Chemical composition and antimicrobial activity of *Artemisia monosperma* and *Salvia officinalis L.* plants

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Abstract

This study was aimed to estimeated at chemical composition and evaluating the effectivenes of the extract of *Artemisia monosperma* and *Salvia officinalis L.* against four species of illness bacteria that causing and food spoileg. The result were refer to found a number of effective compounds in plant extract like Glycosides , Flavonoids , Resins, Alkaloids, Phenols and Trace elements (Co, Zn, Fe, Pb, Cd and Cu). The results of Microbiological tests were showed some difference between the effect of this plants on the declining of the bacterical species growth. Inhibition zone against bacteria was (11-23.5)mm and the (MIC) were 10 - 30 mg/ml for alcoholic extracts against becterial species tested.

Keywords: *Artemisia monosperma*; *Salvia officinalis L.*; Antibacterial activity; Trace element.

Introduction:

One approach to the discovery of new drugs is the study of the bioactive constituents of higher plants. The investigation of plants used as remedies in traditional folk medicine can be an interesting tool to identify several biologically active molecules from the 250,000 higher plant species [1]. Plants and plant products have been used extensively throughout history to treat medical problems in civilization of Chinese, Egyptians, Greeks, and Romans as long as 2,600 years ago [2,3]. Numerous studies have been carried out to extract various natural products for screening antimicrobial activity[2].

The genus *Artemisia L.*(family Asteraceae, tribe Anthemideae), comprises a variable number of species (from 200 to over 400) found throughout the northern half of the world [4]. *Amonosperma* was investigated previously for flavonoids[5,6], acetylenes[7,8], *p*-hydroxyacetophenone derivatives[5], coumarins[9], *p*-coumaric acid derivatives[5], tetrahydrofuran-type terpenoids derived from davanone, in addition to cycloartenol, β-sitosterol and

stigmasterol[9]. Artemisia species are popular plants which are used for the treatment of diseases such as hepatitis, cancer, inflammation and infection by fungi, bacteria, and viruses [10].

Salvia officinalis L., the common sage is the most representative species within the genus of Labiatae. The plant is mostly diffuse in the Mediterranean Basin, in South East Africa and in Central and South America, where it is largely cultivated for culinary and medicinal purposes[11]. Curative properties of sage are particularly recognized since earliest times and its use as a tonic, stimulant, carminative, antiseptic and antihydrotic is reported [11,12]. More recently, sage antioxidant effects have also been demonstrated[13]. The prevailing components in the extract obtained by ultrasound extraction [14] were alpha –thujone 48.4% and camphor 14.2%, in the methylene chloride extract [15]were alpha- thujone 15.7-59.3%, 1.8- cineole 10.9- 43.1, beta- thujone 4.9-25.8% ,and *S. officinalis* contains 1.0 - 2.8% volatile oil[16].

Materials and methods:

Artemisia monosperma and *Salvia officinalis L.* Powder were get it were purchased locally from locally markets.

Aqueous and alcoholic extracts were obtained by soaking 100g of *Artemisia monosperma* and *Salvia officinalis L.* in 500ml distilled water and ethanol 96% respectively at room temperature, then mixing it by electrical blender for 15 min. and filtered by whatman filter paper NO.1 [17]. Dryness the extracted in oven at $37c^{o}$ and refrigeration until used [18].

Chemical detection of active compounds in *Artemisia monosperma* and *Salvia officinalis L.*:

- **Detection of Resins** detected of resin in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [19].
- **Detection of Tannins** detected of tannins in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [20].
- **Detection of Coumarin** detected of coumarin in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [21].
- **Detection of Saponines** detected of sapnines in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [19].
- **Detection of Glaycosides** detected of glaycosides in *Artemisia monosperma* and *Salvia officinalis L.* by used the Fehling reagent.
- **Detection of Alkaloides** detected of alkaloids in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [22].
- **Detection of Phenoles** detected of phenoles in *Artemisia monosperma* and *Salvia officinalis L.* by used 1% Ferric chloride ($FeCl_3$).
- **Detection of Flavonoides** detected of flavonoides in *Artemisia monosperma* and *Salvia officinalis L.* by used the method in [23].

Preparation of standard solution for Atomic Absorption Spectrophotometer (A.A.S) measurement:

Two gram powder of each plant sample was dissolved in 10 ml of aquaregia was add and heated for 5-10min. to small volume and up to marked 25 ml by adding deionized water.

Microorganisms strains:

The microorganisms that used in this study were to type: Gram - positive bacteria: (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram - negative bacteria: (*Escherichia coli* and *Pseudomonas aeruginosa*). All microorganisms were obtained from stock cultures of the Laboratory of Microbiology College of Science, University of Al-Mustansirya.

Antibacterial activity:

Antibacterial activity was determined by the well diffusion method according to [2]. Petri plates containing 20 ml of Nutrient agar medium were seeded with a 24 h culture of the bacterial strains. Wells (6mm diameter) were cut into the agar and 50 µl of the plant extracts were tested in a concentration of (50, 75, 100) mg/ml. The inoculum size was adjusted so as to deliver a final inoculum of approximately 10^8 colony-forming units CFU/ml. Incubation was performed at 37 ºC for 24 h. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zone formed around the well.
Minimum inhibitory concentration (MIC) was determined by the micro-dilution method using serially diluted (2-fold) plant extracts according to [2]. A final concentration from 10 to 30 mg/ml was used for each plant sample. The following ethanol extracts were tested: *(Salvia officinalis L. & Artemisia monosperma)*. Bacteria inocula were adjusted to contain approximately 105 CFU/ml. The test plates were incubated at 37 ºC for 18 h.

Results and Discussion:

Chemical detection of active compounds and trace elements in *Artemisia monosperma* and *Salvia officinalis L.*:

Results in table (1) were showed that the Aqueous and alcoholic extracts of *Artemisia monosperma L.* were contained glaycosides, saponines, flavonoides, resins, coumarin, and phenols as the other studies find [5,6,7,9]. While give negative test for tannins and alkaloids .

The extracts of *Salvia officinalis L*. give positive test for glaycosides, aponines, flavonoides, resins, alkaloids, and phenols as the other studies find [11,13, 16] While give negative test for tannins and coumarin.
Flavonoides compounds are high antioxidant activity, phenols is antimicrobial activities, antibacterial, fungistatic and virustatic [4].

Table (1): Chemical detection of active compounds in *Artemisia monosperma* and *Salvia officinalis L.*

Active compounds	*Artemisia monosperma*	*Salvia officinalis L.*
Tannins	-	-
Glaycosides	+	+
Saponines	+	+
Flavonoides	+	+
Resins	+	+
Coumarin	+	-
Alkaloids	-	+
Phenols	+	+
pH	6.2	5.1

The different concentration of Cu, Fe, Co, Zn, Pb, Cd in the *Artemisia monosperma* and *Salvia officinalis L.* are shown in table (2).

The values for Fe, Pb, and Cd in *Salvia officinalis L.* are higher compared to the *Artemisia monosperma L*. The concentration of Co and Zn in *Salvia officinalis L.* are less as compared to the *Artemisia amonosperma*, while Pb was found absent in *Artemisia monosperma* .

Table(2): Concentrations (ppm) of trace element in *Artemisia monosperma* and *Salvia officinalis L.*

Element	*Artemisia monosperma*	*Salvia officinalis L.*
Co	2	1
Zn	0.04	0.03
Fe	0.65	0.9
Pb	N.L	0.45
Cd	0.05	0.085
Cu	16	30

The biological effects of the trace elements in living system strongly depend upon their concentration and thus should be carefully controlled especially when herbs and drugs are used in human so different trace elements in the different medicinal plants will have their definite role for smooth functioning of our body [24]. The roles of the detected elements are given below.

Pb causes both acute and chronic poisoning, and also poses adverse effects on kidney, liver, vascular and immune system, Fe is necessary for the formation of hemoglobin and also plays an important role in oxygen and electron transfer in human body severe iron deficiency results in anemia and red

blood cells that have a low hemoglobin concentration, In young children, iron deficiency can manifest in behavioural abnormalities(including reduced attention) reduced cognitive performance and slow growth. In adults, severe iron deficiency anemia impairs physical work capacity [25]. Cd is nephrotoxic and can cause renal dysfunction with proteinuria [26]. Zinc is essential to all organism and it is an important trace element having a definite role in the metabolism , growth and development. It is an essential component of over 200 enzyme having both catalytic and structural roles. Zinc deficiency is characterized by recurrent infection, lack of immunity and poor growth. Low intake of Zn may cause coronary artery disease. Clinical materials prove that Zn can have good effect on eliminating ulcer and promoting healing wounds[27]. Cu is universally important cofactor for many hundreds of enzymes. It functions as a cofactor and activator of numerous enzymes which are involved in development and maintenance of the cardiovascular system. A Cu deficiency can result in a decrease in the tinsel strength of arterial walls, leading to aneurysm formation and skeletal maldevelopment[24]. There are no established criteria for Co in medicinal plants [25].

Antibacterial activity :

The antibacterial activities of the extracts obtained from the plants under study by the diffusion method are shown in (table 3). The water extracts of all the plants screened showed don't inhibitory effects against all Bacterial species. The ethanolic extracts of all the plants screened showed various inhibitory effects (11 – 23mm/50 µl inhibition zone) against all Bacterial species. The largest zone of inhibition was observed from ethanolic extracts against *Bacillus subtilis and Escherichia coli*. *Staphylococcus aureus* was more resistant to most extracts used in this study.

The MIC of the ethanol extracts fell in the range of 10 to 30 mg/ml for all bacterial species (table 4).

Table (3): Inhibitory properties (inhibition zone diameter in mm) of plant ethanol extracts on different bacteria.

Test agents	Bacterial species											
	Bacillus subtilis			***Staphylococcus aureus***			***Pseudomonas aeruginosa***			***Escherichia coli***		
	A	B	C	A	B	C	A	B	C	A	B	C
Salvia officinalis L.	16	20	23.5	11	15	20	12	16	21	14.25	19	23
Artemisia monosperma	15	17	22	12.25	15	17	11.5	15	19	12	18.5	22

Includes diameter of well (6 mm).
A: 50 mg/ml.
B: 75 mg/ml.

C: 100 mg/ml.

Table (4): Antibacterial activity (MIC in mg/ml) of the ethanol extracts and their combinations.

Test agents	Bacterial species			
	Bacillus subtilis	***Staphylococcus aureus***	***Pseudomonas aeruginosa***	***Escherichia coli***
Salvia officinalis L.	**10**	**30**	**20**	**10**
Artemisia monosperma	**15**	**30**	**25**	**10**

The results indicated that the crude extracts of all the species studied showed antibacterial activities towards the Gram-positive bacteria and Gram-negative bacteria. These results are consistent with previous reports on related plants regarding Gram-positive bacteria and Gram-negative bacteria [2]. It appears that overall the microorganisms were not as sensitive to the water extract compared with the other extracts as determined by diffusion. The reasons for this could be that all of the identified components from plants active against microorganisms, aromatic or saturated organic compounds, are most often obtained through initial ethanol or methanol extraction [28]. *Salvia officinalis L.* and *Artemisia monosperma* are a plants which has been used in a variety of food preparations. In this work we showed the significant antibacterial activity of the aqueous extract of S. officinalis. Some earlier studies have demonstrated sage antibacterial activity against foodborne bacteria [13, 29, 30, 31, 32]. Among the bacteria tested *Bacillus subtilis* was the most sensitive to all the crude extracts. Other Gram-positive bacteria show lower susceptibility to the essential oils. From Gram-negative bacteria only *Escherichia coli* was more susceptible to extract of *Salvia officinalis L*. According to [33, 34]. Oils from *Salvia officinalis L.* and *Artemisia monosperma* on Gram-negative bacteria. However, most studies investigating the antibacterial effects of essential oils confirmed, that they are more active against gram-positive than gram-negative bacteria [10, 35, 31] Our results indicate that this effect differs due to different chemo type of plant, as is state later.

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التركيب الكيميائي والفعالية المضادة للأحياء المجهرية لنباتي للشيح *Artemisia monosperma* والمريمية *Salvia officinalis L.*

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الخلاصة

هدفت الدراسة الى تحديد التركيب الكيميائي وتقييم فاعلية المستخلصات النباتية للشيح *Artemisia monosperma* والمريمية *Salvia officinalis L.* تجاه أربع سلالات من البكتريا المرضية والمسببة لتلف وفساد الأغذية بطريقتي الانتشار بالحفر والتركيز المثبط الأدنى (MIC) ، واسفرت نتائج الاختبار المبدئي للمستخلصات النباتية عن وجود عدد من المركبات الفعالة في النباتات قيد الدراسة مثل الكلايكوسيدات والفلافونويدات والراتنجات، فضلا عن وجود مواد قلويدية وفينولية، وقد اوضحت النتائج عن وجود عدد من العناصر المعدنية في النباتات المدروسة لاسيما الكوبالت والزنك والحديد والرصاص والكادميوم و النحاس، فيما بينت نتائج الاختبارات الميكروبية وجود فرق بين تأثير هذه النباتات في قدرتها على تثبيط نمو السلالات البكترية المختبرة، اذ تراوح قطر منطقة التثبيط بين 11 – 23.5 ملم للمستخلصات الكحولية، في حين تراوح التركيز المثبط الأدنى (MIC) للمستخلصات الكحولية بين 10 - 30 ملغم / مل ضد السلالات البكترية المختبرة.