



Research Article

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Isolation and identification of six terpenes extracted from leaves of *Inula viscosa* (L.) grown in Syria

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ABSTRACT

Leaves of *Inula viscosa* (L.) Were collected from fields in rural Damascus in Syria. Extracts of leaves were obtained by the classical method of continuous extraction using Soxhlet apparatus. The chemical composition was analyzed by semi-preparative HPLC and then identified by GC/MS. The analysis led to the identification of 68 components in leaves of which six were isolated and identified. Namely, Limonene, α -Pinene, Caryophyllene, α -Terpinene, Neryl Acetate, Geraniol, Extracts of leaves were characterized by a content of Terpenes

Keywords: Terpenes, *Inula Viscosa*, HPLC, Limonene, Caryophyllene, Syria.

INTRODUCTION

Large numbers of medicinal plants are constantly being screened for their possible pharmacological value. One of these plants is *Inula viscosa* Aiton. Which belongs to the family of Asteraceae. Syrian flora is very well known for its diversity and richness of medicinal plants. It contains a large number of pharmacological active species. The Asteraceae family is considered one of the most important members of the Syrian Flora. *I.viscosa* is mainly spread in the eastern Mediterranean countries and grown in southern and western regions of Syria. *I.viscosa* plant in particularly leaves and flowers is used widely in Syria as an herbal tea for its antiseptic and diuretic effects and in addition to its benefits in the treatment of stomach, intestines, bladder and some respiratory and skin diseases. Amin and co-worker reported a review on the medicinal importance of all genus *Inula* [1]. It has very effective properties ranging from anticancerous to antibacterial, hepatoprotective, cytotoxic and anti-inflammatory properties. Zhao and co-worker reported chemical constituents of plants from genus *Inula* and found that the plant is very rich with many alkaloids and sesquiterpene lactones, sesquiterpene acids, triterpenes, sterols, allantolactones, isoolantolactones, and flavonoids supporting the idea that *Inula* is a the natural potential source for isolation of a variety of chemically defined compounds for their many medicinal applications [2]. Al-Dissi,et. al. reported the effects of *I.viscosa* leaf extract on abortion and implantation in rats. They found that the aqueous extract administered i.p. on day 1–6 of gestation, totally diminished fetal implantation and caused a significant ($P<0.05$) reduction in the number of corpora lutea and blood progesterone levels [3].

Shah Biren reported a review about medicinal plants as a source of anti-inflammatory and anti-arthritis agents and found that *Inula viscosa* (Asteraceae) is a very important candidate. Three flavonoids, isolated from *I.viscosa* dichloromethane extract were 7–O-methylaromadendrin, rhamnocitrin, and 3–O-acetylpadmatin along with a sesquiterpene lactone inuvisolide, a sesquiterpene acid; ilicic acid, and a diagalactosyl–diacylglycerol; inugalactolipid A and shown to have 12–O-tetradecanoylphorbol–13–acetate induced ear edema inhibitory activity in mice [4,5].

For our knowledge, the chemical composition of Syrian *I.viscosa* Aiton. has not been reported before. In addition, due to the medicinal importance of genus *Inula*, this paper reports the isolation and identification of six major constituents' fractions from extracts of leaves of Syrian *Inula viscosa* L. using semi-preparative HPLC and GC/MS analyses.

EXPERIMENTAL SECTION

1-Plant material:

Aerial parts (Leaves) of *I.viscosa* L. were collected between 1st of January 2012 and 31 of December 2013, from Kafer-Hoor, El-Shekh Mountain in Syria. The plant was identified by Prof. Emad Al-Kady, the Faculty of Science at Damascus University.

2-Reagents and Apparatus:

It should be pointed out here that all used chemicals and solvents in the extraction processes and analysis were of HPLC and GR grades and were purchased from Merck and Aldrich and used as received. Homemade extraction apparatus designed on the principle of steam distillation processes, semi preparative HPLC model JASCO-LC-1500 equipped with UV/VIS detector and ODS C18 preparative column and GM/MS, 6869N system from Agilent.

3-Extraction Process, Separation and Identifications:

All leaves' samples of plants used in this study were mixed thoroughly and then cleaned and air-dried for ten days at room temperature, after that they were crisped and grounded to be ready for hydro distillation. An amount of 150 grams of the dried leaves were placed in the distillation flask (one-liter capacity, homemade Clevenger-type apparatus) with about 300 mL of distilled water and extracted for about four hours. This process was repeated until the entire essential oil existence was extracted. The isolated oil from each distillation was added to each other, dried over anhydrous sodium sulfate, and stored in dark bottles in a fridge at 3 to 5°C.

4-Extraction for semi preparative separation:

The extraction of active materials in leaves were carried out as follows prior to semi preparative analysis and isolation of six chemical fractions. The extraction was made using three solvents: methanol, dichloromethane and N-hexane. The obtained sample was then cleaned from the presence of chlorophyll using a column of silica gel (90-220 mesh). After that, the column was cleaned up and reactivated with a mixture of chloroform and hexane at a ratio of 15:85 v/v. The obtained sample once more, passed through the silica-gel column and the final obtained sample was dried with nitrogen gas (99.999%). Then the sample was diluted with methanol and subjected for HPLC analysis. The optimized analysis conditions were adjusted in order to eliminate any interference during the fractions isolation.

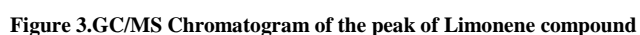
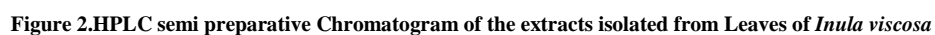
Therefore we used the following operation conditions: Tetrahydrofuran / Acetonitrile /H₂O as a mobile phase, a flow rate of 1.3 mL per minute, an injected sample volume of 250µL, and an analysis time of 95 minutes. Column is ODS- C18 and the wavelength at 205 nm were used. Retention times of some individual constituents were compared to those of available authentic samples in order to check the credibility of the determinations. Six major chemical fractions were isolated using the semi-preparative HPLC system.

5-Identification with GC/MS:

In order to be sure of what have been done, the retention time of some individual component was compared to reference compounds, the identification of each separated compound was investigated by using GC-MS (Agilent 6869) in the following operating conditions: column db-5-ms, injection temperature was set at 275°C, ion source temperature 250°C, the ion fragmentation energy was around 70 e.v, and the mass spectra was obtained by using ionization source of about 70 e.v., The initial temperature of the column was 50° for 2 minutes, then lifted (at a rate of 2 degrees per minute) to 170° (lasted for 7 minutes), then lifted to 250° at a rate 4 degrees per minute (lasted for 10 minutes).

RESULTS AND DISCUSSION

Six major chemical constituents were obtained. Neryl Acetate, Caryophyllene, α -Terpinene, Geraniol, α -Pinene and Limonene. Some of them are being used as following: Caryophyllene: antinociceptive [6], Terpinene: antibacterial, antifungal [7], Geraniol: anti hepatoma activity [8]. α -pinene: a broad-spectrum antibacterial activity [9], Limonene: anticancer [10].



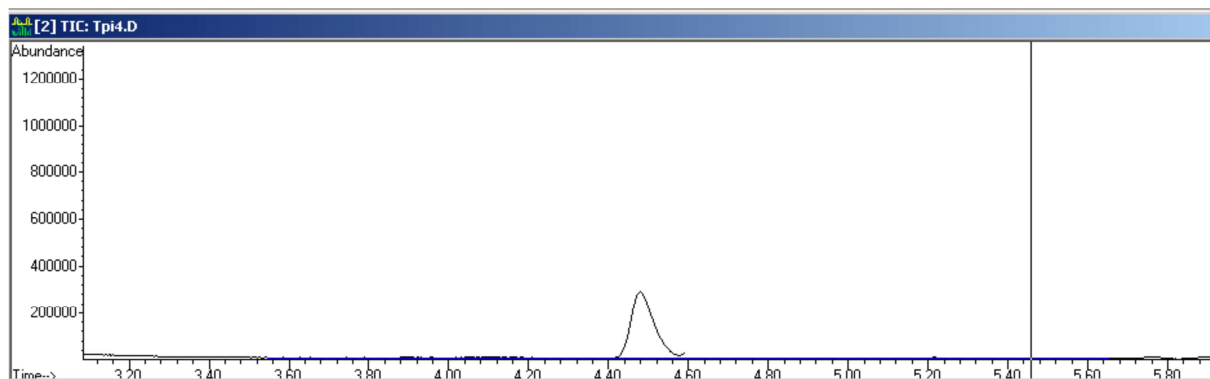
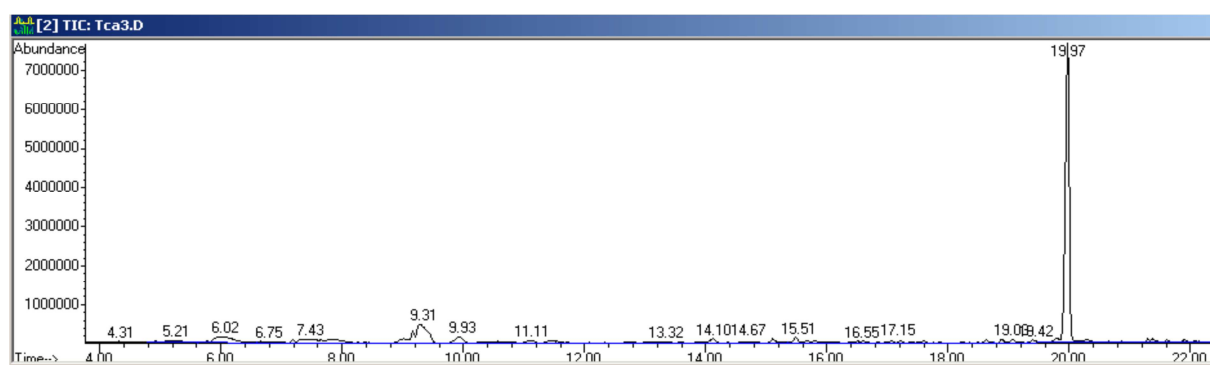
Figure 4.GC/MS Chromatogram of the peak of α -pinene compound

Figure 5.GC/MS Chromatogram of the peak of Caryophyllene compound

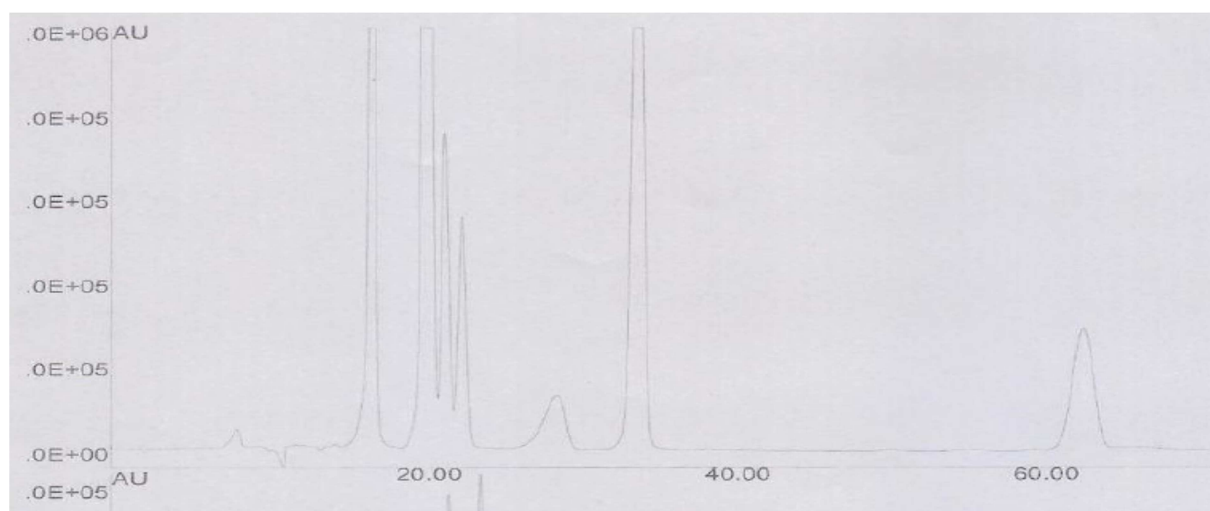
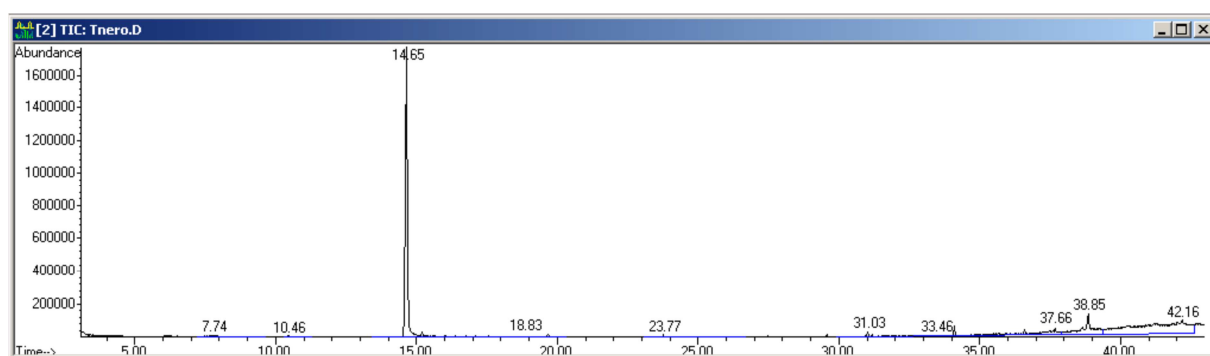
Figure 6 .GC/MS Chromatogram of the peak of α -terpinene compound

Figure 7 .GC/MS Chromatogram of the peak of Neryl-acetate compound

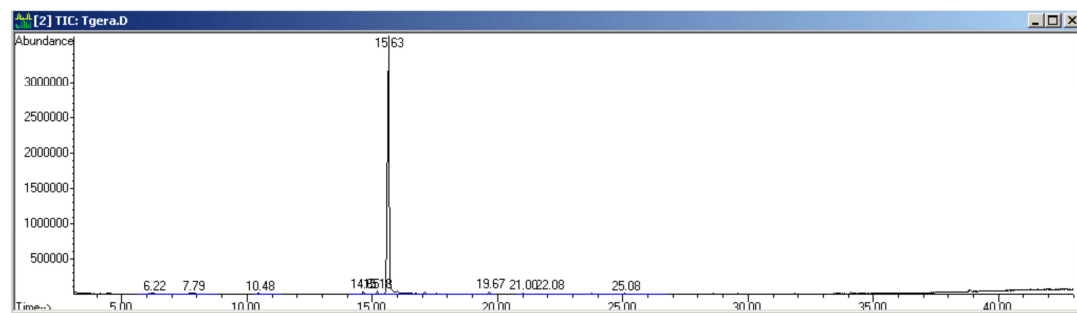


Figure 8.GC/MS Chromatogram of the peak of Geraniol compound

CONCLUSION

The chemical fractions of Syrian *I. viscosa* Aiton have been reported for the first time and it can be concluded that the reported results support the view that *I. viscosa* L. leaves are promising source of natural medicinal product. The considerable reported biological activities of *I. viscosa* (L) extracts make them good candidates to develop natural derived therapeutics; they might be also alternative additives for food and pharmaceutical preparations. For future work, we will study the effect of the above-mentioned fractions on bacteria, fungi and cancer cell lines.

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