala* and *Oxalis corniculata*. Fresh leaf of three plants was collected from a local farm in Lahijan City, Guilan Province, Iran, during the months of spring and summer in 2016 and the plants were identified by the Botany Department of Islamic Azad University of Lahijan, Iran. Exactly, 250 g of the pulverized plant material was cold-extracted in ethanol and methanol, separately. Another 250 g of plant material was extracted in water for 4 days with occasional shaking. The separated extracts were then filtered through Whatman's No. 1 filter paper and the ethanol and methanol filtrate were separately concentrated on rotary evaporator to remove the ethanol and methanol. The aqueous extract was lyophilized to obtain a dry powder extract and for collection of tomato (*Lycopersicon esculentum* Mill.) fruits, both healthy and diseased tomato fruits were prepared from greengrocers of Lahijan markets in Guilan Province, Iran.

Isolation and identification of fungi from naturally infected tomatoes

The fungi associated with diseased tomato fruits were isolated using the blotter method. The tomato fruits were cut into sections with a sterilized knife. The sectioned fruits were surface sterilized with 70% ethanol for three minutes and rinsed three times by distilled water to remove surface contaminants. The sterilized sectioned fruits were dried between filter papers and plated on three layers of filter papers moistened with sterile distilled water in sterilized Petri-dishes and incubated at room temperature (25 ± 2 °C) for 7 days. After incubation, the plates were observed under binocular microscope for fungal growth. The isolates were identified to species level using the compound microscope using standard references (5-6). The identification of the various moulds was done based on their colony and cell morphologies

such as color, mycelia, conidia and sporulating structures. To obtain pure cultures of the isolated fungi, a sterile inoculating needle was used to transfer the fungal spores from filter papers in the Petri-dish and inoculated on acidified potato dextrose agar medium (APDA). Each fungus was plated at the center of the Petri dishes. They were incubated at room temperature (25 ± 2 °C) in a laminar flow for 7 days and their growth observed.

Antifungal screening test of plant extracts against tomato phytopathogenic fungi

The effect of the extract was determined by measuring the mycelial dry weight. Fifty ml of potato dextrose broth (PDB) was poured into each flask containing concentrations 0, 50, 10 and 20 of the respective extracts (2ml each). With a sterile cork borer (3mm) mycelia mass of 7 day old cultures of the isolates were inoculated in the flask and incubated at 28± 2°C. After 10 days the different fungi from the different broths, were taken on dried and weighted filtered papers in desiccators. Then, fungal mycelia were dried at 70 °C for 24 hours and the weight was recorded. Inhibition of fungi by different concentrations was calculated as below (7):

100 – Weight of fungus in extract (%)
Weight of fungus in PDB

Fungicidal effect of plant extracts compared with reference fungicide (carbendazim)

Different concentrations of these effective plant extracts including *S. officinalis*, *O. corniculata* and *P. harmala* (0.0, 2.0, 4.0, 8.0 and 16.0 mg/ml) were prepared separately by dissolving their requisite amount in 50 ml of methanol, sterilized through millipore filter and mixed with PDA medium to obtain the final concentrations. To compare efficacy of plant extracts with that of Carbendazim in controlling the tomato phytopathogenic fungi, different concentrations, 0.0, 2.0, 4.0, 8.0 and 16.0 ppm, of carbendazim of 98% active ingredients were prepared by mixing weighted powder of fungicide with a known volume of sterile (PDA). Fungal plugs (0.7 mm in diameter) were obtained and placed at the center of Petri dish in potato dextrose agar medium with plant extracts of various concentrations and fungicide. The cultures were incubated at 25 ± 2 °C and radial growth of mycelia was measured after 6 days.

Isolation and identification of the bacteria and yeasts from clinical specimens

Isolates used in this study (*Staphylococcus aureus*, *Staphylococcus epidermitidis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Bacillus cereus*, *Bacillus subtilis*, *Proteus vulgaris*, *Serratia marcescens* and *Candida albicans*) were obtained from 2 hospitals in Lahijan during 2016–2017. All the bacteria and yeast were isolated and identified using morphological, microscopy and biochemical tests in the clinical microbiology laboratories of the participating hospitals. The bacterial isolates were first sub-cultured in a nutrient broth (Oxoid) and incubated at 37 °C for 18 h while *C. albicans* isolate were sub-cultured on SDA (Oxoid) for 72 h at 25 °C.

Determination of the Phytochemical on the three plant samples

The extracts were subjected to phytochemical tests for plant secondary metabolites, tannins, saponins, steroid, alkaloids and glycosides in accordance with Trease and Evans (8) and Harborne (9) with little modification.

Table 1. The ethnobotanical data of the plant parts employed.

Plant species	Family	Common name	Plant part used
Salvia officinalis	Lamiaceae	Sage	Leaf
Peganum harmala	Zygophyllaceae	Syrian Rue	Leaf
Oxalis corniculata	Oxalidaceae	yellow wood sorrel	Leaf

Table 2. Frequency of isolated fungi associated with diseases of tomato (%).

Fungal isolates	Frequency of Isolation
Alternaria alternata	29.13
Alternaria solani	18.45
Fusarium solani	11.42
Fusarium oxysporum f. sp. *lycopersici*	9.18
Phytophthora infestans	7.28
Rhizoctonia solani	6.51
Botrytis cinerea	5.78
Phytophthora infestans	5.68
Colletotrichum coccoides	3.37
Verticillium albo-atrum	3.20

Table 3. Preliminary phytochemical analysis of *S. officinalis* L., *P. harmala* L. and *O. corniculata* L. Leaf extracts.

Solvents / Phytoconstituents	*S. officinalis*			*P. harmala*			*O. corniculata*		
	Ethanol	**Methanol**	**Water**	**Ethanol**	**Methanol**	**Water**	**Ethanol**	**Methanol**	**Water**
Carbohydrate	+	+	++	+	+	++	+	+	++
Steroids	++	++	+	-	-	-	+	+	+
Tannins	++	++	++	+	++	++	+	++	++
Alkaloids	-	-	-	++	++	-	-	-	-
Saponins	+	+	-	+	+	-	+	+	-
Cardiac Glycosides	+	+	++	-	-	+	-	-	+
Terpenoids	++	++	+	++	+++	+	++	+++	+
Flavonoids	+	++	-	++	+++	-	++	++	-

Table 4. Mycelial dry weight (gm) of fungi isolated from infected tomato when exposed to varying concentrations of various plants leaves extract.

Concentrations of Extract (%) / Microorganism	*S. officinalis* (methanol)				*P. harmala* (water)				*O. corniculata* (methanol)			
	0	**5**	**10**	**20**	**0**	**5**	**10**	**20**	**0**	**5**	**10**	**20**
Alternaria alternata	0.00	53.5	62.4	74.6	0.00	63.3	77.8	91.3	0.00	46.6	64.4	72.5
Alternaria solani	0.00	44.8	50.6	74.5	0.00	75.8	77.4	92.7	0.00	52.3	64.8	71.3
Phytophthora infestans	0.00	61.4	74.8	91.8	0.00	64.5	77.4	85.6	0.00	66.3	78.8	89.1
Fusarium oxysporum* f. sp. *lycopersici	0.00	64.8	77.5	79.2	0.00	52.3	86.3	95.5	0.00	54.3	66.5	78.9
Verticillium albo-atrum	0.00	66.2	76.8	81.3	1.70	73.3	84.5	84.7	1.90	54.6	66.2	78.7
Botrytis cinerea	0.00	53.3	66.4	93.6	0.00	72.1	87.4	83.4	0.00	53.9	66.3	78.9
Colletotrichum coccoides	0.00	63.6	75.3	82.5	0.00	60.2	76.3	85.5	0.00	52.5	64.5	69.4
Rhizopus stolonifer	0.00	45.4	57.3	78.50	0.00	52.5	76.5	84.8	0.00	70.3	86.8	88.9
Rhizoctonia solani	0.00	54.3	66.5	71.4	0.00	61.4	73.3	93.5	0.00	64.9	87.1	88.7
Fusarium solani	0.00	56.4	67.3	78.9	0.00	72.8	75.5	94.2	0.00	62.4	66.5	79.3

Table 5. Percentage growth inhibition of fungal isolates from infected tomato after exposure to varying concentrations of leaf extract of various plants after 10 days.

Concentrations of Extract (%) / Microorganism	*S. officinalis* (methanol)				*P. harmala* (water)				*O. corniculata* (methanol)			
	0	**5**	**10**	**20**	**0**	**5**	**10**	**20**	**0**	**5**	**10**	**20**
Alternaria alternata	2.20	1.92	1.42	0.7	2.12	1.11	0. 6	0.0 2	2.12	1.08	0. 6	0.33
Alternaria solani	2.91	2.07	1.04	0.52	2.31	1.08	0. 6	0.0 2	1.95	0.85	0. 5	0.32
Phytophthora infestans	1.49	1.8	1.05	0.21	1.49	0.74	0.53	0.2	2.18	0.95	0.52	0.33
Fusarium oxysporum* f. sp. *lycopersici	2.10	1.61	1.07	0.67	1.40	0.95	0.35	0.01	1.75	0.73	0.23	0.24
Verticillium albo-atrum	2.30	1.96	1.06	0.83	1.70	0.67	0.34	0.05	1.90	0.65	0.34	0.21
Botrytis cinerea	2.00	1.90	1.16	0.13	2.20	0.83	0.24	0.09	1.50	0.64	0.54	0.21
Colletotrichum coccoides	1.30	0.80	0. 5	0.32	1.90	0.69	0.35	0.08	1.40	0.44	0.32	0.11
Rhizopus stolonifer	2.30	1.20	0.7	0.55	2.40	1.20	0.19	0.08	1.55	0.74	0.44	0.22
Rhizoctonia solani	2.10	1.30	0.6	0.42	1.90	1.12	0.45	0.03	1.30	0.61	0.53	0.21
Fusarium solani	2.00	1.2	0.7	0.51	2.00	0.7	0.37	0.02	1.90	0.63	0.36	0.02

Table 6. Zone of inhibition exhibited by *S. officinalis* (Leaf methanol extract), *P. harmala* (Leaf water extract) and *O. corniculata* (Leaf methanol extract) on fungi (mm).

Concentration (mg/ml) / Fungi	*S. officinalis* (methanol)				*P. harmala* (water)				*O. corniculata* (methanol)			
	25	**50**	**100**	**200**	**25**	**50**	**100**	**200**	**25**	**50**	**100**	**200**
A. alternata	2	4	9	10	5	7	9	13	3	5	6	7
A. solani	3	6	8	11	5	8	10	14	2	3	4	6
P. infestans	3	6	10	17	2	4	5	7	3	6	8	13
F. oxysporum f. sp. lycopersici	4	5	8	13	4	7	12	16	2	4	5	7
V. albo-atrum	4	2	9	7	1	2	4	6	1	2	4	6
B. cinerea	3	4	9	13	4	6	8	6	2	5	9	15
C. coccoides	3	5	8	5	2	3	4	5	1	2	4	6
R. stolonifer	2	6	9	11	3	5	7	9	2	4	6	7
R. solani	2	5	8	11	2	6	9	12	2	3	5	7
F. solani	2	6	9	14	2	7	10	15	3	4	5	7

Table 7. Effect of different concentrations of reference fungicide (Carbendazim) on mycelial growth of the most common phytopathogenic fungi of tomato.

Carbendazim Concentration (ppm)	**Mean diameter of mycelial growth (mm)**			**Percentage of mycelial growth inhibition**		
	A. alternata	***A. solani***	***F. solani***	***A. alternata***	***A. solani***	***F. solani***
0	91.5±0.2*	87.8±0.7*	82.4±0.1*	0	0	0
2	78.3±0.1*	64.3±0.1*	59.3±0.4*	26.20	38.01	29.21
4	43±0.2*	31.2±0.3*	23.2±0.3*	53.31	80.01	71.23
8	0.00	0.00	0.00	100	100	100
10	0.00	0.00	0.00	100	100	100

Values in the same column followed by asterisk (*) are significant different at (P= 0.05). Data are means (n=4) ± Standard error of four replicates.

Table 8. Screening of primary antimicrobial activity of *S. officinalis* L. Leaf extracts on selected microbial isolates (mm)

Microorganism	Miconazole mg/ml	AMP µg/ml	ST (mg/ml)	Water	Methanol	Ethanol	Amphotericin (mg/ml)
S. aureus	–	4	25	5	20	10	–
P. aeruginosa	–	3	20	4	17	12	–
E. coli	–	19	0	7	14	11	–
E. faecalis	–	18	11	7	12	11	–
S. epidermidis	–	6	22	2	14	11	–
K. pneumonia	–	5	11	3	8	5	–
B. cereus	–	3	20	5	13	10	–
B. subtilis	–	4	22	5	14	12	–
P. vulgaris	–	22	18	7	14	10	–
S. marcescens	–	15	25	3	12	9	–
C. albicans	29	–	–	4	18	13	27

ST: Streptomycin, AMP: Ampicillin

Table 9. Screening of primary antimicrobial activity of *P. harmala* L. Leaf extracts on selected microbial isolates (mm).

Microorganism	Miconazole mg/ml	AMP µg/ml	ST (mg/ml)	Water	Methanol	Ethanol	Amphotericin (mg/ml)
S. aureus	–	4	20	12	13	11	–
P. aeruginosa	–	3	20	13	12	9	–
E. coli	–	19	3	9	11	10	–
E. faecalis	–	18	23	15	20	17	–
S. epidermidis	–	6	22	12	16	14	–
K. pneumonia	–	5	11	7	10	9	–
B. cereus	–	3	20	14	17	16	–
B. subtilis	–	4	22	15	19	18	–
P. vulgaris	–	19	21	11	13	11	–
S. marcescens	–	17	25	9	13	12	–
C. albicans	29	–	–	5	11	7	27

ST: Streptomycin, AMP: Ampicillin

Table 10. Screening of primary antimicrobial activity of *O. corniculata* L. Leaf extracts on selected microbial isolates (mm).

Microorganism	Miconazole mg/ml	AMP µg/ml	ST (mg/ml)	Water	Methanol	Ethanol	Amphotericin (mg/ml)
S. aureus	–	4	20	18	15	11	–
P. aeruginosa	–	3	20	11	8	5	–
E. coli	–	19	3	16	13	11	–
E. faecalis	–	18	23	10	7	6	–
S. epidermidis	–	6	22	18	16	15	–
K. pneumonia	–	5	11	7	10	9	–
B. cereus	–	3	20	5	12	10	–
B. subtilis	–	4	22	6	13	11	–
P. vulgaris	–	22	18	11	10	8	–
S. marcescens	–	17	25	12	11	10	–
C. albicans	29	–	–	11	7	5	27

ST: Streptomycin, AMP: Ampicillin

Table 11. Minimum inhibitory concentration and minimum bactericidal concentration of *S. officinalis* (Methanol), *P. harmala* (Methanol) and *O. corniculata* (water) leaf extract (mg/ml).

Extracts / Microorganism	*S. officinalis*			*P. harmala*			*O. corniculata*		
	Methanol		ST MIC	Methanol		ST MIC	Water		ST MIC
	MIC	MBC		MIC	MBC		MIC	MBC	
S. aureus	5	10	0.065	5	15	0.065	5	10	0.065
P. aeruginosa	10	20	0.05	5	20	0.05	5	10	0.05
E. coli	5	20	0.065	5	15	0.065	5	15	0.065
E. faecalis	5	15	0.065	5	10	0.065	5	15	0.065
S. epidermidis	5	15	0.065	10	20	0.065	10	20	0.065
K. pneumonia	10	20	0.05	10	20	0.05	10	15	0.05
B. cereus	5	15	0.065	5	20	0.065	10	20	0.065
B. subtilis	5	10	0.065	6.25	10	0.065	6.25	12.5	0.065
P. vulgaris	5	15	0.25	5	15	0 .25	5	10	0 .25
S. marcescens	5	20	0 .05	10	20	0 .05	10	20	0 .05

Results

Botanical and common name of the plants used and the extract percentage yield of the selected plant species are illustrated in Table 1.

Fungal isolates were isolated from naturally infected tomatoes at the Experimental Farm of Faculty of Agriculture, Lahijan University, Iran in 2016 and 2017 seasons. According to the results, 10 of the isolated fungi from naturally infected tomatoes were *Alternaria alternata* which occurred more frequently with 29.13%, followed by *Alternaria solani* with 18.45%, *Fusarium solani* with 11.42% and the least was *Verticillium albo-atrum* with 3.20% (Table 2).

Investigations on the phytochemical screening of aqueous and alcoholic leaf extracts of the selected three plants revealed the presence of saponins, steroids, tannins, glycosides, alkaloids and flavonoids (Tables 3).

Glycosides and steroids were not found in *P. harmala* and *O. corniculata* leaf extracts, respectively. Also, all three extracts of the plants tested showed varying degree of antifungal activities against the test fungal species. The present results showed that the mycelial growth of

the ten fungi decreased with increase concentrations. All plant extracts tested significantly ($p<0.05$) reduced the mycelial growth of all the fungi in the broth medium at all concentrations. However, the effectiveness of the *P. harmala* leaf water soluble extract increased with increased concentration more than that of methanol extracts of *S. officinalis* and *O. corniculata* on fungal isolates of *F. oxysporum*, *F. solani*, *R. solani*, *A. solani* and *A. alternata*, respectively, and this was also statistically significant ($p<0.05$). In contrast, methanol extract of *Oxalis corniculata* was only more effective on fungal isolates of *P. infestans* and *R. stolanifer* while the effectiveness of the *S. officinalis* increased with increased concentration only on *B. cinerea* and *P. infestans*, respectively (Table 4).

At any concentrations, the extracts did not inhibit growth of *V. alba-atrum* and *C. coccoides*, completely. However, the toxicity of *P. harmala* was greater than that of *O. corniculata* and *S. officinalis* in the percentage growth inhibition of the isolates (Table 5).

For all the concentrations of plant extracts used in this work, there was not a constant 100% inhibition. Inhibitory effects of the most effective plants extract at various concentrations 25, 50, 100 and 200 mg/ml in comparison with carbendazim as a reference fungicide. Carbendazim shows various capabilities to suppress tomato phytopathogenic fungi on solid medium. All the fungal isolates were sensitive to carbendazim and its mycelial growth was inhibited at 8 ppm. To compare efficacy of plant extracts with that of fungicide (Carbendazim) in controlling the tomato phytopathogenic fungi, different concentrations (0.0, 2.0, 4.0, 8.0 and 10.0 ppm) of Carbendazim of 98% active ingredients were used in this study (Table 7). *P. harmala* extract was the most effective in suppressing the mycelial growth of phytopathogenic fungi followed by *S. officinalis* and *O. corniculata*. The *P. harmala* extract showed the highest zones of inhibition on *F. oxysporum f.* sp. *Lycopersici*, *F. solani*, *R solani*, *A. solani* and *A. alternata*, respectively at 200 mg/ml. *S. officinalis* showed maximum % inhibition of *P. infestans* and *B. cinerea* at maximum concentration while *O. corniculata* were inhibitory against *P. infestans* and *R. stolonifer* at 200 mg/ml. all the extracts appeared to have mild activity against *V. albo-atrum* and *C. coccoides* (Table 6).

All three extracts of leaves of *S. officinalis* showed varying degree of antimicrobial activities against the test bacterial species and *C. albicans*. The antibacterial activities of the ethanol and methanol extracts of *S. officinalis* compared favourably with that of three standard antibiotics (streptomycin and ampicillin) and have appeared to be broad spectrum as its activities were free on gram reaction for tested bacteria. In contrast; bioactivity of three extracts of *S. officinalis* for *C. albicans* was not significant. The inhibition zone for *K. pneumonia* was much less (3 - 8 mm), *S. marcescens* (3-12) as compared to other bacteria, respectively. The methanol extract with inhibition zone 8 - 20 mm was found to be more effective than the ethanol extract with inhibition zone 5-12 mm against all the organisms, especially *P. aeruginosa* and *S. aureus*. The methanol extract of *S. officinalis* exhibited good activity against *C. albicans* (inhibition zone 18mm) while bioactivity of other extracts was not significant. The aqueous extract showed low antimicrobial activity with inhibition zones ranging between 3 and 10 mm for different microorganisms tested (Table 8). The minimum inhibitory concentration (MIC) of the methanol extract for different organisms ranged between 5 and 10 mgml-1, while the MIC of streptomycin control ranged between 0.065 and 0.05 mgml-1. The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 10 and 20 mgml-1. Water extract showed minimum activity against any of the bacteria. Generally, the methanol extract was more active than other extracts. This may be attributed to the presence of soluble phenolic and polyphenolic compounds. However, anti-Candida activity of water extract of *S. officinalis* was stronger other than alcoholic extract. Also, in this investigation three extracts of leaves of *P. harmala* indicated varying degree of antimicrobial activities against the test bacterial species and *C. albicans*. The

antimicrobial activities of the ethanol and methanol extracts of *P. harmala* compared an average reaction with that of three antibiotics. In contrast; the highest diameter of the inhibitory zone was recorded with *C. albicans* 11 mm, for the methanol extract of *P. harmala* and *O. corniculata* water extract (Tables 9 and 10). The inhibition zone for *K. pneumonia* ranged between 7 and 10 mm, *E. coli* (9-10) and *P. aeruginosa* (9-13) as compared to other bacteria, respectively. The methanol extract with inhibition zone 10 - 20 mm was found to be more effective than the ethanol extract with inhibition zone 9-18 mm against the tested bacteria. The water extract showed low antibacterial activity with inhibition zones ranging between 2 and 7 mm for different bacteria tested. While the highest diameter of the inhibitory zone was registered with *E. faecalis* 20 mm, for methanol extract of *S. officinalis* was recorded with S. aureus 12 mm.

MIC of the methanol extract for different organisms between 5 and 10 mgml-1, while the MIC of streptomycin control ranged between 0.065 and 0.25 mgml-1. The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 10 and 20 mgml-1 (Table 11). On the basis of the obtained results that are shown in Table 9, with the exception of *K. pneumoniae*, *B. cereus* and *B. subtilis*, the leaf extracts of *Oxalis corniculata* have a good antimicrobial activity so that all of the tested clinical isolates were considerably sensitive to water extract while the alcoholic leaf extract of this species were presented a relatively poor antibacterial activity. Water extract exhibited similar inhibition zone against *K. pneumoniae*, *B. subtilis* and *B. cereus* where it was around 7 mm, 6 mm and 5 mm, respectively. Moreover, MIC values for *Oxalis corniculata* L. leaf water extract ranged between 5 and 10 mgml-1, while the MIC of streptomycin control ranged between 0.065 and 0.05 mgml-1. MBC values for *Oxalis corniculata* L. leaf water extract ranged between 10 and 20 mgml-1 (Table 11).

Discussion

Studies have referred antimicrobial activities of three plant alcoholic and aqueous extracts (*S. officinalis*, *P. harmala* and *O. corniculata*) against phytopathogenic fungi and clinical bacteria. Antibacterial and phytochemical study of *S. officinalis* in Iraq by Muttalib and et al indicated that Chloroform extract 100 mg/ml was found active against two types of bacteria, *S. aureus* with MIC of 90 mg/ml and *Proteus* spp., with MIC of 80 mg/ml (10). Antimicrobial activity of the essential oil from leaves of *S. officinalis* L. was studied in Serbia. Both concentrations of oil showed antibacterial activity against *B. subtilis*, *S. aureus* 6538, *E. coli* 95 and *S. enteritidis*, as well as antifungal activity against *A. niger*. The oil was found to have significant antibacterial and antifungal activity and therefore can be used as a strong antimicrobial agent (11). In study Shiri et al., to determine the antibacterial effect of crude methanol extracts of six selected medicinal plants against *S. aureus* and *E. coli* showed that *P. harmala* possessed significant antibacterial activities against the two tested strains (25 mm *S. aureus*, 17 mm *E. coli*) (12). An ethanol *P. harmala* extract has been shown to have high antibacterial activity against MRSA (methicillin resistant *S. aureus*) (13) and CRSA (cefixime resistant *S. aureus*) (14). It has been also found that *P. harmala* have high antimicrobial activity against *C. albicans* at MIC values ranged from (0.2-2.5) mg\ml (15). Shahedur Rahman, et al showed antimicrobial effect and MIC of *O. corniculata* leaf extract against clinical isolates. The results indicated that among the solvent extracts tested, methanol extract of *O. corniculata* leaf had higher antibacterial activity compared to Erythromycin and Nalidixic acid against *Staphylococcus* sp. whereas the methanol extract of *O. sanctum* showed higher antibacterial activity compared to CIP-5 against *S. aureus*. The best MIC values were recorded to be 256 µg mL-1 against *S. typhi* for ethanol extract of *O. corniculata* leaf and 128 µg mL-1 against *S. aureus* for the methanol extract of O. sanctum leaf (16).

Satish and et al, indicating their potential use of *O. corniculata* in the management of diseases caused by *Xanthomonas* species as a seed protectant (17). In a study, antimicrobial efficiency of 20 ethnomedicinal plants (crude leaf extracts) were examined using water, benzene and acetone as solvents and tested against seven human pathogens like *E. coli* (MDR), *S. aureus* (MDR), *K. pneumoniae*, *B. cereus*, *V. cholerae* and *C. albicans*. Among the tested plants *O. corniculata* L., showed profound antimicrobial activity (> 11 mm inhibition zone), MIC (0.35-0.80 mg / ml) and MBC (0.45 – 1.0 mg / ml) values. The organic extracts of this plant could be a possible source to obtain new and effective herbal medicines to treat infections, which may cause by multi-drug resistant (MDR) strains of microorganisms from community as well as hospital settings (18). Also, the activity of the alcoholic extract against most bacterial strains investigated in this study is in agreement with previous works which show that aqueous extracts of plant generally showed little or no antibacterial activities, exception of *O. corniculata* L. leaf water extract. This is similar to the findings of Obi and Onuoha, who reported alcohol to be best solvent for the extraction of most plant active principles of medical importance (19).

Conclusion

According to results obtained from this study, all extracts of the plants tested showed varying degree of antimicrobial activities against phytopathogenic fungi and clinical isolates. Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antimicrobial activity.

Conflict of interest

The authors declare no conflict of interest.

References

1. Littleton J, Rogers T, Falcone D. Novel approaches to plant drug discovery based on high throughput pharmacological screening and genetic manipulation. *Life Sci* 2005; **78**: 467-75.
2. Duffy CF, Power RF. Antioxidant and antimicrobial properties of some Chinese plant extracts. *Int J Antimicrob Agents* 2001; **17**(6):527-9.
3. Eldeen I, Elgorashi E, Van Staden J. Antibacterial, anti-inflammatory, anti-cholinesterase and mutagenic effects of extracts obtained from some trees used in South African traditional medicine. *J Ethnopharmacol* 2005; **102**(3):457-64.
4. Dellavalle PD, Cabrera A, Alem D, et al. Antifungal activity of medicinal plant extracts against phytopathogenic fungus Alternaria spp. *Chil. J. Agr. Res* 2011; **71**(2):231.
5. Barnett HL, Hunter BB. Illustrated General of Imperfect fungi, t3rd edition. Burgess Publishing Co. Minnesota. 1972, pp.241.
6. Mathur SB. Kongsdal SO. Common laboratory seed health testing methods for detecting fungi. International Seed Testing Association, Copenhagen, Denmark, 2003, pp.357-64.
7. Adejumo TO, Ikotun T, FIorin DA. In-vitro anti-fungal properties of some botanicals on *Protomycopsis phaseoli* p. 13 in: Book of Abstract, 28 Annual Conference of Nigerian Society for Plant Protection at Cocoa Research Institute of Nigeria (CRIN), Idi-Ayunre Ibadan, 24th-28th 2000; pp.33.
8. Trease GE, Evans WC. Pharmacognosy, 13th edition, Balliere Tindall, London, 1989; pp.176-80.
9. Harborne JB. Phytochemical methods, 2nd edition, Chapman and Hall publications, London, Newyork. 1984; pp.288.
10. Muttalib LY, Naqishbandi AM. Antibacterial and phytochemical study of Iraqi *Salvia officinalis* leave extracts. *Iraqi J*

Pharm Sci 2012; **21**(1):93-7.
11. Miladinović D, Miladinović L. Antimicrobial activity of essential oil of sage from Serbia. *FU Phys Chem Technol* 2000; **2**(2):97-100.
12. Shiri Y, Solouki M, Saeidi S. Activity of some Iranian plant extracts against multi-drug resistant human pathogens isolated from urinary tract infections. *Zahedan J Res Med Sci* 2014; **16**(10):50-4.
13. Moghadam MS, Maleki S, Darabpour E, et al. Antibacterial activity of eight Iranian plant extracts against methicillin and cefixime restistant *Staphylococcous aureus* strains. *Asian Pac J Trop Dis* 2010; **3**(4):262-5.
14. Lopez A, Hudson J, Towers G. Antiviral and antimicrobial activities of Colombian medicinal plants. *J Ethnopharmacol* 2001; **77**(2):189-96.
15. Rahman MS, Khan MMH, Jamal M. Anti-bacterial evaluation and minimum inhibitory concentration analysis of Oxalis corniculata and *Ocimum santum* against bacterial pathogens. *Biotechnology* 2010; **9**(4):533-6.
16. Satish S, Raveesha K, Janardhana G. Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Lett Appl Microbiol* 1999; **28**(2):145-7.
17. S Maji S, Dandapat P, Ojha D, et al. In vitro antimicrobial potentialities of different solvent extracts of ethnomedicinal plants against clinically isolated human pathogens. *J. phytol* 2010; **2**(4):57-64.
18. Obi VI, Onuoha C. Extraction and characterization methods of plants and plants products. In: Biological and Agricultural Techniques. Ogbulie JN., Ojaiko OA (eds). Websmedia publishers, Owerri. 2000; pp.271-86.