

## Study of essential oil composition and antibacterial activity of *Artemisia scoparia* extraction in the region of Baladeh- Noor, Mzandaran, Iran

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### ARTICLE INFO

#### Article history:

Received 23 December 2013

Accepted 17 May 2014

Available online 1 June 2014

#### Keywords:

Essential oil, Anti- bacterial, Eastern Sagebrush, MIC, MBC, *Artemisia scoparia*

### ABSTRACT

Sagebrush genus (*Artemisia scoparia*) is the one of the most important genus of Asteraceae family. It has numerous values because of medicine properties. Sagebrush genus has numerous application in traditional medicine from ancient times. In this research, the isolation and identification of essential oil and antibacterial activity of *Artemisia scoparia* have been studied. Samples were collected from the region of Baladeh, Noor, Mazandaran, Iran in middle of June 2013. The essential oil derivation was extracted after drying plant under shadow by Hydro-distillation method using Clevenger apparatus. Isolation and identification of essential components was done by GC-MS. Antibacterial activities of essential oil were assessed by diffusion disc method. Four bacteria used in this research were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella typhimurium* apparatus. Isolation and identification of essential components was done by GC-MS. Antibacterial activities of essential oil were assessed by diffusion disc method. Four bacteria used in this research were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Salmonella typhimurium*. The results showed that essential oil of *Artemisia scoparia* in both broth method microdilution and disc diffusion, had the most inhibition diameter zone in *Staphylococcus aureus* than *Staphylococcus epidermidis*. Among negative gram bacterias, *E.coli* had inhibition diameter zone of higher in disc diffusion method. *E. coli* with the most MIC (Minimum Inhibitory Concentration) was the most resistant bacteria than essential oil of *Artemisia scoparia*. According to this results, essential oil of *Artemisia scoparia* can be used as a protector combination, natural flavoring in food.

### 1. Introduction

Natural drugs have been shown to be considered as the basis and even in some cases the only treatment methods. The components of the natural drugs have been used in pharmacy industry for many years. Early of this century, the progress in sciences including organic chemistry led to development of pharmacy

industry and chemotherapy substitution. In doing so, modern physician could treat a lot of incurable illnesses. This fact applies more specially about infectious sickness that has cured with sulfamids, antibiotic and other chemical combinations. Immunotherapy (stimulation and harshness of body defensive system) play great outline in ruining in many infectious illnesses.

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Discovery of new plants among that brought samples from distant lands, new applications of plants as helping drugs in chemotherapy or antibiomatic, finding to plant health value and at last discovery of new material such as vitamins, hormones, antimicrobial, antiviral, antitumor among identified plants have discovered recently, have helped greatly to improve the plants medicine (Zaman, 2003). Eastern Sagebrush genus has medical application and economic value for exporters. It has great expansion in Iran (Zargari, 1992). Eastern Sagebrush genus has powerful antibacterial features and it has also been used for jaundic, hepatitis, blood sugar, inflammation, and liver impairment cures (Zargari, 1996; Tan et al., 1998). Shoot parts of Eastern Sagebrush plant is used for sugar blood and inflammation treatment in Iranian traditional medicine. Fresh leaves of plants as vegetables and dry leaves as spice have been used by local people in Khorasan province (Mirjalili et al., 2007).

Scientific knowledge study on available plant in country and consideration of how its material impact on bacteria can be great help to proper usage of this combination for illness treatment. In this research it is paid to consideration of essence combination and antibacterial properties of Eastern Sagebrush plant. A lot of researches have been done on essence consideration of Sagebrush genus in Iran and other parts of world. Basher et al., (1997), Rabiee et al., (2003), Sefidkon et al., (2003), Kapoor et al., (2004), Ghasemi et al., (2005), Farzaneh et al., (2006), Mirjalili et al., (2006), Hadian et al., (2007), Saedi et al., (2008), have performed research on essence combination of *A. scoparia* genus. Baykan et al., (2012), and Cha et al., (2005) examined the essence and antimicrobial activity of *A. scoparia* genus.

## 2. Materials and Methods

### 2.1. Botanical Properties

*Artemisia scoparia* is a two- years old plant with vertical root, stem with many branches, up to 30-70 cm height, legged leaves on fluffy heaped or none fluffy to 3-5 cm length, with long leafstalk or inclined, oval width or nearly circle, twice cutter branches, panicle

inflorescence with vertical branches and with smaller peduncle from bract.

It is distributed in Iran including North Eastern, North, North Western, West, South, South Eastern and the Central part (Ghahreman,1994).

### 2.2. Accumulation and essential oil extract of *Artemisia scoparia*

Plant was collected from heights of Baladeh of Noor in province of Mazandaran, Iran in the middle of June 2013. Isolation of essential oil performed after drying of plant at shadow through Hydro-distillation and by Clevenger apparatus. Essential combination identification was performed by using of GC/MS .

### 2.3. Antibacterial Activity

Four bacteria applied for the antibacterial activity of essential oil of *Artemisia scoparia* were shown in Table 1.

### 2.4. MIC Appointment (Minimum Inhibitory Concentration) and MBC Appointment (Minimum Bactericide Concentration)

For appointment of MIC rate used nutrient broth medium. At least inhibition density appointed after adding essence to microplates and put them at incubator in 37°C for 24 hours. In fact tarnish of microplates is explanatory of inducer of the growth of bacteria. The first didn't tarnished and microplates were clear completely, that considered as MIC. MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. After determine MIC, in order to identity of MBC rate in steril conditions, some of the content of sumps were taken and moved to microplates, then cultured surfacely and placed in incubator for 24h in 37°C. Afterwards growth of bacteria was studied. The first concentration at wich no growth was observed as MBC. MBC is the lowest concentration of an antibacterial agent required to kill a particular bacterium (Saharkhiz et al., 2008; Shahnazi et al., 2007; Mahbubi et al., 2008; Mortazavi et al., 2009)

### 2.5. Appointment of inhibitor diameter using of diffusion disc method

For appointment of antimicrobial activity, certain volumes of plant essence added to blank discs, then the discs were placed on Moller Hintun Agar medinum, and incubated at 35-37 °C for 18-24 hour and evaluated (Shahnazi et al., 2007).

### 3. Results

Essential oil obtained from structure of *Artemisia scoparia* is yellow and has incisive smell. Chemical analysis of essential oil *Artemisia scoparia* showed that 74 compounds forms %97.83 of total essence. Main combination of this essential oil include 1,8-Sinoel (%13.08); Camphene (%6.74); Bicyclo 2.2.1 Heptan-2-L. 7.7.1 (%5.2); Spatolenul (%3.84); Cyclo Hexen 1-methyl-3-(1-methy) (%3.74); and Germakrendi (%3.68) that constitute %36.28 of total essence. In both methods, broth microdilution and diffusion disc method of *Staphylococcus aureus* had more sensitive with smaller MIC and more inhibition diameter than *Artemisia scoparia* essential oil. In disc diffusion method *Staphylococcus epidermidis* with the least inhibition diameter was more resistant than *Staphylococcus aureus*. Altogether *E.coli* with the most MIC is the most resistant bacteria than essential oil of *Artemisia scoparia*. This research showed that essential oil of *Artemisia scoparia* is effective on both positive and negative gram bacteria, but the effectiveness level is different in dependence on organism (Table 3 and Figures 2 and 3). Positive gram bacteria in comparison of negative gram bacteria were more sensitive for essential oil of *Artemisia scoparia* in disc diffusion method. Among the positive gram bacteria, *Staphylococcus aureus* showed least MIC and inhibitor diameter of higher and *Staphylococcus epidermidis* was more sensitive than essential oil of plant. Among negative gram bacteria, *E.coli* had inhibition diameter of higher in disc diffusion method and *Salmonella typhimurium* with least MIC was the most sensitive bacteria (Table 4 and Figure 5).

### 4. Discussion

Many of the compounds that identified in genus of *Artemisia scoparia* hasn't been reported yet. Compound of 1,8 cineol was reported for the first time in the genus.

Compounds of Camphene (%6.74); Bicyclo 2.2.1 Heptan-2-L. 7.7.1; Cyclo Hexen 1-methyl-3-(1-methy...) (%3.74); and Germakrendi (%3.68) were identified in Eastern Sagebrush that didn't reported in performed studies by other researchers. This may be due to season changes, growth stage, collection time of plant, climate conditions and plant growth place (Mahbubi and Ghazian, 2009; Mirjalili et al., 2008).

Therefore in this research we showed that the positive gram bacteria are more sensitive than negative gram bacteria. It might be due to membrane of polysaccharide of cell walls. These bacteria show less sensitive to antibacterial effects of essence. Among gram positive bacteria direct connection of essence hydrophobic combination perform with two layer phospholipid. This impact takes place whether increasing ions penetrating or leaking cell crucial combination, and occur on inability system of bacterial enzyme (Sandri et al., 2007). Baykan et al., (2012). According to essence consideration and antimicrobial activity of *A.scoparia*, *Staphylococcus aureus* was the most sensitive bacteria to essential oil. Antibacterial activities showed in consistent with results to other bacteria while main components of essential oil showed different degree of growth inhibition (Cha et al., 2005).

### Conclusion

According to increasing limitation on using chemical material of antimicrobial due to spreaders of drug resistance, it is appeared volatile oils are better antimicrobial resource in keeping nutritive material and human illnesses control. The usage of essential oil of plant can be used for infectious substance in medical science, and food industry.

**Table 1.** Bacteria of gram positive and gram negative used in this study

NO.	Microorganism	Microorganism Code	Classification
1	<i>Staphylococcus aureus</i>	PTCC1189	Gram Positive
2	<i>Staphylococcus epidermidis</i>	PTCC1435	Gram Positive
3	<i>Escherichia coli</i>	PTCC1399	Gram Negative
4	<i>Salmonella typhimurium</i>	PTCC1609	Gram Negative

**Table 2.** Chemical Constitutive of *Artemisia scoparia*

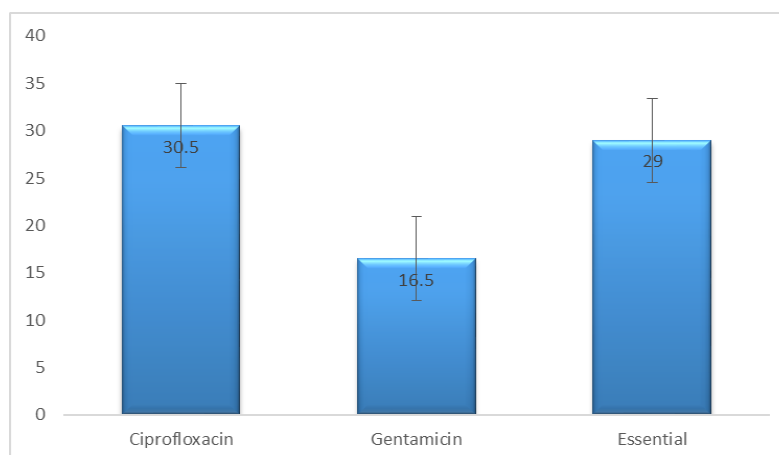
No	Constitutive Name	Inhibitory Value	Constitutive percentage
1	Santolina triene	962	3.36
2	Camphene	998	6.74
3	1,8-Cineole	1070	13.08
4	Cyclohexene,1-methyl-3-(1-methy...	1217	3.74
5	Bicyclo[2.2.1]heptan-2-ol,1,7,7...	1243	5.2
6	Germacrene-D	1413	3.68
7	Spathulenol	1488	3.84
8	Diepi-alpha-cedren I	1523	3.39

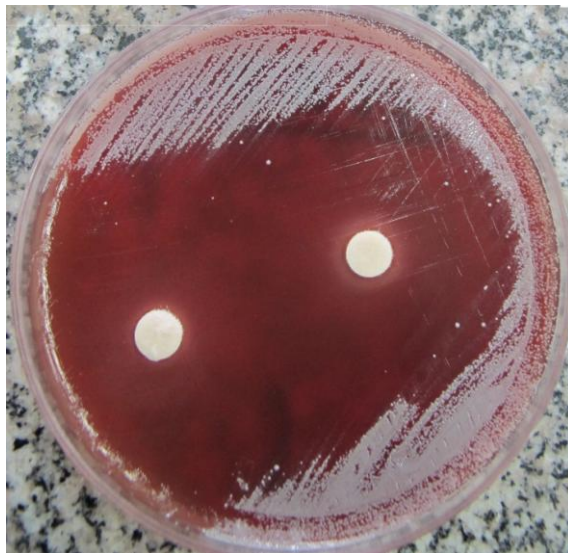
**Table 3.** Antibacterial effects of *Artemisia scoparia* on gram positive bacteria

Bacteria Genus	Average of inhibition Diameter			MIC	MBC
	Essential	Gentamicin	Ciprofloxacin		
<i>S.aureus</i>	39	12	30	0.2	0.46
<i>S.epidermidis</i>	19	21	31	0.93	1.86
Average	29	16.5	30.5		

**Table 4.** Antibacterial effects of *Artemisia scoparia* on gram negative bacteria

Bacteria Genus	Average of inhibitor diameter			MIC	MBC
	Essential	Gentamycin	Ciprofloxacin		
<i>E.coli</i>	21	22	32	3.73	7.46
<i>S.typhimurium</i>	20	19	28.25	0.2	0.46
Average	20.5	20.5	30.125		

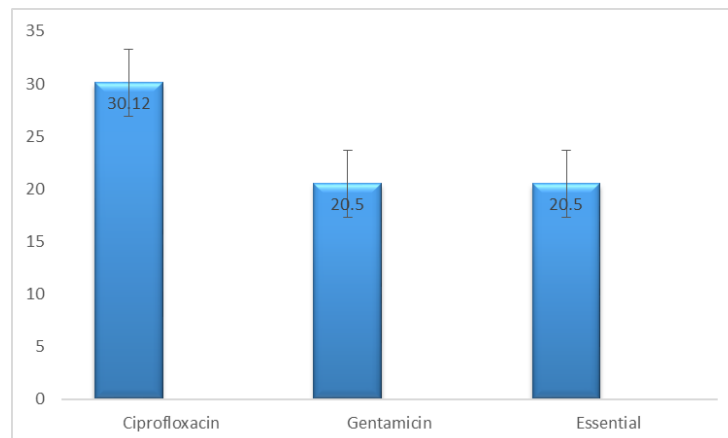
**Figure 1.** Comparison of antibacterial effects of *Artemisia scoparia* and antibiotics on gram positive bacteria



**Figure 2.** Inhibition diameter of *Staphylococcus epidermidis* in disc diffusion method



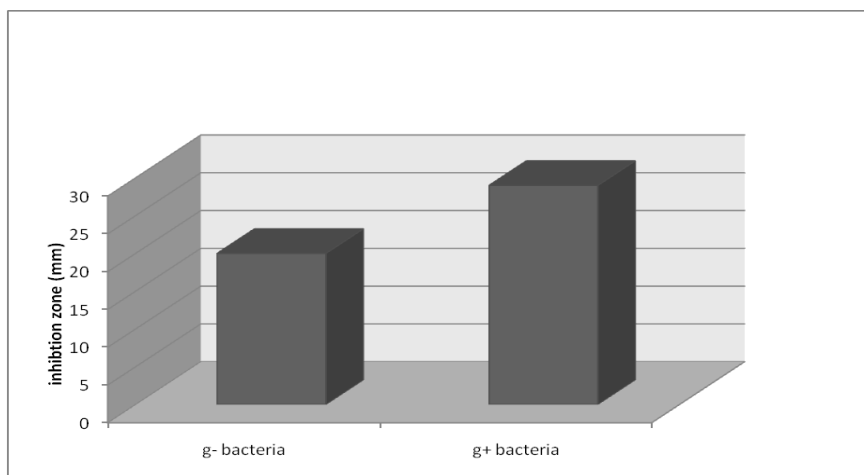
**Figure 3.** Inhibition diameter of bacteria *Staphylococcus aureus* in disc diffusion method



**Figure 4.** Comparison of antibacterial effects of *Artemisia scoparia* and antibiotics on gram negative bacteria



**Figure 5:** Inhibition diameter of *Escherichia coli* in disc diffusion method



**Figure 6:** Comparison of effect of essential oil on gram positive and negative bacteria

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