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INHIBITORY EFFECT OF ARTEMISIA SCOPARIA ESSENTIAL OIL AND METHANOLIC EXTRACT ON THE GROWTH OF FOOD CONTAMINATED MICROORGANISMS

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ABSTRACT

This study was carried out to evaluate the chemical composition of essential oil and *in vitro* antibacterial activity of the essential oil and methanolic extract of *Artemisia Scoparia* against 3 bacterial (1 gram-negative and 2 gram-positive bacteria) species using dick-diffusion method and Agar well diffusion (AWD) assay. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were quantified by micro-dilution method. The essential oil was obtained by hydro-distillation. The inhibition zones of essential oil of *Artemisia Scoparia* was 32, 31 and 30 mm respectively against *S. aureus, L. monocytogenes* and *E. coli*. The lowest MIC (obtained against *E. coli* respectively*)* and MBC (obtained against *E. coli*) was determined for the essential oil at concentrations: 12.5 and 3.125 mg/ml respectively. The sensitivity of *E. coli* was more than *S. aureus, L. monocytogenes.* Results showed significant antibacterial activity for this herbal essential oil and extract which suggest its capacity as a natural food preservative against bacteria in the food industry by considering the organoleptic properties and probable interactions.

Keywords: *Artemisia Scoparia*, Antimicrobial Activities, Essential Oil and Methanolic Extract

INTRODUCTION

Spoilage of food due to the presence of bacterial and fungal infection has been a major concern for decades and it causes a considerable loss worldwide. The demand for non-toxic, natural preservatives has been rising with increased awareness and reports of ill-effects of synthetic chemicals present in foods. Furthermore, emergence of food-borne pathogens has lately become a major public health concern **[1]**. The increasing incidence of food-borne diseases,

coupled with the 'resultant' social and economic implications, means that there is a constant striving to produce safer food and to develop new natural antimicrobial agents. There is, therefore, still a need for new methods of reducing or eliminating food spoilage and food food-borne pathogens, possibly in combination with the existing approaches. Plant secondary metabolites, such as essential oils and plant extract, are **[2]** used in food industry as flavoring agents and as pharmaceuticals due to their functional properties, as well as bio-preservatives to prolong the shelf life of foods, by reducing or eliminating pathogenic bacteria and increasing the overall quality of food products. It is well known that some essential oils exert antimicrobial and antioxidant properties **[3]**. Essential oils and extracts obtained from many plants have recently gained popularity and scientific interest **[4]**. Plant extracts and essential oils, as well as their con-stituents, are used in the food, cosmetics, and pharmaceutical industries **[5]**. Mostly, plant derived essential oils consist of chemical components such as terpenoids including mono-terpenes, sesquiterpenes and their oxygenated derivatives. These compounds have the ability to easily diffuse across cell membrane to induce biological reactions **[6]**. The antimicrobial activities of plant oils and extracts have formed the foundation of many applications such as in raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies **[7]**. The genus *Artemisia* (family: *Asteraceae*) is a source of valuable drugs and essential oils because of its intricate chemical composition comprising several chemotypes **[8]**. This plant is one of the diverse genera of *Asteraceae* family with many important medicinally valuable essential oils and secondary metabolites **[9]**. The genus *Artemisia* comprises over 500 species that are mainly found in Asia, Europe and North America **[10]**. *A. scoparia* is distributed from central Europe to western Asia. The plant has medicinal properties like anticholesterolemic, antipyretic, antiseptic, antibacterial, cholagogue, diuretic and vasodilator. The essential oil has strong insecticidal activity against stored-product insects **[11]**. The aim of the present study was to determine antibacterial activity essential oil and methanolic extract of *Artemisia Scoparia* against different food-borne pathogens for their potential as a natural preservative and for nutraceutical formulations.

MATERIALS AND METHODS

Chemicals and Plant materials

Gentamicin (Sina daroo, Iran), Muller Hinton agar (Merck, Germany), BHI (Brain-heart infusion broth) (Merck, Germany), methanol and Dimethyl sulfoxide (DMSO) (Merck, Germany) were purchased. The aerial part (leaves) of plant was collected in October 2014 from the

mountains of North Khorasan Province in Iran. The plant was identified by the Research Center of Natural Products Health (NPH), North Khorasan University of Medical Sciences (Iran).

Extraction of the essential oil

The dried and powdered plant sample (80 g) was subjected to hydro-distillation using a Clevenger-type apparatus for 4h. The oil was extracted with $CHCL_3$ and then were dried over anhydrous sodium sulfate (Na_2 SO_4) and stored under N_2 atmosphere at 20 ºC in a selected vial until use **[12]**.

Extraction of the methanolic extract

Plant were air-dried at room temperature **[13]** and the powdered material was then weighed (150 g), soaked in 250 CC of methanol (MeOH) **[14]** at room temperature over a period of 3 days **[13]** (72 hrs) and filtered using Whatman No1 filter paper. The filtrate obtained was concentrated under reduced pressure (at 68°C) in a rotary evaporator to obtain the crude extracts were kept at 4°C until further uses **[14]**.

Organisms and Inoculation Conditions

Authentic pure cultures of bacteria were obtained from Persian Type Culture Collection (PTCC). They included gram positive bacteria; *Staphylococcus aureus* (PTCC 1431) and *Listeria monocytogenes* (PTCC 1298) and gram-negative bacteria; *Escherichia coli* (PTCC 1399). The bacteria strains were first grown on [4] BHI broth (Brain Heart Infusion broth) **[15, 16, 17]** medium at 37°C for 24 hrs prior to seeding on to the nutrient agar **[4]**. Finally, suspensions were adjusted to 0.5 Mc-Farland standard turbidity. Bacterial suspensions were standardized to concentrations of 1.5×10^8 CFU/ml **[18]**.

Antimicrobial assay

The antibacterial activity of the essential oil and methanolic extract were studied using the disc diffusion **[19]**, Agar well diffusion (AWD) assay **[17]** and micro-dilution methods **[20]** against three laboratory standards, including *Staphylococcus aureus* (PTCC 1431), *Listeria monocytogenes* (PTCC 1298) and *Escherichia Coli* (PTCC 1399) **[19]**.

Disk-diffusion method

A modification of the Kirby-Bauer disc diffusion assay was used in which the Mueller Hinton (MH) agar plates were swabbed **[21, 22]**. The bacterial cell suspensions were prepared from a 24 hrs culture and adjusted to an inoculation of 1.5×10^8 cells ml^{-1} using Mc-farland method **[21, 23, 24]**. Both dried extract and essential oil were dissolved in the same solvent (DMSO) (Merck, Germany) to a final concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390, 0.195 and sterilized through by 0.45 µm Millipore filters. The discs (6 mm in diameter) impregnated with 20 µm of essential oil or methanolic extract from each concentration placed on the inoculated agar **[25]**. DMSO was taken as the negative control **[9]**. These plates were kept 30 min at room

temperature to allow the diffusion of the oil and extract, and then they were incubated at 37°C for 24 hrs **[26]**. After incubation, antibacterial effectiveness of *A. Scoparia* extract and essential oil were evaluated by measuring the diameter (mm) of zone of inhibition around the disc using transparent millimeter scale. Each cycle was carried out in triplicate **[27]**.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Test

The micro-dilution method was used to determine the minimum inhibitory concentrations (MIC) of the plant extract and essential oil using 96 well micro-titration plates as previously described by Samie et al. (2005) **[20]**. The 96-well plates were prepared by dispensing into each well 95 µl of [28] BHI broth (Brain Heart Infusion broth) **[15, 16, 17]** and 5 µl of the inoculum **[28]** (standardized at 1.5×10^6 CFU/ml by adjusting the optical density to 0.1 at 600 nm by Shimadzu UV-120-01 spectrophotometer) **[29]**. One hundred microliter aliquot from the stock solutions of the EOs and their serial dilutions initially prepared was transferred into wells. The final volume in each well was 200 µl. The plates were covered with sterile plate sealer and then incubated at 37 °C for 24 h **[28]** afterward the methanolic extract was serially diluted **[29]** in (DMSO) **[30, 31]** and inoculated with 20 µl of the standard inocula (1.5×10^6 CFU ml^{-1}) [29]. After that, 4 mL of the *A. Scoparia* solution at different concentrations (100, 50, 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.390 and 0.195 mg mL^{-1}) was added and followed by shaking for 30 s **[32]**. Micro titer plates were then incubated at 37°C for 24 hours. After incubation, wells were examined for microbial growth **[6, 30]**. MIC was defined as the lowest concentration of the EO in the medium in which there was no visible growth after incubation (no red color signifying live growth) **[28]**.

The MBC was defined as the lowest concentration which no growth was noted on Muller-Hinton agar. Control flasks without *A. Scoparia* were tested in the same way **[32]**. The tests were done in triplicate.

Agar well diffusion (AWD) assay

A cotton wool swab was dipped into a bacterial suspension adjusted to 0.5 McFarland Standard **[29]** and spread evenly over the entire surface of the BHI agar plate by swabbing in three directions. Then, three 4 mm-diameter wells were punched into the BHI agar, and 50 µL of methanolic extract and essential oil (100 mg ml^{-1}) were loaded into one of the wells. The remaining wells contained 50 µL **[17]** of DMSO **[30, 31]** (negative control) and 50 of Gentamicin (positive control), respectively. The plates were incubated overnight at 37 ºC and the radial zones of inhibition (mm) were measured on the following day **[17]**. The tests were done in triplicate.

RESULTS AND DISCUSSION

Disc-diffusion test

EO showed moderate *in vitro* antimicrobial activity against all tested bacteria, including gram-positive and gram negative ones with diameter zones of inhibition 30 to 32 mm. However, among the 3 bacterial species, *E. coli* was more resistant against the extract and essential oil rather than the *S. aureus* and *L. monocytogenes*, at all the concentrations (**Table 1**).

Results of MIC and MBC

In vitro antibacterial potential of *A. Scoparia* essential oil and methanolic extract against a panel of microorganisms is shown in Table 2. Antimicrobial activity of essential oil of *A. Scoparia* was determined via the micro well dilution method at 10 concentrations against three bacteria, two gram-positive and one gram-negative species. The results of in vitro antimicrobial activity assay showed that the essential oil possessed broad antimicrobial activity against the microorganisms tested. Results show that the extract and essential oil are able to prevent the growth of all the 3 tested bacteria but the essential oil was stronger than methanolic extract. The MIC values are between 12.5-25 mg/ml. The lowest MIC was observed for the essential oil at 3.125 mg ml^{-1} for *E. coli* while *S. aureus and L. monocytogenes* were the most resistant bacteria against the essential oil (MIC=25 mg ml^{-1}).

It seems reasonable to assume that their antimicrobial mode of action from methanolic extract and essential oils might be related to the phenolic compounds present. Most of the studies on the mechanism of phenolic compounds have focused on their effects on cellular membranes. Phenolic compounds not only attack cell walls and cell membranes. Thereby effecting their permeability and release of intracellular constituents. But they also interfere with the membrane functions (electron transport, nutrient uptake, protein, nucleic acid synthesis and enzyme activity). Thus, active phenolic compounds might have several invasive targets which could lead to the inhibition of bacterial pathogens [2].

The MBC values are summarized in **Table 3**. Results show that the essential oil of *A. Scoparia* was stronger than the methanolic extract and *E. coli* was sensitive bacteria (MBC=3.125). Figures of 1 and 2 also confirm these results.

Agar well diffusion (AWD) assay

These results indicated that the diameters of inhibition zones varied from 24-28 mm and 29-33 mm for the various concentration of extract and gentamycin respectively. Among the three bacteria, *S. aureus* and *L. monocytogenes* were the most sensitive to the extract and essential oil (the diameter of inhibition zone was 27mm for

methanolic extract and 28 mm for essential oil). However, among the 3 isolates, one bacteria (*E. coli*) were resistant to the extract and essential oil at all the concentrations.

Some studies show that gram-negative bacteria are more resistant to essential oil others claim the same for gram-positive bacteria. In our study the gram-negative bacteria were in both categories [33]. High proportion of monoterpenoids and sesquiterpenoids present in *A. scoporia* has [11] shown to led to a strong antibacterial activity resulting in destabilization of the microbial membrane [34, 35]**.** Deans et al (1955) investigated the susceptibility of gram-positive and gram-negative bacteria to plant volatile oils and found no evidence for a difference in sensitivity between gram-negative and gram-positive organisms. However, some oils appeared more specific, exerting a greater inhibitory activity against gram-positive bacteria. It is often reported that gram-negative bacteria are more resistant to the plant-based essential oils [2, 36]**.** The hydrophilic cell wall structure of gram-negative bacteria is constituted essentially of a lipo-polysaccharide (LPS) that blocks the penetration of hydrophobic components of oil [3] and avoids the accumulation of essential oils in target cell membrane. This is the reason that gram-positive bacteria were found to be more sensitive to the essential oil of *A. scoporia* than those of gram-negative bacteria [2, 36]. Plant-derived essential oil enjoy a natural status and, for this reason, are generally recognized as safe by consumers, which accept well their antimicrobial potential, essential oils are considered with attention by the food scientists. Furthermore, also the interest of the food industries is on the increase, thanks to the consumer demand for effective nature products [37].

Table 1: Results of disc-diffusion test and inhibition zones (mm) for methanolic extract and essential oil of *A. Scoparia*

Treatments	Microorganisms		
	S. aureus	***L. monocytogenes***	***E. coli***
Methanolic extract	**31**	**30**	**29**
Essential oil	**32**	**31**	**30**
Positive control Gentamicin	**32**	**35**	**31**
Negative control DMSO	**6**	**6**	**6**

***Values are the mean of three replicate**

Table 2: MIC (mg ml^{-1}) of methanolic extract and essential oil from *A. Scoparia*

Treatments	Microorganisms		
	S. aureus	***L. monocytogenes***	***E. coli***
Methanolic extract	**25**	**25**	**12.5**
Essential oil	**6.25**	**6.25**	**3.125**

***Values are the mean of three replicate**

Table 3: MBC (mg ml^{-1}) of methanolic extract and essential oil from *A. Scoparia*

Treatments	Microorganisms		
	S. aureus	*L. monocytogenes*	*E. coli*
Methanolic extract	50	50	50
Essential oil	6.25	6.25	3.125

***Values are the mean of three replicate**

Table 4: Results of Agar well diffusion (AWD) assay and inhibition zones (mm) for methanolic extract and essential oil of *A. Scoparia*

Treatments	Microorganisms		
	S. aureus	*L. monocytogenes*	*E. coli*
Methanolic extract	27	27	24
Essential oil	28	28	26
Positive control Gentamicin	33	29	32
Negative control DMSO	6	6	6

***Values are the mean of three replicate**

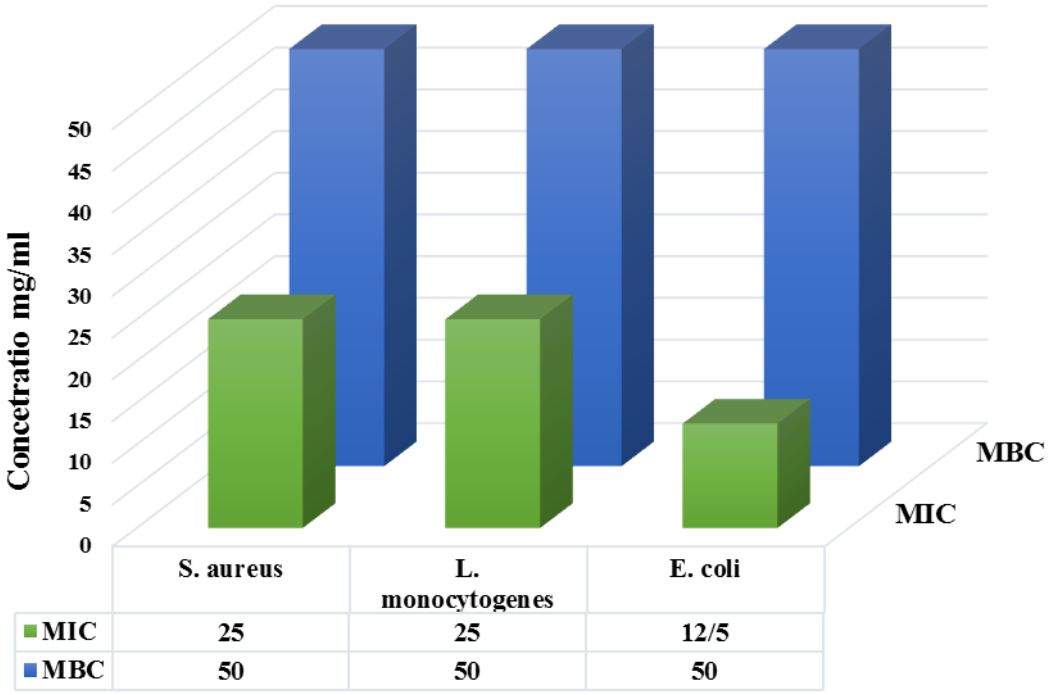

Figure 1: MIC and MBC (mg ml^{-1}) of methanolic extract from *A. Scoparia*

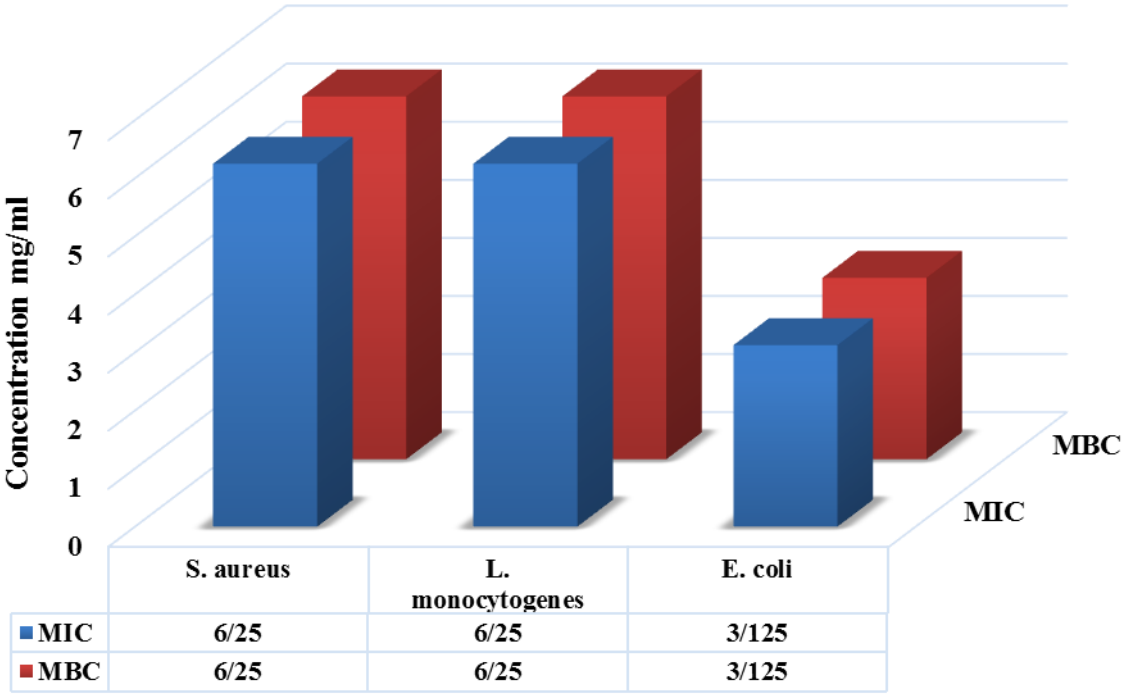

Figure 2: MIC and MBC (mg ml^{-1}) of essential oil from *A. Scoparia*

CONCLUSION

This study demonstrated the potential use of *A. scoporia* Eos as well as their components as antibacterial agents, in particular against gram-negative bacteria, such as *E. coli* and gram-positive bacteria such as *S. aureus* and *L. monocytogenes*, providing an explanation for the reported traditional use of this plant. Our findings may indicate that the methanolic extract and essential oil of *A. scoporia* to be used as a natural preservative in food systems against the well-known causal agents of foodborne diseases and food spoilage such as *S. aureus, L. monocytogenes* and *E. coli*. However, further studies are needed to evaluate the organoleptic and pharmaceutical effects and practical effectiveness of this application.

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