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A comparative evaluation of *Juniperus* species with antimicrobial magistrals

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Abstract: The objective of this study was to evaluate the *in vitro* bioactivity of the active ingredient in selected antimicrobial magistral drug formulations and plant extracts used in folk medicine, comparatively. The active ingredients of magistrals such as; boric acid, balsam of Peru, zinc oxide, *Calendula* tincture, thymol, resorcinol, crystal violet were used as well as fruit or leaf extracts of *Juniperus excelsa* (*Je*), *J. sabina* (*Js*), *J. foetidissima* (*Jf*), *J. communis ssp. nana* (*Jcsn*), *and J. oxycedrus* spp. *oxycedrus* ripe (*Joso*) to determine the antimicrobial activity against gram positive bacteria (*S. pyogenes*, *S. aureus*, *S. epidermidis*, *E. faecalis*), gram negative bacteria (*K. pneumoniae, H. influenza*, *P. aeruginosa*, *A. baumannii, E. coli)*, and fungi (*Candida. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*) by using microdilution method. The inhibition end point of the minimum inhibition concentrations (MICs) were determined as µg mL^{-1}. The active ingredient and plant extracts have shown antibacterial and antifungal activities with a MIC values of 1->128µgmL^{-1}. The active ingredient crystal violet (MIC; 1 µg mL^{-1}) as well as *Je*- fruit ethanol, *Jf*-leaf and fruit ethanol, *Joso*-leaf and fruit ethanol extracts (MIC; 16µg mL^{-1}) have exhibited the highest antimicrobial activities (MIC; 16µg mL^{-1}). Although ingredients of magistrals seem to exert similar antifungal activity against *C. albicans, C. tropicalis, and C. parapsilosis* (MIC; 32µgmL^{-1}), thymol and resorcinol were observed to be more active against *C. krusei* (MIC; 16µg mL^{-1}). Extracts were more pronounced against *P. aeruginosa, A. baumannii,* and *S. epidermidis* (MIC ranging from 16 to 32). In the mine time, the extracts showed equal antifungal activity against *C. albicans and C. parapsilosis* (MIC; 16µg mL^{-1}). In our study, antimicrobial activity of the natural compounds and ingredients of selected magistrals have found to be promising with MIC values of 16-32µg mL^{-1}. According to the results of our antimicrobial activity studies, utilization of *Juniperus* extracts in antimicrobial magistral formulations can be suggested.

Keywords: Medicinal plant, antibacterial, antifungal, magistrals, *Juniperus.*

INTRODUCTION

Magistral drugs are the preparations, mixed, assembled, packed and labeled drugs prepared by a licensed pharmacist to meet the unique needs of an individual patient when commercially available drugs do not meet those needs. They can also contain and provide compounds that are not available and involved in commercial medicinal formulations. Pharmaceutical formulations as magistral drugs can play a vital or important role to find an appropriate treatment for a patient. Despite the large role of technology and manufacturers play in the development and production of pharmaceuticals from natural or synthetic compounds, many pharmacists continue to work with researchers to come up with improved or entirely new medicinal compounds (Allen, 2012).

Plants are also well-known to be rich sources of bioactive compounds. Traditional healers have long used plants to prevent or cure infectious diseases. Many of these agents appear to have structures and modes of action that distinct from those of the antibiotics in current use. So, it is worthwhile to study plants and plant products for their activities against microorganisms. One approach that has been used for the discovery of antimicrobial agents from plants based on the evaluation of traditional medicinal plant extracts. Throughout history books arranged for drugs and pharmacopeia, which includes the magistral drugs were discovered (Allen, 2012).

The active substances that are used in antimicrobial magistrals in Turkey are boric acid, balsam of Peru, zinc oxide, *Calendula* tincture, thymol, resorcinol, and crystal violet etc. Boric acid is odorless, slight pearl sheen colorless crystals or white powder that is soluble in water, ethanol and glycerin. It has a weak topical bacteriostatic and fungistatic activity (Prutting and Cerveny, 1998). Balsam of Peru is a dark brown, viscous liquid that is non-drying. It is practically insoluble in water but freely soluble in dehydrated alcohol. It is used for topical treatment of superficial skin lesions because of its high cinnamic and benzoic acid contents which have antiseptic activity (Ph. Eur., 2009). Zinc oxide is a white powder that is insoluble in water. It exhibits antimicrobial activity through different mechanisms and used as preservative in topical pharmaceutical formulations (Pasquet *et al*., 2014). *Calendula officinalis* is a medicinal plant belonging to the family Asteracea. It is used in phytotherapy because of its antibacterial, anti-

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inflammatory and wound healing properties (Ferreira *et al.*, 2014). Thymol (2-isopropyl-5-methylphenol) obtained from thyme has a broad antimicrobial activity spectrum against Gram positive bacteria (*Staphylococci, Enterococcus* spp.), Gram negative bacteria (*E. coli, Pseudomonas aeruginosa*) as well as *Candida* species (JP 15, 2006). Resorcinol, freely soluble in water, glycerin, ethanol, slightly soluble in chloroform, is an odour, white powder or crystals becomes red on exposure to light and air. It is used as antiseptic and disinfectant and in the treatment of skin infections such as acne and seborrheic eczema (PPRC, 1992). Crystal violet is a triphenylmethane dye that has antimicrobial activity and used to prevent fungal growth in poultry feed, as a bacteriostatic agent in medical solutions and to treat skin infections in humans and animals (Jones and Falkinham, 2003).

Juniperus is a large genus of *Cupressaceae* family that is about 70 species. *Juniperus* is widely used in traditional medicine to decrease blood pressure in dysmenorrhea, cough, bronchitis, and colds (Moein *et al*., 2010). *Juniperus communis ssp. nana* is known in Turkey as "Bodur ardıç" and it is a small tree about 4m height. In traditional medicine *J. communis* has been used to treat wounds and inflammatory diseases as well as abortion (Chandra and Prasad, 2010). *Juniperus foetidissima* is called "Kokulu ardıç" In Turkey and it is a high tree ranging from10 to 20 m. It is used in the treatment of colds, urinary tract infections, dermatological diseases, pneumonia, rheumatic diseases and stomach pain (Lesjak *et al.,* 2013). *Juniperus oxycedrus* (katran ardıcı) is a tree having antiparasitic, antimicrobial and antiseptic activities (Karaman *et al.,* 2003). Additionally, leaves, fruits, tars and volatile oils of other *Juniperus* species are used as traditional medicine in different regions of Anatolia.

Since the increasing need of the broad spectrum antimicrobial magistral drugs, it is now become essential to look for newer pharmaceutical products. Therefore, nowadays many researchers have been making efford to find new antimicrobial and antifungal agents from natural or synthetic origins for a variety of newly active compounds with different molecular targets against bacteria and yeast like fungi.

This study is aimed to compare the similarities and differences between the antimicrobial activity of *Juniperus* extracts (ethanol and water extracts of fruits and leaves of *Je, Jcsn, Js, Jf*, and *Joso*) and active ingredients in magistral drugs (boric acid, balsam of Peru, zinc oxide, *Calendula* tincture, thymol, crystal violet) against gram positive bacteria *(S. pyogenes, S. aureus, S. epidermidis, E. faecalis),* gram negative bacteria (*K. pneumoniae, H. influenza, P. aeruginosa, A. baumannii, E. coli*), and fungi (*Candida albicans, C. tropicalis, C. parapsilosis, C. krusei*) by using the micro dilution method. Besides, the study was also focused on the use of natural compounds instead of the active ingredients of magistral drugs by determining minimal inhibition concentrations (MIC; $\mu g\ mL^{-1}$).

MATERIALS AND METHODS

Plant material

Juniperus species were collected in 2007 from the vicinities of Yozgat province, Turkey and were identified by Assoc. Prof. Dr. Nilüfer ORHAN. The voucher specimens have been stored in the Herbarium of Faculty of Pharmacy (GUEF), Gazi University, Ankara, Turkey. The herbarium (GUEF) numbers of the plants are given as follows; *Juniperus oxycedrus L.* ssp. *oxycedrus* (GUEF 2616), *J. communis* ssp. *nana* (Willd.) Syme (GUEF 2617), *J. sabina* L. (GUEF 2618), *J. foetidissima* Willd. (GUEF 2619), *J. excelsa* M. Bieb. (GUEF 2754).

Preparation of the extracts

Preparation of the ethanol extracts (EtOH Ext.): The chopped dried leaves (100g) and fruits (100g) were extracted with ethanol 80% (1 and 2L) by mixer for 8h individually. The following day, the extracts were filtrated and the residues were extracted by the same procedure with ethanol again. The filtrates were pooled and evaporated to yield dry extracts under reduced pressure.

Preparation of the aqueous extracts (H_2O Ext.): the chopped dried leaves (100g) and fruits (100g) were extracted with 5L distilled water by mixer for 8h individually. The following day, the extracts were filtrated and evaporated to yield dry extracts under reduced pressure. The yield percentages of the ethanol and aqueous extracts were given in table 1.

Active ingredients in magistral formulations

Boric acid, balsam of Peru, zinc oxide, *Calendula* tincture, thymol, crystal violet, and brilliant green were purchased from Botofarma Co.

Antimicrobial activity

Four Gram positive bacterial strains (*Streptococcus pyogenes* ATCC49766, *Staphylococcus aureus* ATCC 10145, *Staphylococcus epidermidis* ATCC02026, *Enterococcus faecalis* ATCC29212), five gram negative bacterial strains (*Klebsiella pneumonia* ATCC07005, *Haemophilus influenza* ATCC6633, *Pseudomonas aeruginosa* ATCC25923, *Acinetobacter baumannii* ATCC12228, *Escherichia coli* ATCC25922) and four fungi (*Candida albicans* ATCC10231*, Candida tropicalis* ATCC13803*, Candida parapsilosis* ATCC90028, *Candida krusei* ATCC6258), were used for the determination of antimicrobial activity. Culture suspensions, stock solutions, and inoculums were prepared according to the methods of the Clinical and

Table 1: Yields% (w/w) of the H_2O and EtOH extracts from the leaf and ripe fruits of the studied *Juniperus* species.

Plant Name	Leaf		Ripe Fruit
	H_2O Ext.	EtOH Ext.	EtOH Ext.
J. oxycedrus ssp. oxycedrus	17.1	35.2	33.3
J. communis ssp. nana	16.6	29.1*	36.2
J. sabina	16.3*	27.6	19.8
J. foetidissima	16.3	32.5	22.5
J. excelsa	22.2	33.6	33.2

*: Not tested

Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards (NCCLS). All materials were dissolved in dimethylsulfoxide (DMSO) and sterilized by filtration using 0.22 mm Millipore (MA 01730, USA) and used as the stock solutions. Mueller-Hinton Broth (MHB; Difco) and Mueller-Hinton Agar (MHA; Oxoid) were applied for growing and diluting of the bacteria suspensions. 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 *et al.,* 2011; Özçelik *et al.,* 2012; Piraset *al*., 2013). Reference antimicrobial agents were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and dissolved in phosphate buffer solution (ampicillin, pH 8.0; 0.1mol/mL), dimethylsulphoxide (ketoconazole), or in water (gentamicin, fluconazole, meropenem).

Broth microdilution assay was carried out for antibacterial and antifungal activity tests. Media was placed into each 96 wells of the microplates. Solutions to be tested at 512 μgmL^{-1} were added into first rows of microplates and twofold dilutions of the solutions (256-0.125μgmL^{-1}) were made by dispensing the solutions to the remaining wells. The lowest concentration of the compounds that completely inhibit macroscopic growth was determined and MICs were (Özçelik *et al.,* 2011; Özçelik *et al.,* 2012; Piraset *al*., 2013).

RESULTS

The minimum inhibition concentration (MIC $\mu g\ mL^{-1}$) of the *Juniperus* water and ethanol extracts (prepared from *Jcsn, Je, Jf, Joso, Js* fruits and leaves) and active ingredients in some magistral formulations (boric acid, balsam of Peru, zinc oxide, *Calendula* tincture, thymol, resorcinol, crystal violet), which were tested by using microdilution method to determine antimicrobial activity against bacteria (*Streptococcus pyogenes, Staphylococcus aureus, S. epidermidis, Enterococcus faecalis, Klebsiella pneumonia, Haemophilus influenza, Pseudomonas aeruginosa, Acinetobacter baumannii*, *Escherichia coli*) and fungi (*Candida albicans, C. tropicalis, C. parapsilosis*, *C. krusei*) are shown in table 2 and table 3.

According to the results the highest antibacterial activity was detected against gram positive bacteria (*S. pyogenes, S. aureus, S. epidermidis, E. faecalis*) with a MIC values of $1\mu gmL^{-1}$; while the lowest antibacterial activity were seen with a MIC values of $32\mu g\ mL^{-1}$. As shown table 2 and table 3 active magistral ingredient crystal violet showed high activity against gram positive bacteria and fungi with a MIC values of $1\mu gmL^{-1}$. On the other hand, plant extracts *J. excelsa* ripe fruit-EtOH, *J. foetidissima* leaf-EtOH, *J. foetidiss.* ripe fruit-EtOH, *J. oxycedrus* spp *oxycedrus* fruit-EtOH, and *J. oxycedrus spp. oxycedrus* leaf-EtOH (MIC; $16\mu g\ mL^{-1}$) showed the highest antimicrobial activity. Boric acid, balsam of Peru, zinc oxide *Calendula tincture,* timol and resorcinol possessed the highest antibacterial activity against *A. baumannii* and *S. epidermidis* (MIC; $16\mu g\ mL^{-1}$). Although ingredient of magistrals seems to exert similar antifungal activity against *Candida albicans*, *C. tropicalis* and *C. parapsilosis* (MIC; $32\mu g\ mL^{-1}$), thymol and resorcinol were observed to be more active against *C. krusei* (MIC; $16\mu g\ mL^{-1}$). *Juniperus* water and ethanol extracts (prepared from *Jcsn, Je, Jf, Joso, Js* fruits and leaves) were more pronounced against *P. aeruginosa*, *A. baumannii*, and *S. epidermidis* (MIC ranging from 16 to 32). In the mine time, the fractions showed equal antifungal activity against *C. albicans* and *C. parapsilosis* (MIC; $16\ \mu g\ mL^{-1}$) (table 2, table 3).

On the other hand, active ingredient of magistral and plant fractions were shown antifungal activity against fungi *C. albicans, C. tropicalis, C. parapsilosis,* and *C. krusei* with MIC ranging from 1 to $128\mu gmL^{-1}$. The highest antifungal activity was detected by crystal violet with MIC values of $1\mu gmL^{-1}$ (table 3).

DISCUSSION

Antimicrobial activity of a number of medical plant extracts (*Je* leaf and fruit EtOH, *Je* leaf H_2O*, Jcsn* fruit EtOH, *Jcsn* leaf H_2O*, Js* leaf and fruit EtOH*, Jf* leaf and fruit EtOH*, Jf* leaf H_2O, *Joso* leaf and fruit EtOH*, Joso* leaf H_2O extract), which are used for the prevention and in the treatment of many diseases and the active ingredient of magistral (boric acid, balsam of Peru, zinc

oxide, *Calendula* tincture, thymol, resorcinol, crystal violet)were evaluated against *Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Klebsiella pneumoniae, Haemophilus influenza, Pseudomonas aeruginosa, Acinetobacter baumannii*, *Escherichia coli*, and four fungi *Candida albicans, C.tropicalis, C. parapsilosis*, *C. krusei* by using the microdilution method. Minimum inhibition concentrations (MICs; µg mL^{-1}) were reported. Results of comparative antibacterial and antifungal activities of medical plant extracts and in table 2, and table 3 antimicrobial activity were seen with a MIC ranging from 16 to 128µg mL^{-1}.

The antibacterial effects of different concentration of boric acid in root canals that were infected with *E. faecalis* biofilms were investigated. 6% concentration of boric acid showed the highest antibacterial activity (69%) (Zan *et al*., 2013). According to our results obtained from antibacterial activity test boric acid was found to be moderately effective against *A. baumannii* and *S. epidermidis* (16µg mL^{-1}), while there were less active against *K. pneumoniae, H. influenza, P. aeruginosa, E. coli*, *S. pyogenes, S. aureus,* and *E. faecalis* (32µg mL^{-1}).

In one study, medicinal plants including balsam of Peru were researched for antimicrobial activity against *E. coli* and *S. aureus* by using an agar diffusion method. Aqueous and ethanol extracts of Peru balsam did not showed activity against *E. coli and S. aureus*. Whereas the ethanol extract showed 12 mm inhibition zone against *S. aureus* (Bussmann *et al*., 2010). In our study, magistral ingredient of Peru balsam possess antimicrobial activity against gram negative bacteria and gram positive bacteria with a MIC value 32µg mL^{-1} and 64 µg mL^{-1}. It showed antifungal activities against Candida genus with a MIC value of 32µg mL^{-1} apart from *C. krusei* with a MIC value 128 µg mL^{-1}.

In another study, the antimicrobial activity of zinc oxide nanoparticle was investigated against *S.aureus.* The MIC for the small size of zinc oxide nanoparticles was determined to be 80 mg mL^{-1}, while the big size of zinc oxide nanoparticles was 1.2 mmL^{-1} (Jones *et al.,* 2008). In current study, zinc oxide exhibit more antibacterial activity with a MIC value 16µg mL^{-1} against *A. baumannii* and *S. epidermidis* than against *K. pneumoniae, H. influenza, P. aeruginosa, E. coli*, *S. pyogenes, S. aureus,*and *E. faecalis* (32µg mL^{-1}).

In one of the earliest study, the antimicrobial effect of ethanol crude extract of petals and reproductive parts of flowers derived from *Calendula officinalis* in different concentrations were determined against *S. aureus, S. epidermidis*. *P. aeruginosa*, *E. coli, K. pneumonia* by using agar diffusion method. The inhibition zone for extracts of petals part against *P. aeruginosa* was 25 mm in the concentration 100 mg mL^{-1}, whereas the inhibition zone against *S. aureus* was 14 mm in the concentration 50 mg mL^{-1}. The extracts of reproductive parts were less effective than the petal extracts (Hamad *et al.,* 2011). In our study, *Calendula* tincture exhibit antimicrobial activity with a MIC ranging from 16 to 64 µg mL^{-1}

The antimicrobial activity of thymol was tested against *C. albicans* using the broth dilution method in one previous study. The minimum inhibitory concentration for thymol indicated to be MIC value of 0.625 µgmL^{-1} (Botelho *et al.,* 2007). However, thymol was shown antimicrobial effective with a MIC ranging from 16 to 32 µg mL^{-1}, in our study.

Dibrominated resorcinol dimers were synthesized to evaluate the antimicrobial activity by using broth dilution assay against *S. aureus, E. coli* and *C. albicans*. It is reported that the MICs against bacteria were ranging between 0.78->100 µg $mL^{-1}$and the MIC against fungi was ranging between 12.5 ->100 µg mL^{-1} (Jones *et al.,* 2008). The data obtained from our study showed that resorcinol had antimicrobial activity with a MIC ranging from 16 to 32 µg mL^{-1}.

Moreover, the effect of topical gentian violet against methicillin-resistant *S. aureus* (MRSA) was by using the broth macrodilution method and MIC was determined to be 0.0225-0.0096 µg mL^{-1} (Okano *et al.,* 2000). In our study liquid crystal violet (gentian violet) exhibit significant antimicrobial activity with a MIC value 1µg mL^{-1} which are close to effective with control agents (meropenem, gentamicin, ketoconazole).

The antimicrobial activities of methanol, acetone and hexane extracts of six different species of *Juniperus* fruits was evaluated by using disc-diffusion method against 29 bacterial strains including multiple-antibiotic-resistant bacteria, and two yeasts. The inhibitory effect of acetone extract of *J. Sabina* against bacterial strains was ranging between 7.0-9.5 mm, while the methanol extract showed activity ranging between 6.5–8.5 mm. Both extracts showed inhibition against multiple antibiotic resistant *Staphylococci*. Methanol extracts of *J. foetidissima*, *J. communis* spp. nana, and the hexane extracts of *J. communis* spp. *nana* and only one acetone extract belongs to *J. excelsa* indicated antifungal activity against *C. albicans* (Öztürk *et al.,* 2011). In our study among all tested *Juniperus* extracts, the ethanol extracts of fruits of *J. excelsa*, *J. foetidissima*, and *J. oxycedrus* ssp. *oxycedrus* & leaves of *J. foetidissima* and *J. oxycedrus* ssp. *oxycedrus* (MIC; 16 µg mL^{-1}) showed the highest antimicrobial activity against *A. baumannii* and *S. epidermidis* as well as fungi *C. krusei*.

Table 2: Antibacterial activity against of compounds and references and the control drugs as minimum inhibition concentration (MICs; in μgmL^{-1})

Extract/ Compound/ References	*Microorganisms*								
	Gram negative bacteria					*Gram positive bacteria*			
	K. pneumonia ATCC 07005	*H. influenza* ATCC 6633	*P. aeruginosa* ATCC 25923	*A. baumanii* ATCC 12228	*E. coli* ATCC 25922	*S. pyogenes* ATCC 49766	*S. aureus* ATCC 10145	*S. epidermidis* RSKK 02026	*E. faecalis* ATCC 29212
Je Leaf EtOH	32	32	16	16	32	32	32	16	32
Je Fruit EtOH	32	32	16	16	32	32	32	16	32
Je Leaf H_2O	64	64	32	32	64	64	64	32	64
Jcsn Fruit EtOH	32	32	16	16	32	64	32	16	64
Jcsn Leaf H_2O	64	64	32	32	64	64	64	32	64
Js Leaf EtOH	32	32	16	16	32	32	32	16	32
Js Fruit EtOH	32	32	16	16	32	32	32	16	32
Jf Leaf EtOH	32	32	16	16	32	32	32	16	32
Jf Leaf H_2O	64	64	32	32	64	64	64	32	64
Jf Fruit EtOH	32	32	16	16	32	32	32	16	32
Joso Fruit EtOH	32	32	16	16	32	32	32	16	32
Joso Leaf H_2O	64	64	32	32	64	64	64	32	64
Joso Leaf EtOH	32	32	16	16	32	32	32	16	32
Boric acid	32	32	32	16	32	32	32	16	32
Balsam of Peru	32	32	32	16	32	32	32	16	32
Zinc oxide	32	32	32	16	32	32	32	16	32
Calendula tinc.	32	32	32	16	32	32	32	16	32
Thymol	32	32	32	16	32	32	32	16	32
Resorcinol	32	32	32	16	32	32	32	16	32
Crystal violet	1	1	1	1	1	1	1	1	1
AMX-CLA	<0.12	-	1	<0.12	-	1	<0.12	<0.12	<0.12
MRP	-	-	1	-	0.12	1	0.25	0.25	-
GEN	-	0.5	1	-	-	-	-	-	-

*Je: J. excelsa; Jcsn: J. communis*ssp. *nana; Js: J. sabina; Joso: J. oxycedrus*ssp.*oxycedrus; C: Calendula;* **AMX-CLA:** Ampicilline-Clavunate; **MRP:** Meropenem; **GEN:** Gentamicin; EtOH: Ethanol extract; H_2O: Water extract; -: not done.

Table 3: Antifungal activity against yeast like fungi of compounds and references and the control drugs as minimum inhibition concentration (MICs; in µg mL^{-1})

Extract/Compound/References	*Yeast like fungi*			
	C. albicans ATCC 10231	*C. tropicalis* ATCC 13803	*C. parapsilosis* ATCC 90028	*C. krusei* ATCC 6258
Je Leaf EtOH	32	32	32	128
Je Leaf H_2O	64	64	64	128
Je Fruit EtOH	16	16	16	128
Js Ripe Fruit EtOH	32	16	32	128
Js Leaf EtOH	32	16	32	128
Jf Leaf EtOH	32	16	32	128
Jf Leaf H_2O	32	32	32	128
Jf Ripe Fruit EtOH	32	16	32	128
Jcsn Fruit EtOH	32	16	32	128
Jcsn Leaf H_2O	32	32	32	128
Joso Fruit EtOH	32	16	32	128
Joso Leaf H_2O	32	32	32	128
Joso Leaf EtOH	32	16	32	128
Boric acid	32	32	32	128
Balsam of Peru	32	32	32	128
Zinc oxide	32	32	32	32
Calendula tincture	32	32	32	64
Thymol	32	32	32	16
Resorcinol	32	32	32	16
Crystal violet	1	1	1	1
FLU	2	2	2	64
KET	1	1	1	2

Je: J. excelsa; Jcsn: J. communis ssp. nana; Js: J. sabina; Joso: J. oxycedrus ssp. oxycedrus; FLU: Fluconazole; KET: Ketoconazole; EtOH: Ethanol extract; H_2O: Water extract.

CONCLUSION

In our study, the relationship between antimicrobial activity of the natural compounds and magistral ingredient showed a correlation at MIC values of 16-32 µg mL^{-1}. The results of the antimicrobial activity supported the utilization of plant extract in magistral formulation.

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