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Full Length Research Paper

Antimicrobial activity of some medicinal plants against multidrug resistant skin pathogens

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The development of new antimicrobial agents against multidrug resistant pathogens for the treatment of skin infections is of increasing interest. Therefore, the aqueous and ethanolic extracts from different parts of five medicinal plants used locally in folk medicine were evaluated for antimicrobial activity against the most frequent skin pathogens. It was found that most plant extracts studied had antibacterial and antifungal activities. The antibacterial activities with the best minimum inhibitory concentration (MIC) values were significantly produced by the aqueous extracts of *Eminium spiculatum* stems and *Lupinus varius*, seeds against *Pseudomonas aeruginosa* and by the ethanolic extracts of *Mandragora autumnalis*, fruits against *Escherichia coli*, and Methicillin-resistant *Staphylococcus aureus* (MRSA). Whereas, the highest significant antifungal activity with the best MIC value was produced by aqueous extracts of *L. varius* seeds against *Candida albicans*. However, leaf extracts of the tested plants were appeared to produce the least antimicrobial activity. It was concluded that the antimicrobial activity is associated with the used part of plant in addition to the type of solvent used for extraction. The antimicrobial effects of some plant extracts, in particular aqueous seed extracts of *L. varius* and ethanolic fruit extracts of *M. autumnalis*, may be used for the topical treatment of skin infections.

Key words: Antimicrobial activity, methicillin-resistant *Staphylococcus aureus*, plant part, skin pathogens.

INTRODUCTION

Many efforts have been made to discover new antimicrobial compounds from various species of medicinal plants. Medicinal plants are heavily and worldwide used in folk medicine. Screening of such plants may result in the discovery of novel effective compounds against pathogenic microorganisms. The compounds that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developing new antimicrobial drugs. Jordan has a rich flora and a wide knowledge of its indigenous medicinal plants. Medicinal plants constitute an important component of flora and are widely distributed in different floristic regions of Jordan because of its geographic location, climate, and the presence of nearly 2,500 natural plant species. More than 500 species are classified as medicinal plants which are widely distributed all over the country and used for the treatment of various diseases (Al-Eisawi, 1982; Oran and Al-Eisawi, 1998; Afifi and Abu-Irmaileh, 2000).

In recent years, drug resistance to human pathogenic bacteria and fungi has been commonly reported from all over the world. Therefore, the increasing prevalence of multidrug resistant strains of microorganisms and the recent appearance of strains with reduced susceptibility to antibiotics raises an urgent need to search for new sources of antimicrobial agents (Sieradzki et al., 1999). Human infections, particularly those involving the skin and mucosal surfaces constitute a serious problem, especially in tropical and subtropical developing countries (Falahati et al., 2005). Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* were observed to be the most frequent skin pathogens. MRSA gained much attention in the past decade, as it is a major cause of hospital-acquired infections.

In this study, ethanolic and aqueous extracts of different plant parts (roots, stems, leaves, flowers, fruits, and seeds) of five medicinal plants, including; *Ecbalium elaterium*, *Eminium spiculatum*, *Gundelia tournefortii*, *Lupinus varius*, and *Mandragora autumnalis*, were screened for the presence of antimicrobial activity against

the multidrug resistant Gram-positive bacterium *S. aureus* (MRSA), Gram-negative bacteria *E. coli* and *P. aeruginosa*, and the fungus *C. albicans*. In the present study, the selection of medicinal plant parts is based on the fact that most of these plant parts were not previously screened against multidrug resistant pathogenic microorganisms.

MATERIALS AND METHODS

Preparation of plant materials and extracts

Plant parts, used in folk medicine, of five medicinal plant species were air-dried in the shade and powdered into a fine powder in a blender. The powdered plant material was soaked in water and ethanol (plant material to solvent ratio was 1:10, m/v) and extracted for 24 h at room temperature with shaking at 150 rpm. Aqueous extracts and ethanolic extracts were filtered through Whatman No. 1 filter paper. The filtrate was evaporated in a hot oven at 40°C until dry. The dried extracts were resuspended in phosphate buffer saline (500 mg/ml).

Test microorganisms and preparation of inocula

The four test microorganisms used in this study are multidrug-resistant skin pathogens; three bacteria [*E. coli* (resistant to ampicillin, chloramphenicol, erythromycin, novobiocin, and penicillin G), *P. aeruginosa* (resistant to ampicillin, chloramphenicol, erythromycin, penicillin G, and vancomycin), and *S. aureus* (MRSA) (resistant to ampicillin , erythromycin, penicillin G, and vancomycin) and one fungus [*C. albicans* (resistant to ampicillin, erythromycin, penicillin G, and vancomycin)]. Microbial cultures were grown in Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated for 24 h at 37 and 30°C, respectively. The cultures were adjusted to achieve $2x10^6$ colony forming units (CFU/ml) for bacteria and $2x10^5$ spore/ml for fungal strain. All test microorganisms are clinical strains obtained from the Hospital of Jordan and identified by conventional methods.

Antimicrobial screening tests

The antimicrobial activity of aqueous and ethanolic plant extracts was screened against frequent skin pathogens by using the agar well diffusion assay (Bauer et al., 1969). An inoculum suspension was swabbed uniformly to solidified 20 ml Mueller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for the fungus and the inoculum was allowed to dry for 5 min. Holes of 6 mm in diameter were made in the seeded agar using sterile cork borer. Aliquot of 50 µl from each plant crude extract (500 mg/ml) was added into each hole on the seeded medium and allowed to stand on the bench for one hour for proper diffusion, and thereafter incubated at 37°C for 24 h for bacteria and at 30°C for 24 h for the fungus *C. albicans*. The antimicrobial activity was evaluated by measuring the inhibition zone diameter in millimeters (mm) around the wells. Aliquots of 50 µl of phosphate buffer saline were used in the same manner as negative control. These studies were accomplished in triplicate.

Determination of minimum inhibitory concentration and minimal microbial concentration

The minimum inhibitory concentration (MIC) and minimal microbial concentration (MBC) were determined for the highly active plant parts that showed significant antimicrobial activity against the test microorganisms according to the methods of Nakamura et al. (1999) and Dulger and Aki (2009), with some modifications. A dilution series of the extract was prepared and 100 µl from each dilution transferred to 96-well microplate well. Before inoculation of 900 µl of the test organisms, the bacterial and the fungal strains were adjusted to $1x10^8$ CFU/ml in MHB and $1x10^7$ spore/ml in SDB, respectively. After inoculation, the final concentrations were in the range 248 to 1 mg/ml. The microplates were incubated for 24 h at 37 and 30°C for bacteria and for the fungus, respectively. MIC and MMC values were determined by plating 50 µl from clear wells onto MHA for bacteria and SDA for the fungus. The MIC was considered the lowest concentration of the sample that prevented visible growth. The MMC was defined as the lowest concentration yielding negative subcultures or only one colony. All samples were examined in triplicate.

Statistical analysis

The mean values (mean ± standard deviation) were statistically analyzed with SPSS Release 19.0 program by the general one-way analysis of variance (ANOVA) using Tukey's studentized range. Significant differences were considered significant at $P < 0.05$.

RESULTS

The antimicrobial activity of plant parts used in folk medicine in Jordan from five medicinal plant species has been evaluated *in vitro* against four pathogens including three bacterial species (*E. coli*, *P. aeruginosa* and *S. aureus* (MRSA)) and one fungus (*C. albicans*) that are known to cause dermic and mucosal infections besides other infections in humans.

In general, most plant extracts of the different plant parts exhibited broad spectrum of antimicrobial activity (Table 1). This was not true for plant leaves; leaves showed no or weak antimicrobial effects. All aqueous and alcohol leaf extracts, except alcohol extract of *M. autumnalis*, of the medicinal plants investigated in this study exhibited no antifungal activity against *C. albicans*. Likewise, aqueous and alcoholic leaf extracts of *E. elaterium* appeared to exhibit neither antibacterial nor antifungal activities. In comparison to other leaf extracts, leaf extract of *L. varius* showed antibacterial activity toward all test bacteria, including MRSA, ranging from 15.0±1.0 to 20.0±1.7 mm.

Table 1 illustrated that fruit extracts of *E. elaterium* (unlike leaf extracts) showed antimicrobial activity against all test microorganisms except MRSA. On the other hand, aqueous extracts of *E. spiculatum* exhibited antibacterial activity against all test microorganisms. It was observed that aqueous extracts of *E. spiculatum* stems and *L. varius* seeds exhibited the highest significant antibacterial activity against *P. aeruginosa* with mean inhibition zone equal to 29.3±1.5 and 28.7±1.5 mm, respectively. Furthermore, aqueous seed extracts of *L. varius* exhibited antimicrobial activity against all test microorganisms with the maximum significant antifungal

Table 1. Antimicrobial activity of alcohol and aqueous extracts of different plant parts of the selected plant species on skin pathogenic microorganisms.

Plant species	Plant part	Solvent	Diameter of Inhibition Zone (mm)[a]			
			E. coli	*P. aeroginosa*	MRSA	*C. albicans*
Ecbalium elaterium	Leaf	Ethanol	0^{a}	0^{a}	0^{a}	0^{a}
		Water	0^{a}	0^{a}	0^{a}	0^{a}
	Fruit	Ethanol	10.7 ± 1.5^{b}	10.0 ± 2.0^{b}	0^{a}	11.7 ± 1.2^{b}
		Water	10.0 ± 1.0^{b}	12.3 ± 1.5^{b}	0^{a}	16.3 ± 0.6^{c}
Eminium spiculatum	Leaf	Ethanol	0^{a}	0^{a}	0^{a}	0^{a}
		Water	23.3 ± 1.5^{f}	0^{a}	0^{a}	0^{a}
	Stem	Ethanol	0^{a}	17.3 ± 1.5^{c}	0^{a}	25.0 ± 2.0^{f}
		Water	20.3 ± 0.6^{e}	29.3 ± 1.5^{f}	21.7 ± 0.6^{c}	0^{a}
Gundelia tournefortii	Leaf	Ethanol	0^{a}	12.7 ± 1.2^{b}	0^{a}	0^{a}
		Water	0^{a}	0^{a}	0^{a}	0^{a}
	Root	Ethanol	10.7 ± 1.5^{b}	13.0 ± 2.0^{b}	15.7 ± 1.5^{b}	17.0 ± 1.0^{cd}
		Water	0^{a}	0^{a}	0^{a}	0^{a}
Lupinus varius	Leaf	Ethanol	$18.7 \pm 1.5d^{e}$	20.0 ± 1.7^{cd}	15.0 ± 1.0^{b}	0^{a}
		Water	17.0 ± 1.0^{d}	0^{a}	20.0 ± 2.0^{c}	0^{a}
	Seed	Ethanol	9.3 ± 1.5^{b}	$22.3 \pm 3.1d^{e}$	15.3 ± 1.5^{b}	0^{a}
		Water	13.7 ± 0.6^{c}	28.7 ± 1.5^{f}	17.0 ± 2.0^{b}	31.3 ± 0.6^{g}
	Flower	Ethanol	$19.0 \pm 1.0d^{e}$	0^{a}	24.0 ± 2.0^{d}	20.7 ± 1.5^{e}
		Water	0^{a}	$22.7 \pm 1.5d^{e}$	0^{a}	0^{a}
Mandragora autumnalis	Leaf	Ethanol	0^{a}	0^{a}	0^{a}	18.7 ± 1.5^{d}
		Water	0^{a}	0^{a}	24.7 ± 2.5^{d}	0^{a}
	Fruit	Ethanol	26.0 ± 1.0^{g}	25.3 ± 1.5^{e}	28.7 ± 1.5^{e}	25.3 ± 0.6^{f}
	plant part	Water	0^{a}	0^{a}	0^{a}	0^{a}

[a]Inhibition zone diameters are expressed as Means±SD. The means±SD within column followed by the same letter are not significantly different (Tukey's studentized range test: $\alpha = 0.05$).

effect (31.3±0.6 mm) against *C. albicans*. Interestingly, alcohol extracts of *G. tournefortii* roots and *M. autumnalis* fruits exhibited antibacterial and antifungal activity against all test microorganisms. In addition, alcohol extracts of *M. autumnalis* fruits showed the highest significant antibacterial activity against *E. coli* (26.0±1.0 mm) and MRSA (28.7±1.5 mm). Remarkably, MRSA and *E. coli* were found to be susceptible to all *L. varius* parts; both alcohol and aqueous extracts of leaf and alcohol extracts of flower showed inhibition zone values ranging from 15.0±1.0 to 24.0±2.0 mm for MRSA and from 9.3±1.5 mm to 19.0±1.0 mm for *E. coli*.

Significant antimicrobial effects, expressed as MIC and MMC of crude extracts against *E. coli*, *P. aeruginosa*, MRSA, and *C. albicans*, are shown in Table 2. Extracts of selected plants were among the most active with the MIC values ranging from 4 to 64 mg/ml and MMC values ranging from 8 to 128 mg/ml. Generally, plant extracts that were significantly exhibited the maximum inhibition zone showed the strongest MIC (the lowest) and the lowest MMC. For example, alcohol extracts of *M. autumnalis* fruits showed very strong activity against MRSA with the best MIC (4 mg/ml) and the lowest MMC value (8 mg/ml).

DISCUSSION

The current study was initiated because of the increasing resistance to antibiotics of many skin pathogens including bacteria and fungi. Plant extracts and compounds are of new interest as antiseptics and antimicrobial agents in dermatology (Augustin and Hoch, 2004). As a result, the antimicrobial activity of different medicinal plant parts extracts of five plants was screened against the most common skin pathogens. In general, leaf extracts of the selected plants appeared to be the least effective source of active antimicrobial agents. However, extracts

Table 2. Minimum inhibitory concentration and minimum microbial concentration of the highly significant active extracts of the appropriate plant part.

Name of plant	Plant part	Solvent	MIC (MMC)[a] in mg/ml			
			E.coli	***P. aeroginosa***	**MRSA**	***C. albicans***
E. elaterium	Fruit	Ethanol	64 (64)	32 (32)	-	64 (128)
		Water	64 (128)	32 (64)	-	32 (64)
E. spiculatum	Stem	Ethanol	-	16 (64)	-	16 (64)
		Water	16 (32)	8 (8)	16 (32)	-
G. tournefortii	Root	Ethanol	64 (128)	32 (32)	32 (128)	16 (128)
L. varius	Seed	Water	32 (32)	4 (8)	16 (64)	8 (32)
M. autumnalis	Fruit	Ethanol	8 (16)	8 (8)	4 (8)	16 (64)

[a]MIC is the minimum inhibitory concentration. MMC is the minimum microbial concentration.

of other plant parts showed broad-spectrum of anti-microbial activity against test microorganisms. Therefore, the antimicrobial activity is correlated to the plant part used for extraction, the extracting solvent, and the target microorganism. This finding is in agreement with Dogruoz et al. (2008) who reported that the bacterial inhibition can vary with the plant extract, the solvent used for extraction, and the organism tested. In previous studies (Aburjai et al., 2001; Darwish and Aburjai, 2010) it was reported that methanolic extracts of the whole plant material of *G. tournefortii* exhibited antibacterial activity against multidrug-resistant *E. coli* and *P. aeruginosa*. However, the results of the current study demonstrated that only root extracts of *G. tournefortii*, but not the whole plant parts, are responsible for antimicrobial properties. Therefore, the results of this study illustrated that the plant part used for extracting antimicrobial agents is important and played a significant role in production of antimicrobial effects.

It was observed that the Gram-positive bacterium MRSA and the eukaryotic fungus *C. albicans* were the least sensitive microorganisms to leaf extracts of selected plant species compared with the other test bacteria (Gram-negative *E. coli* and *P. aeruginosa*). This variation in sensitivity between those microorganisms may be due to their differences in cell wall composition and in ribosomal proteins. Similar observation was reported by Oskay and Sar (2007). It was interesting to note that MRSA and *C. albicans* showed more sensitivity to the investigated extracts of the other plant parts (stem, flower, fruit, and seed). This has clearly indicated that extracts of those plant parts might have different modes of action on test organisms. In comparison to extracts of other plant species investigated in this study, extracts of *L. varius* were found to exhibit a wide spectrum of antimicrobial activity. Thus, extracts of *L. varius* appeared to be the best source for antimicrobial effects on bacteria and yeasts with dermatological relevance.

In conclusion, the results of this study clearly indicated that the antibacterial and anticandidal activity vary with the species of the plants, plant part used, solvent type, and microorganism tested. Moreover, this study established a good base for developing future drugs for treatment of infectious diseases caused by *C. albicans* and some pathogenic bacteria such as MRSA, *P. aeruginosa*, and *E. coli*. Further, the active phyto-compounds of these plants against multidrug-resistant bacteria and *C. albicans* should be characterized and their toxicity should be evaluated *in vivo*.

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