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**Research Article**

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# Antibacterial activity of essential oil and two of its components extracted from Syrian *Inula viscosa* against some Syrian gram positive and negative bacteria isolates

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**ABSTRACT**

*The antimicrobial activity of essential oil and two compounds ,namely Caryophylene and Limonene , isolated from aerial parts of Syrian Inula viscosa(l.) were tested against two gram positive bacteria, (Listeria Monocytogeneses & Staphylococcus Aureus)and two gram negative (Salmonella Typhimurium & Proteus Mirabilis).The results stated that the $MIC_{50}$ of essential oil were amounted to about 12.5 µl/ml and 6.3 µl/ml for gram positive and negative bacteria respectively ,whereas the $MIC_{50}$ of Caryophhylene and Limonene were amounted respectively about 50 µl/ml and 25 µl/ml for both gram positive and negative bacteria respectively .*

**Key words:** Essential oil, Antimicrobial, Chemical compounds, *Inula visoca*, Syria

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## INTRODUCTION

In the demand for new therapeutics, plants are considered to be one of the main sources of biologically active materials. It has been estimated that about 50% of the prescription products in USA and Europe are originated from natural products, including plants or their derivatives [1,2]. Treatment of microbial infections have drawn the attention of researchers due to their great impact on the population's health. On the other hand and based on the random use of antibiotics, the bacteria and fungi have evolved new mechanisms to evade old and new antimicrobial agents [3].

*Inula viscosa*(L.) is a plant widely spread in different regions of the Mediterranean basin [4]. In traditional medicine this plant has several uses, anti-inflammatory [5], anthelmintic, lung disorders [6], antipyretic, antiseptic, and antiphlogistic activities [7,8] in addition to treating gastroduodenal disorders [9] , antifungal [10], antioxidant [11], antiulcerogenic [12] and anthelmintic [13] properties and prevent zygote implantation [14], potential antimicrobial activities [15].

## EXPERIMENTAL SECTION

### 1-Plant materials

The aerial parts of *Inula viscosa(l)* were collected from fields in Kafr Hoorvillage in western south of Syria during the flowering season ,namely October 2013 . The sample was cleaned from any strange plants, dust or any other contaminants. The collected plants were air dried and cut to pieces.

### 2-Essential oil extraction

Extraction of essential oils was conducted using a water steam distillation device (Clevenger-type apparatus) according to the manufacturer's instructions **[16 ,17**], the device was attached to a condenser and cold water recycler (hydrodistillation technique). distilled water was added (1:10 v/v), and each sample was distilled for 2 h. The

supernatant contained essential oil, which was dehydrated by filtering through anhydrous na2so4. The essential oil was prepared and collected in airtight vials and stored in refrigerator.

**3-Identification and isolation of the main components of essential oil**

The identification of each pure component was accomplished by the GC-Fid technique [18] .The GC analysis was carried out using a 30-m column hp-5 (0.25 mm i.d 0.4 μm film thickness) with helium as carrier gas. The oven temperature was kept at 50°C for 2 min, programmed to 110°C at a rate of 2°C/min, and kept constant for 3 min. Subsequently, it was programmed to 175°Cat a rate of 4°C/min, kept constant for 2 min programmed to 250°C at a rate of 5°C/min and kept constant for 5 min. The injection mode was split less, the injector temperature was 250°C,and the detector temperature was 275°C. Chromatograms of the essential oils were computed by the normalization method from the GC peak areas was calculated as the mean value of two injections. Confirmation of the components of essential oils was performed using the GC-MS technique, and isolation was conducted using a preparative HPLC(Jasco),equipped with a Uv/Vis detector and aliquots collector. The solvents were purchased from Merck [Germany]. GC-MS conditions were comprised of a mass range of 36 -300 Amu, sample rate of 65,and source temperature of 260°C.The HPLC analytical conditions were optimized to have the best separation conditions and to avoid any adjacent peaks. The best HPLC separation conditions were seen as follows: mobile phase of Thf/Can.; mobile phase flow rate of 1.3 ml/min; sample volume of 150 µl; analysis time of 90 min; and detector conditions of response=fast, range=0.32.

**4-Microorganisms and growth conditions**

Local isolates of listeria Monocytogeneses, *Staphylococcus Aureus, Salmonella Typhimurium*, and *Proteus Mirabilis* were grown within 24-48 h in 2yt agar (peptone, 16 g/liter; yeast extract, 10 g/liter; NaCl, 5 g/liter; and agar, 13 g/liter [difco, bd, spars, md])[19]. The bacteria were suspended in a sterile phosphate buffered saline (Pbs). Bacteria abundance in Bbs was monitored by recording the optical density (OD) at 590 NM. The exact counts were assessed respectively by viable counts on 2yt agar plates.

**5-Determination of minimum inhibitory concentration**

The microdilution broth susceptibility assay was employed [20].Three replicates of serial dilutions of the essential oils and their components were prepared in an Lb broth medium in 96-well microtiter plates, using a range of concentrations for each essential oil and its components from0.375 to 50 µl/ml, and 100 µl of freshly grown bacteria standardized $10^6$Cfu/Ml in the Lb broth were added to each well. Positive control was done with the same conditions but without essential oils, while the negative control was done with the same conditions but without adding the bacteria. The plate was incubated with shaking for 24 h at 37°C. The lowest concentration that completely inhibited visual growth was recorded and interpreted as the minimum inhibitory concentration (MIC).A mean value of each test was obtained by averaging the triplicate values with log conversion.

## RESULTS

Regarding to dried sample ,the results showed that the percentage of essential oil ranging from 1.7 % to2.2 % during the flowering season. On the other hand there were about fifty one components of essential oil extracted from Syrian *Inual Viscosa*, however the percentage of each ranging from 0.1(Vulgarolb ) as the minimum to 9.2 (Azulene) as the maximum of the total area which amounted to about 87 %.**Table 1.**

The results also showed that the $Mic_{50}$ of essential oil were amounted to about 12.5 µl/ml and 6.3 µl/ml for gram positive and gram negative bacteria respectively whereas the $MIC_{50}$ of Caryophyllene and Limonene were respectively amounted to about 50 µl/ml and 25 µl/ml for both gram positive and gram negative bacteria respectively. **TABLE 2**

**Table1: Essential oil composition of Syrian *Inula viscosa* (l). aerial parts obtained by steam distillation**

| Component name | percentage |
|---|---|
| Caryophyllene oxide | 1.9 |
| Eucalyptol | 0.3 |
| Linalool | 4.6 |
| 1-Limonene | 1.3 |
| a-terpineol | 1.6 |
| Edulan 1 dihydro | 2.2 |
| Eugenol | 2.7 |
| Copaene | 0.8 |
| b-Damascenone | 1.2 |
| caryophyllene | 1.3 |
| Gama-selinene | 1.4 |
| Valencene | 1.6 |
| b-cadinene | 1.2 |
| a-cadinene | 0.8 |
| 1.8-Cineol | 1.9 |
| Benzaldehyde | 2.1 |
| cyclooctane | 2.7 |
| Hotrienol | 1.5 |
| 3.5-Octadien-2-one | 0.9 |
| 6-Methyl-3.5-Heptadien-2-one | 1.6 |
| 1-undecanol | 2.4 |
| 1-Boreneol | 1.7 |
| 1-Terpinen-4-ol | 1.5 |
| Alpha -Terpineol | 1.1 |
| p-cymene-8-ol | 2.1 |
| Trance-Geraniol | 2.4 |
| Edulan I .dihydro | 2.7 |
| Methyl eugenol | 1.3 |
| Theaspirane B | 1.6 |
| Cyclosativene | 1.1 |
| a-Ylangene | 0.6 |
| a-Terpineyl Acetate | 0.8 |
| Longifolene | 1.3 |
| Trans-caryophyllene | 1.4 |
| Phytol | 3.9 |
| 1H-Indene 1 | 1.8 |
| ethylideneoctahydro | 2.1 |
| Beta-Damascenone | 2.1 |
| Aromadendrene | 1.7 |
| - Eudesma-4(14).11-diene | 1.3 |
| Alpha-Amorphene | 1.1 |
| B=Eudesmene | 1.2 |
| Nerolidol | 1.1 |
| Azulene | 9.2 |
| Cedren-13-ol | 1.3 |
| Pentadecenol | 0.6 |
| Ascabin | 0.1 |
| Retene | 1.3 |
| VulgarolB | 0.1 |
| 12-carboxyeudesina-3-11(13)diene | 1.3 |
| Ethyl palmitate | 1.2 |
| Total Area % | 87 |

**Table 2 : Minimum inhibitory concentration ($MIC_{50}$) in µl/ml of essential oil and two of its components extracted from Syrian *Inula viscosa***

| Compounds | *Listeria Monocytogeneses* | *Staphylococcus Aureus* | *Salmonella Typhimurium* | *Proteus Mirabilis* |
|---|---|---|---|---|
| Essential Oil | 12.5 µl/ml | 12.5 µl/ml | 6.3 µl/ml | 6.3 µl/ml |
| Limonene | 50 µl/ml | 50 µl/ml | 25 µl/ml | 25 µl/ml |
| Caryophyllene | 50 µl/ml | 50 µl/ml | 50 µl/ml | 50 µl/ml |

## CONCLUSION

Based upon above mentioned results, the essential oil showed higher antibacterial activity compared to the Caryophyllene and limonene that all were extracted form Syrian *Inula viscosa* which suggests that the effect of essential oil isolated from this plant on bacteria should be investigated further .

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