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In Vitro Antibacterial, Antifungal and Anti-Mycobacterium Activity of Selected Lamiaceae and Asteraceae Species

Berrin Özçelik[1*], Rachid Belhattab[2], İlkay Erdoğan Orhan[3]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Gazi University 06330, Ankara, Turkey.

[2]Department of Biochemistry, Faculty of Nature and Life Sciences, Ferhat Abbas University, 19000, Setif, Algeria.

[3]Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, 06330, Ankara, Turkey.

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Abstract

In this study, six extracts obtained from various plants of Lamiaceae and Asteraceae families were screened *in vitro* against Gram negative and Gram positive bacteria, as well as *Mycobacterium tuberculosis* and two yeasts. Acetone extracts of *Origanum glandulosum, Marrubium vulgare* and *Artemisia herba alba* as well as ethanol extracts of *Artemisia herba alba*, *A. absinthium* and *A. fragrance* were used. Activity of the extracts were screened against *Escherichia coli, Salmonella enteritidis; Staphylococcus aureus, Enterococcus faecalis; Candida albicans, Candida krusei* and *Mycobacterium tuberculosis* H37Rv using micro-dilution method according to the Clinical and Laboratory Standards Institute. All the extracts showed antimicrobial activity against Gram positive and Gram negative bacteria at concentrations ranging between 64-256 µg/mL. Anti-Candida activities of the extract developed the same MIC values towards all the extracts tested (MIC: 128 µg/mL) whereas *A. herba alba* ethanol extract was more effective against *C. krusei* at the same potency as the standard fluconazole (MIC: 64 µg/mL). Anti-mycobacterium activity was at the same MIC values of 128 µg/mL for all the extracts tested. Tested total extracts showed antimicrobial activity with higher MIC values than standard antimicrobial agents. Fractions of extracts or isolated metabolites could prove to be more potent against pathogenic microorganisms.

1. Introduction

Numerous works dealt with the antimicrobial activity of essential oils and extracts obtained from different plant origins (Lopez-Lutz, Alviano, Alviano & Kolodziejczyk, 2008). Generally, those dealing with essential oils are the plants belonging to the Lamiaceae family since they are known to be rich in monoterpene fraction which bears the antimicrobial activity. According to Lawrence, plant species can be divided in two groups: rich and poor containing essential oils (Lawrence, 1992). *Origanum* genus, comprises 38 species widespread in the Mediterranean basin and Eurasia belongs to the former whereas the genus *Marrubium* which comprises more than 30 species belongs to the latter. *Origanum glandulosum* is endemic to North Africa and largely used in folk medicine against cold and rheumatism. *Marrubium vulgare* known as horehound is used in Algerian folk medicine for the treatment of pain, especially those related to the digestive tract.

Artemisia species (Asteraceae family) are widespread over the world; there are about 22 species of *Artemisia* genus in Turkish flora (Kordali, Cakir, Mavi, Kilic & Yildirim, 2005) and 13 species in Algerian flora (Ozenda, 2004). They have a characteristic scent or taste due to the monoterpenes and sesquiterpenes. *Artemisia* species are not only added to beverages but also used to cure several illnesses such as digestive disorders and diabetes. Many studies have been carried out using essential oils from these plants to evaluate their antimicrobial activities (Bendahou et al., 2009), little is known about the potential of their organic extracts.

Due to the limited antimicrobial potential of antibiotics and in some cases to the side effects they develop, much attention has been paid during the last decades to new antimicrobials of plant origin. Thus, the aim of this work was to investigate the potential of extracts obtained from several species belonging to the Lamiaceae family such *Origanum glandulosum* Desf. and *Marrubium vulgare* L. and Asteraceae family such as *Artemisia herba alba* Asso. and *A. absinthium* growing wild in Algeria and *A. fragrance* from Turkey to inhibit microorganisms growth.

2. Material and Methods

2.1. Plant collection and preparation of extracts

Origanum glandulosum, Marrubium vulgare and *Artemisia herba alba* used for the acetone extract were collected at flowering stage (June 2008) in Setif region (Algeria); *Artemisia herba alba* as well as *A. absinthium* used for the ethanol extract were collected at the flowering period from the same origin too but one year later (June 2009). *A. fragrance* was collected in Turkey. A voucher specimen of each plant was deposited at the Department of Biology, F.A.University, Setif (Algeria).

The plant samples were air dried at room temperature before being pulverized. For the extraction with acetone a Soxhlet apparatus was used. Plant material from *Origanum glandulosum, Marrubium vulgare* and *Artemisia herba alba* was exhaustively extracted. The remaining extracts were obtained from *Artemisia* species, *A. herba alba, A. absinthium* and *A. fragrance* by maceration in the ethanol. All the extracts were evaporated to dryness in a rotary evaporator (Büchi) to determine the yields.

***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com

2.2. Microbiological studies

The extracts were dissolved in dimethylsulfoxide (1:1) at a final concentration of 1024 µg/mL and used as stock solutions. Ampicillin, ofloxacine, amphotericin B and fluconazole were used as the standard antibacterial and antifungal drugs. Reference antibacterial agents of ampicillin (AMP), ofloxacin (OFX) reference antifungal agents of amphotericin (AMP) and fluconazole (FLU) were purchased from Sigma Chemical Co. (St.Louis, MO, USA) and were dissolved in phosphate buffer solution (ampicillin**,** pH: 8.0; 0.1 µmol/mL), dimethylsulphoxide (amphotericin B), or in water (ofloxacin, fluconazole). The stock solutions were dissolved in liquid media according to the Clinical and Laboratory Standards Institute (CLSI, 2006; CLSI, 2008).

Bioactivity tests were carried out against standard (ATCC; American Type Culture Collection, RSKK; Culture Collection of Refik Saydam Central Hygiene Institute) strains. As standards; Gram negative strains of *Escherichia coli* ATCC 25922, *Salmonella enteritidis* RSKK, and as Gram positive strains of *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212 were used for the determination of antibacterial activity. *C. albicans* ATCC 10231 and *C. krusei* ATCC 6258 were used for the determination of antifungal activity. Mueller Hinton Broth (MHB; Difco) and Mueller Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria suspensions (Özçelik, Kartal & Orhan, 2011). The synthetic medium RPMI-1640 with L-glutamine was buffered to pH: 7 with 3-[N-morpholino]-propansulfonic acid and culture suspensions were prepared as described previously (Özçelik, Orhan, Kartal & Konuklugil, 2010). The microorganism suspensions used for inoculation were prepared at 10^5CFU (colony forming unite/mL) by diluting fresh cultures at McFarland 0.5 density (10^8 CFU/mL) (Orhan, Özçelik, Ozgen & Ergun, 2010). Suspensions of bacteria and fungi were added in each well of the diluted extracts, density of 10^5 CFU/mL, both fungi and for bacteria. The bacterial suspensions used for inoculation were prepared at 10^5 CFU/mL by diluting fresh cultures at McFarland 0.5 density (10^8 CFU/mL). The inhibitory effects were visually checked and minimal inhibitory concentration corresponds to the lowest concentration which led to an absence of growth. Microplate Alamar Blue Assay was used for determine anti-mycobacterium activity. *Mycobacterium tuberculosis* H37Rv ATCC 27294 (American Type Culture Collection) was subcultured on Middlebrook 7H11 agar (Becton Dickinson). Suspensions were prepared in 0.04% (vol/vol) Tween 80–0.2% bovine serum albumin so that their turbidities matched that of a McFarland no. 1 turbidity standard. Controls isoniazid (INH) and ethambutol (EMB) were obtained from Sigma. Stock solutions of INH and EMB were prepared in deionizer water. Sterile deionizer water was added to all outer-perimeter wells of sterile 96-well plates to minimize evaporation of the medium in the test wells during incubation. 100 microliters of 6 drug solutions were added to the wells in rows B to G in columns 2 and 3, by using a multichannel pipette, 100 µl was transferred from column 3 to column 4, and the contents of the wells were mixed well. Identical serial 1:2 dilutions were continued through column 10 and 100 µl of excess medium was discarded from the wells in column 10. Final drug concentration ranges were as follows for INH, from 0.031 to 8.0 µg/mL, for EMB

***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com

Table 1. Antimicrobial activity as MICs (µg/mL) of the extracts

	Microorganisms						
	Gram negative bacteria		**Gram positive bacteria**		**Yeast like Fungi**		**Mycobacterium**
Extracts	***E. coli* ATCC 25922**	***S. enteritidis* RSKK 538**	***S. aureus* ATCC 25923**	***E. faecalis* ATCC 29212**	***C. albicans* ATCC 10231**	***C. krusei* ATCC 6258**	***M. tuberculosis* H37Rv ATCC 27294**
Og-EtOH	128	128	256	128	128	128	128
Mv-EtOH	128	128	256	128	128	128	128
Aa-EtOH	128	128	256	64	128	128	128
Af-EtOH	128	128	256	64	128	128	128
Ah-ac	128	128	256	128	128	64	128
Ah-EtOH	128	128	256	128	128	128	128
AMP	1	0.5	0.5	0.25	-	-	-
OFX	0.25	0.25	0.25	1	-	-	-
AmpB	-	-	-	-	<0.25	<0.25	-
FLU	-	-	-	-	4	64	-
INH	-	-	-	-	-	-	0.125
EMB	-	-	-	-	-	-	4

Og: *Origanum glandulosum* Desf.; *Mv*: *Marrubium vulgare* L.; *Aa*: *Artemisia absinthium*; *Af*: *Artemisia fragrance*; *Ah*: *Artemisia herba alba* Asso.; AMP: ampicillin, OFX: ofloxacin, FLU: fluconazole, Amp B: amphotericin B, INH: Isoniazid, EMB: ethambutol; -: not tested; EtOH: ethanol; *ac*: acetone.

0.5 to 128 µg/mL. One hundred microliters each of *M. tuberculosis* inoculum was added to the wells in rows B to G in columns 2 to 11. The plates were sealed with parafilm and were incubated at 37°C for 5 days. 50 microliters of a freshly prepared 1:1 mixture of 10X Alamar Blue (Invitrogen) reagent and 10% Tween 80 was added to well B11. The plates were reincubated at 37°C for 24 h. If well B11 turned pink, the reagent mixture was added to all wells in the microplate. The microplates were resealed with parafilm and were incubated for an additional 24 h at 37°C, and the colors of all wells were recorded. A blue color in the well was interpreted as no growth, and a pink color was scored as growth. A few wells appeared violet after 24 h of incubation, but they invariably changed to pink after another day of incubation and thus were scored as growth. The MIC was defined as the lowest drug concentration which prevented a color change from blue to pink (Franzblau et al.,1998). All were tested in triplicate in each run of the experiments.

3. Results

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Many of screening efforts in pharmaceutical fields have been made to find new antimicrobial natural or synthetic compounds that control infectious caused by bacteria, fungi and other microorganisms.

***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com

Bioactivity as antibacterial, antifungal, and anti-mycobacterium susceptibility screening test was employed to evaluate the activity extracts of *Origanum glandulosum* Desf., *Marrubium vulgare* L., *Artemisia herba alba* Asso., *A. absinthium*, and *A. fragrance* obtained by different procedures against *E. coli*, *S. enteritidis*, *S. aureus*, *E. faecalis*, *C. albicans*, *C. krusei*, *M. tuberculosis* of clinical importance. The medicinal plants belong to Lamiaceae and Asteraceae families. The results of the antibacterial, antifungal and anti-mycobacterium activity of all the extracts tested are presented in Table 1. As shown in Table 1, the MIC values range from 64 µg/mL as the most potent to 128 µg/mL as the least potent.

The extracts showed same antimicrobial activity against Gram positive bacterium *S. enteritidis*, Gram negative bacterium *E. coli* as well as fungus *C. albicans* at MIC values of 128µg/mL. Although the inhibition concentration was high all the extracts showed weak activity against *S. aureus* (MIC: 256 µg/mL). The most potent plant extracts with MIC values of 64µg/mL were ethanol extracts of *A. absinthium* and *A. fragrance* against Gram positive strain of *E. faecalis.*

The same effect was developed with *A. herba alba* ethanol extract against *C. krusei* (MIC 64µg/mL). *Artemisia* species show the same effects towards all the microorganisms tested with some exceptions. *A. absinthium* and *A. fragrance* ethanol extracts demonstrated the same effect against *E. faecalis* (MIC 64 µg/mL), therefore the highest effect exhibited by *A. herba alba* ethanol extract was observed with *C. krusei* (MIC 64 µg/mL). The MICs values developed by the standard ampicillin were at a range of 0.25 to 1 µg/mL. The antimicrobial standard ofloxacin exhibited the same effect on *E. coli*, *S. enteritidis* and *S. aureus* with MIC values of 0.25 µg/mL with the exception of *E. faecalis* (MIC 1 µg/mL).

O. glandulosum and *M. vulgare* on one hand and *A. absinthium* and *A. fragrance* on the other hand developed the same effects against all the microorganisms tested. *A. herba alba* extracts obtained by acetone and ethanol show similar effects with an exception when tested on *C. krusei* (MICs 64 µg/mL and 128 µg/mL respectively). *C. albicans* developed the same sensitivity to all extracts tested (MIC 128 µg/mL). The same results have been obtained with *C. krusei* with an exception with *A. herba alba* ethanol extract (MIC 64 µg/mL). Controls fluconazole was more effective against *C. albicans* than *C. krusei* (MIC: 4 µg/mL and 64 µg/mL respectively), whereas amphotericin B MIC values were <0.25µg/mL for both yeasts.

The results obtained from anti-mycobacterium activity tested against *M. tuberculosis* H37Rv, all the extracts demonstrated the same effect with MIC value of 128µg/mL, since anti-mycobacterium activity values demonstrated for the controls INH and EMB at MIC: 0.125 µg/mL, and 4 µg/mL, respectively.

4. Conclusion

Essential oils of Lamiaceae species have been an interest of study because they are rich in monoterpenes, molecules with proved antimicrobial activity. *O. glandulosum*, an endemic species in North Africa, can be divided in several chemotypes depending on the thymol-carvacrol balance (Ruberto, Baratta, Sari & Kaabeche, 2002). Samples in this study were collected in the same site of that previously used for essential oils and glycosidic bond volatiles (Belhattab et al., 2005).

***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com

The constituents of that oil were dominated by carvacrol. The acetone extract of this plant is also rich in polyphenols such as quercetin and rosmarinic acid (Belhattab, Larous, Kalantzakis, Boskou & Exarchou, 2004). The oils as well as the hexane and water extracts of this plant have already been tested against some saprophytic moulds where they exhibited interesting inhibitory effects (Belhattab et al., 2004). In another previously published study, it was reported that the essential oils from *Tarchonanthus camphoratus* (Asteraceae), were found to be active against all the tested bacterial strains (*S. aureus*, *Bacillus ssp*., *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumoniae*, *P. mirabilis*) except for *Pseudomonas aeruginosa*. Although, it is reported that the extract concentrations generally were in the range of 100 times more than the standard antibiotic chloramphenicol (Matasyoh, Kiplimo, Nicholas & Karubiu, 2007). In another study, antimicrobial activity was researched with disc diffusion test on the extract of *Thymus vulgaris* L. (Lamiaceae), *Rosmarinus officinalis* L. (Lamiaceae), *Melissa officinalis* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae), *Ocimum basilicum* L. (Lamiaceae), and *Achillea millefolium* L. (Asteraceae). This document revealed that all extracts have shown variability in the inhibitory concentrations (50 to 500 µg/mL, and/or; 20 to 250 µg/mL, for tested bacteria (*E. coli*, *P. aeruginosa*, *Shigella spp.*, *K. pneumoniae*, *Proteus spp*., *S. aureus*, *E. aerogenes*). Similarly, in one study it is reported that MIC varying from 12.5 to 1,000 µg/mL of *Rhus glaba* extracts both of tested Gram negative and Gram positive bacteria (Saxena, McCutcheon, Farmer, Towers & Hancock, 1994).

In our study, all the extracts showed inhibition at concentration ranging of 64-256 µg/mL against Gram positive and Gram negative strains tested. To our knowledge, it's the first study dealing with antimicrobial activity of the extracts from *Origanum glandulosum* Desf. (Lamiaceae), *Marrubium vulgare* L. (Lamiaceae), *Artemisia herba alba* Asso. (Asteraceae), and *A. absinthium* (Asteraceae), growing wild in Algeria and *A. fragrance* from Turkey. It can be concluded from the above that acetone and ethanol extracts obtained from these selected medicinal plants have a antimicrobial activity, but further studies by different methodologies are needed to complete the research in the activities and to identify the bioactive constituents of the plants.

Conflicts of Interest

The author declares no conflicts of interest related to this study.

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***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com

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***Corresponding author:** Berrin Özçelik
E-mail: microberr@yahoo.com