Vol 1, No 1 (2015)

GC-MS composition and antiproliferative activity of *Inula graveolens* (L.) Desf. essential oil

Afifi[a] F. U., Abu-Dahab[b] R., Kasabri[b] V. and Abaza[a] I.M.

[a] *Department of Pharmaceutical Sciences, Faculty of Pharmacy The University of Jordan, Amman, Jordan*
b Departement of Biopharmaceutics and Clinical Pharmacy, Faculty of Pharmacy The University of Jordan, Amman, Jordan

Abstract: The composition of the essential oil isolated from the aerial parts of *Inula graveolens* (L.), (Asteraceae) was determined by GC and GC-MS using two extraction methods, namely hydrodistillation and Solid Phase Micro-Extraction (SPME). Oxygenated monoterpenes had the highest contribution to the hydro-distilled essential oil content (87.32 %) with bornyl acetate being the main component detected in this fraction (70.58 %). Both oxygenated monoterpenes, borneol (11.36%) and bornyl acetate (58.30%) remained as the major constituents while camphene was detected as the major hydrocarbon monoterpene (16.58%) in the SPME extracted oil. The antiproliferative activity of the crude oil and of some pure volatile compounds was evaluated using two breast cancer cell lines (MCF7 and T47D) and compared to reference drugs. The IC_{50} values of reference drugs; cisplatin and doxorubicin were 7.3 ± 1. 9 µM and 0.16 ± 0.0 µM for MCF7 cells, 21.3 ± 9.7 µM and 0.2 ± 0.0 µM for T47D cells, respectively. Caryophylline oxide was the main active compound of the volatile fraction with the IC values (µg/mL) for MCF7 cells and T47D cells, respectively (8.098 ± 0.71; 6.121 ± 0.818).This is the first time to investigate the essential oil composition of the aerial parts of *I. graveolens* using SPME. The findings of the present study demonstrated qualitative and quantitative variation in the composition of the essential oil between the two different extraction methods. The strong antiproliferative activity of caryophyllene oxide justifies the plants' use as an anticancer agent in the traditional medicine.

Keywords: Antiproliferative; Asteraceae; GC-MS; *Inula graveolens*; SPME

Corresponding author: Fatma U. Afifi,Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan, Amman, Jordan, e-mail: fatueafi@ju.edu.jo

Vol 1, No 1 (2015)

Introduction:

The genus *Inula* belongs to the family Asteraceae and comprises about 100 species of flowering plants found in temperate regions of Europe and Asia. In Jordan, this genus is represented by three species, namely *I. crithmoides* L., *I. graveolens* (L.) Desf. (Syn.: *Dittrichia graveolens* (L.) Greuter; *Erigeron graveolens* L.) and *I. viscosa* (L.) Ait. (Syn.: *D. viscosa* (L.) Greuter; *E. viscosum* L.) (Al-Eisawi, 2013). The most commonly distributed species is *I. viscosa*, known in Arabic as "tayyoun" has been widely studied for its chemical and biological properties. In many places, such as road sides, waste lands and pastures this species is accompanied by *I. graveolens* (L.) Desf. (synonym: *Dittrichia graveolens* L. Greuter). The latter species is also native to Southern Europe, North Africa, Mediterranean area and western Asia and has been naturalized to different parts of the world including America, Australia and Africa. This annual species reaches a height of about 80 cm has sticky leaves and bright yellow flowers appearing in September / October (Feinbrunn-Dothan, 1978). Although this species is characterized by a very strong aromatic odor, the volatile oil composition of *I. graveolens*, growing in Jordan has not been reported. Instead, emphasis has been given to the sesquiterpene lactones and flavonoids/phenolics as the major constituents of *Inula* species (Oksuz and Topcu, 1992; Topcu et *al*., 1993; Abou Douh, 2008). Reported ethnopharmacological studies praise *I. graveolens* for its antibacterial, cytotoxic and antioxidant activities (Oksuz and Topcu, 1992; Topcu et *al*., 1993; Braham et *al*., 2001; Abou Douh, 2008). Recently, Abu Irmaileh et *al*. (2014) investigated the phytotoxicity of this species in reducing the seedling development, seed germination, root length and shoot length of different plant species. Along its reputed antimycobacterial and veterinary phytotherapeutic roles; bactericidal effect was reported for *I. graveolens* essential oil involving both, the cytoplasmic membrane and cell wall in this toxic action against *S. aurues* (Pieroni et *al*., 2006; Bamuamba et *al*., 2008; Guinoiseau et *al*., 2010). Allegedly there have been sporadic reports of allergic contact dermatitis from *I. graveolens* (Thong et *al*., 2008). Methylated quercetins isolated from *I. viscosa* proved to have outstanding *in vitro* proapoptotic, antiproliferative and antimicrobial properties (Rozenblat et *al*., 2008; Talib et *al*., 2012).

In continuation of our interest in the composition and antiproliferative activity of aromatic medicinal plants used in the traditional medicine in Jordan, the present study was designed to evaluate the composition of the volatile oil of *I. graveolens* using two extraction methods, namely hydrodistillation and solid phase micro-extraction (SPME) and to screen the *in vitro* antiproliferative effect of its volatile oil and some of the components of the volatile oil against MCF7 and T47D breast cancer cell lines (Abu-Dahab and Afifi, 2007; Abu-Dahab et *al*., 2012, 2014). Already earlier Abu-Dahab and Afifi (2007) revealed that the ethanol extract of this species has antiproliferative activity against MCF7 and T47D cell lines. Based on the promising results of the latter the study the present manuscript was expanded to the volatile fraction of *I. graveolens*.

Materials and methods:

Plant material

Flowering aerial parts of *I. graveolens* were collected from Al-Jubeiha region, in the vicinity of the University of Jordan, Amman (31°57'N and 35°56'E), Jordan, during late summer 2012. The plant was identified by Prof. Barakat Abu Irmaileh (Department of Plant Protection, Faculty of Agriculture, The University of Jordan). The leaves were air dried at room temperature (RT) in the shade until constant weight, and subsequently assayed for essential oil composition. A voucher specimen (AST21/FMJ) has been deposited in the Department Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan.

Hydrodistillation of plant material

Ground air dried aerial parts, each 150 g were hydro-distilled using a Clevenger apparatus for 3 h. The distillation was repeated twice and the oils obtained were pooled separately, dried over anhydrous sodium sulfate (Na_2SO_4) and stored at 4 ºC in amber glass vials until analysis**.**

Solid Phase Micro Extraction of volatile oils (SPME)

The SPME experiments were performed using the fiber assemblies (PDMS/DVB; d_f 65 µm, length 1 cm) for manual sampling (Supelco, USA). About 0.1 g of freshly powdered dried leaves were put into 5.0 mL amber glass vials, tightly capped with PTFE-coated septa, and SPME extraction was performed for 2.0 min at RT. Desorption of the analytes was carried out at 240 ºC for 60 seconds. Each sample was repeated twice.

GC-MS and GC-FID analysis

The analysis was performed using Varian Chrompack CP-3800 GC/MS/MS-200 (Saturn, Netherlands), equipped with DP-5 (5% diphenyl, 95% dimethyl polysiloxane) GC capillary column (30 m × 0.25 mm i.d., 0.25 µm film thicknesses), with helium as a carrier gas (flow rate 0.9 mL/min). The temperature in MS source reached 180 ºC, the ionization voltage was 70 eV. The column temperature was kept at 60 ºC for 1 min (isothermal), and then programmed to 246 ºC at a rate of 3 ºC/min, and kept constant at 246 ºC for 3 minutes (isothermal). About 1 µL aliquot of each oil sample, appropriately diluted to 10 µL in GC grade *n*-hexane, was subjected to GC/MS analysis. A hydrocarbon mixture of *n*-alkanes (C_8-C_{20}) was analyzed separately by GC/MS under the same chromatographic conditions using the same DP-5 column. Identification of compounds was based on the built in libraries (NIST Co and Wiley Co, USA) and by comparing their calculated retention indices (RI) relative to (C_8-C_{20}) *n*-alkanes literature values measured with columns of identical

polarity, or with authentic samples. α- and β-pinenes, *p*-cymene, limonene, linalool (Fluka, Buchs, Switzerland), eugenol, sabinene hydrate (Sigma-Aldrich, Buchs, Switzerland) were used as reference substances in GC/MS analysis (Adams, 2001). GC-grade hexane and analytical reagent grade anhydrous Na_2SO_4 were purchased from Scharlau (Barcelona, Spain) and UCB (Bruxelles, Belgium), respectively.

For the quantitative analysis (% area), a Hewlett-Packard HP-8590 gas chromatograph equipped with a split-splitless injector (split ratio 1:50) and an FID detector was used. The column was an optima-5 (5% diphenyl, 95% dimethyl polysiloxan) fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The temperature of the oven was increased at a rate of 10 ^{o}C/min form 60 ºC to 250 ºC and then held constant at 250 ºC for 5 min. The temperatures of the injector and detector were maintained at 250 ºC and 300 ºC, respectively. The relative peak areas of the oil components were measured and then used to calculate the concentration of the detected compounds.

In vitro assay for cytotoxic activity

Cell culture

Cell lines under investigation were MCF7 and T47D breast cancer cell lines. These were obtained from the European Collection of Animal Cell Culture Collection. The ECACC numbers are 86012803 and 85102201, respectively. Characteristics and properties of the cell lines used are given in Table 1. Cells were culture in RPMI 1640 media supplemented with 10% foetal bovine serum, 1% of 2 mM l-glutamine, 50 IU penicillin and 50 ug/mL streptomycin.

Table 1. Cell lines used and their properties/characteristics.

line Cell	ATCC no	Description	Seeding density	Growth Media	ER status	P53 status	Her level
MCF7	HTB-22	Epithelial adenocarcinoma	5000 cell/well	RPMI 1640	+	Wt	Very low
T-47D	HTB-133	Ductal carcinoma	10000 cell/well	DMEM/F12	+	Mu	Moderate

Cells were seeded with a density of 5000 cell/well (15 000 cell/cm^2) and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 24 h, the cells were treated with the diluted volatile oils, pure compounds and controls. Test compounds were incubated with the cells for 72 h at 37 ºC in humidified conditions containing 5% CO_2.

Crude volatile oils were initially dissolved in dimethylsulfoxide and then diluted with the growth medium and passed through a 0.2 µm filter. The solution was diluted with growth media and serial

dilutions were made (0.1 to 200 µg/mL) before the addition of 100 µL to the cells. Then, incubation followed. At the end of the exposure time, cell growth was measured using the sulphorhodamine B (SRB) assay as described earlier (Abu-Dahab et *al*., 2014). As positive controls, cisplatin and doxorubicin were used.

Results and discussion:

The hydrodistillation of the ground air dried areal parts of *I. graveolens* afforded a colorless oil (1.8 %, v/w). The essential oil components were identified in GC-MS analysis based on the comparison of the obtained RI and MS fragmentation patterns to those of standard compounds and on computer matching with the built-in libraries. The obtained results are presented in Table 2. GC/MS analysis of the hydrodistilled oil resulted in the identification of sixteen components representing 98.21 % of the total oil content. Oxygenated monoterpenes had the highest contribution to the hydrodistilled essential oil content (91.87 %) with bornyl acetate being the main component detected in this fraction (70.58 %). Monoterpene hydrocarbon fraction (2.57 %) was represented mainly by camphene (1.97%). Oxygenated sesquiterpenes accounted for 2.74 % of the total oil content and was predominated only by caryophyllene oxide (1.82%). The remaining sesquiterpenes identified were less than 0.5% in their occurrence. These results were consistent with those of previous reports on the composition of *I. graveolens* oil from other countries (Blanc et *al*., 2004; Dohi et *al*., 2009).

Table 2. Comparison of the essential oils of *Inula graveolens* obtained by hydrodistillation and SPME method.

RI Lit[a].	RI Exp[b].	Compound	Hydrodistilled oil %	Oil from SPME %
902	903	Heptanal	0.06	-
939	943	α-pinene	-	1.34
954	957	Camphene	1.97	16.58
975	979	Sabinene	0.20	2.27
979	982	β- pinene	0.40	0.54
1029	1030	d,l Limonene	-	0.41
1089	1092	Alpha-Terpinolene	-	0.14
1097	1110	unidentified	-	0.67
1146	1146	Camphor	0.50	2.94
1150	1148	Camphene hydrate	0.32	-
1169	1168	Borneol	20.12	11.36
1289	1289	Bornylacetate	70.58	58.30
1286	1285	Isobornylacetate	0.35	-
1419	1422	Caryophyllene (E)	0.11	2.13
1439	1437	Humulene <beta->	0.22	-
1455	1457	Humulene<alpha->	0.18	-
1561	1560	Germacrene B	0.46	-
1583	1582	Caryophyllene oxide	1.82	0.49
1654	1650	Cadinol<alpha->	0.57	-
1670	1672	Caryophyllene<14-hydroxy-9-epi-(E)	0.35	-
		Terpenoids		96.50
		Monoterpenes	94.44	93.88
		Monoterpene hydrocarbons	2.57	21.28
		Oxygenated monoterpenes	91.87	72.60
		Sesquiterpenes	3.71	2.62
		Sesquiterpene hydrocarbons	0.97	2.13
		Oxygenated sesquiterpenes	2.74	0.49
		Miscellaneous	0.06	-
		Total identified	98.21	96.50

Analysis of the SPME obtained volatile oil of *I. graveolens* resulted in the identification of eleven compounds amounting to 96.50 % of the total oil content. Interestingly, the composition of the SPME volatile oil showed quantitative differences as compared to the composition of the hydrodistilled oil. The monoterpene hydrocarbons were detected in the SPME oil about ten times more (21.28 % versus 2.57 %) in their concentration compared to the hydrodistilled oil. Camphene as the major hydrocarbon monoterpene found in concentration of (16.58 %). Nevertheless, the two oxygenated monoterpenes bornyl acetate (58.30%) and borneol (11.36%) remained as the major constituents. This is the first report of applying SPME as a simple, not exhaustive, solvent-free extraction technique to *I. graveolens* oil, hence no comparison of the findings to other studies could be made. The current

investigation revealed that higher amounts of oxygenated monoterpenes are found in the hydrodistilled (91.87 %) oil as compared to the oil obtained by SPME (72.60 %), indicating that hydrodistillation of *I. graveolens* may cause oxidation of some compounds.

The antiproliferative activity of the crude oil was evaluated using two breast cancer cell line models and the results are presented in Figure 1 and Table 3. The IC_{50} values (concentration of the extract needed to reduce proliferation by 50 % after 72 h of incubation compared to control wells) were determined. The IC_{50} values of reference drugs; cisplatin and doxorubicin were 7.3 ± 1. 9 µM and 0.16 ± 0.0 µM for MCF7 cells and 21.3 ± 9.7 µM and 0.2 ± 0.0 µM for T47D cells, respectively.

The crude oil exhibited against both cell lines poor antiproliferative activity. From the major components of the volatile oil, borneol, bornyl acetate, camphene, caryophyllene and caryophyllene oxide were screened for their antiproliferative activity at the concentration of 50 µg/mL. Borneol, bornyl acetate and camphene only minimally reduced the percentage proliferation with values around 100% viability, whereas caryophyllene and caryophyllene oxide reduced the rate to below 20%. Further dilutions were made for both active substances and IC_{50} was calculated for both cell lines tested, as given in Table 3.

Table 3. IC_{50} values in µg/mL for crude volatile oils and major pure components of *I. graveolens.*

	MCF7	**T47D**
Crude oil	51.54 ± 3.34	57.24 ± 1.67
Caryophyllene	14.38 ± 1.62	14.43 ± 1.33
Caryophyllene oxide	8.098 ± 0.71	6.121 ± 0.818

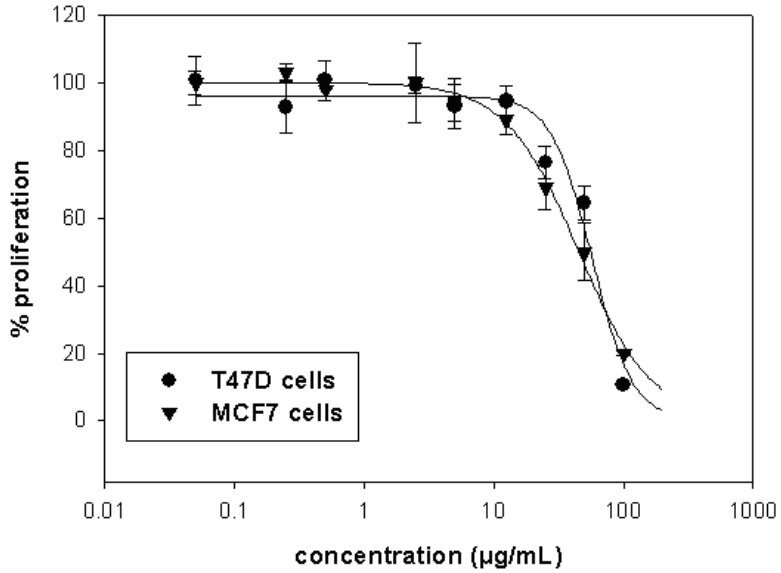
Results present the average and standard deviation of at least two determinations on two cell line passages and each is an average of at least three replicates.

While the antibacterial, antifungal, antiviral, and antioxidant activity of the *Inula* species have been reported for crude extracts, nothing, for the best of our knowledge, has been documented for the biological activity of the extracts or volatile oils of *I. graveolens* up to now.

The antiproliferative activity of *I. viscosa* grown in Jordan has been studied by Talib et *al.* in 2010, and the major components were identified and tested again, nevertheless, none of those was within the volatile oil constituents (Talib et *al*., 2012).

The biological activity of caryophyllene has been approached before and caryophyllene has been found to have antioxidant as well as strong antibacterial and antifungal activity against a large panel of pathogens (Calleja et *al.*, 2013; Dahham et *al.*, 2015). Dahham et *al.*, (2015) have also studied the antiproliferative effect of caryopyllene on different types of cancer cells, where the compound demonstrated selective antiproliferative effect against three cancer cell lines, namely HCT 116 (colon cancer, IC_{50} = 19 µM), PANC-1 (pancreatic cancer, IC_{50} = 27 µM), and HT29 (colon cancer, IC_{50} = 63 µM) cells, whereas it exhibited either moderate or poor cytotoxic effects against ME-180, PC3, K562 and MCF7. The compound displayed low toxicity against the normal cell lines 3T3-L1 and RGC-5 (Dahham et *al.*, 2015). Nevertheless, in contrast to this reported results for IC_{50} against MCF7 cells, the value obtained in the present study is much lower (70 µM).

Figure 1. Antiproliferative activity of crude oil of *I. graveolens* against MCF7 and T47D cells. Results present the average and standard deviation of at least 3 replicates.



Conclusions:

The findings of the present study demonstrated qualitative and quantitative variation in the composition of the essential oil between the two different extraction methods. SPME provides advantages as a simple, rapid, solvent-free, sensitive and inexpensive extraction method that minimizes sample size and sample preparation time. The strong antiproliferative activity of caryophyllene oxide justifies the plants' use as an anticancer agent in the traditional medicine.

Competing interests

The authors declare that they have no competing interests.

Acknowledgement

We gratefully acknowledge the financial support of the Deanship for Academic Research, The University of Jordan for this study.

References

Abu Douh M.A. (2008). New eudesmane derivatives and other sesquiterpenes from the epigeal parts of *Dittrichia graveolens*. *Chem. Pharm. Bull.* 56, pp1535-1545.

Abu-Dahab R., Afifi F. (2007). Antiproliferative activity of selected medicinal plants of Jordan against a breast adenocarcinoma cell line (MCF7). *Sci Pharm*. 75, pp121–136.

Abu-Dahab R., Kasabri V., Afifi F.U. (2014). Evaluation of the volatile oil composition and antiproliferative activity of *Laurus nobilis* L. (Lauraceae) on breast cancer cell line models. *Rec. Nat. Prod*. 8(2), pp136-147.

Abu-Dahab R., Kasabri V., Afifi F.U., Naffa R., Majdalawi L. (2012). Comparison of the antiproliferative activity of crude ethanol extracts of nine *Salvia* species grown in Jordan against breast cancer cell line models. *Pharmacog. Mag*. 8(32), pp319-324.

Adams, R.P. (2001). Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. 2nd ed. Carol Stream, IL, Allured Publ. Corp, Illinois, USA.

Al-Eisawi D.M.H. (2013). Flora of Jordan Checklist. Revised Edition 1, Amman – Jordan.

Bamuamba K., Gammon D.W., Meyers P., Dijoux-Franca M.G., Scott G. (2008). Anti-mycobacterial activity of five plant species used as traditional medicines in the Western Cape Province (South Africa). *J. Ethnopharmacol.* 117(2), pp385-390.

Braham H., Ben J.H., Martin M.T., Mighri Z. (2001). Biological and chemical studies of *Inula graveolens* sesquiterpene structures. Identification of essential oil constituents by GC/MS. *J. Soc. Chim Tunis*. 4, pp1215–1222.

Calleja, M.A., Vieites, J.M., Montero-Meterdez, T., Torres, M.I., Faus, M.J., Gil, A., Suárez A. (2013). The antioxidant effect of β-caryophyllene protects rat liver from carbon tetrachloride-induced fibrosis by inhibiting hepatic stellate cell activation. *Br. J. Nutr*. 109, pp394–401.

Dahham S.S., Tabana Y.M., Iqbal M.A., Ahamed M.B., Ezzat M.O., Majid A.S., Majid A.M. (2015). The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. *Molecules.* 20(7), pp11808-11829.

Dohi S., Terasaki M., Makino M. (2009). Acetylcholinesterase inhibitory activity and chemical composition of commercial essential oils. *J. Agric. Food. Chem*. 57, pp4313-4318.

Feinbrun-Dothan N. (1978). Flora Palaestina Part Three. The Israel Academy of Sciences and Humanitites, Jerusalem.

Guinoiseau E., Luciani A., Paul G.R., Quilichini Y., Ternengo S., Bradesi P., Berti L. (2010). Cellular effects induced by *Inula graveolens* and *Santolina corsica* essential oils on *Staphylococcus aureus. Eur. J. Clin. Microbiol. Infect. Dis*. 29, pp873-879.

Hamdan I., Afifi F. (2010). Capillary electrophoresis as a screening tool for alpha amylase inhibitors in plant extracts. *Saudi Pharm. J.* 18, pp91-95.

Oksuz S., Topcu G. (1992). A eudesmanolide and other constituents from *Inula graveolens*. *Phytochemistry*. 31, pp95-197.

Pieroni A., Giusti M.E., de Pasquale C., Lenzarini C., Censorii E., Gonzáles-Tejero M.R., Sánchez-Rojas C.P., Ramiro-Gutiérrez J.M., Skoula M., Johnson C., Sarpaki A., Della A., Paraskeva-Hadijchambi D., Hadjichambis A., Hmamouchi M., El-Jorhi S., El-Demerdash M., El-Zayat M., Al-Shahaby O., Houmani Z., Scherazed M. (2006). Circum-Mediterranean cultural heritage and medicinal plant uses in traditional animal healthcare: a field survey in eight selected areas within the RUBIA project. *J. Ethnobiol. Ethnomed*. 2, pp16-28.

Rozenblat S., Grossman S., Bergman M., Gottlieb H., Cohen Y., Dovrat S. (2008). Induction of G2/M arrest and apoptosis by sesquiterpene lactones in human melanoma cell lines. *Biochem. Pharmacol*. 75(2), pp369-382.

Seca A.M., Grigore A., Pinto D.C., Silva A.M. (2014). The genus *Inula* and their metabolites: from ethnopharmacological to medicinal uses. *J. Ethnopharmacol*. 154, pp286-310.

Talib W.H., Abu Zarga M., Mahasneh A.M. (2012). Antiproliferative, antimicrobial and apoptosis inducing effects of compounds isolated from *Inula viscosa*. *Molecules.* 17(3), pp3291-3303.

Talib W.H., Mahasneh A.M. (2010). Antimicrobial cytotoxicity and phytochemical screening of Jordanian plants used in traditional medicine. *Molecules.* 15, pp1811-1824.

Thong H.Y., Yokota M., Kardassakis D., Maibach H.I. (2008). Allergic contact dermatitis from *Dittrichia graveolens* (L.) Greuter (stinkwort). *Contact Dermatitis.* 58(1), pp51-53

Topcu G., Oksuz S., Shieh H.L., Cordell G.A., Pezzuto J.M., Bozok-Johansson C. (1993). Cytotoxic and antibacterial sesquiterpenes from *Inula graveolens*. *Phytochemistry*. 33, pp407–410.