GC-MS composition and antiproliferative activity of *Lavandula angustifolia* Mill. essential oils determined by hydro-distillation, SFE and SPME

Afifi[a]* F. U., Abu-Dahab[a] R., Beltrán[b] S., Alcalde[b] B. B. and Abaza[a] I. F.

[a] *School of Pharmacy, The University of Jordan, 11942 Amman, Jordan*
[b] *Faculty of Sciences, University of Burgos, Spain*

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The aim of this study was to compare different sample preparation methods for the gas chromatographic determination of the composition of essential oils of *Lavandula angustifolia* Mill. (Lamiaceae) grown in Jordan. The essential oils obtained by hydro-distillation and supercritical-fluid-extraction (SCFE) from dry flowers and the volatile emitted from the fresh flowers were determined by solid-phase-micro-extraction (SPME) and analyzed by GC and GC-MS. In all applied methods, oxygenated monoterpenes dominated the oils. The linalool was the main constituent in the hydrodistilled-, SCF extracted and in SPME detected oils with 35.65%, 33.63% and 22.10%, respectively. The antiproliferative activity of the ethanolic extract, crude lavender oil and of some pure volatile compounds was evaluated using breast cancer cell lines (MCF7, T47D and ZR-75-1). Findings were compared to reference drugs and normal fibroblasts. The ethanolic extract showed potent antiproliferative activity against all breast cancer cell lines under investigation, with minimum toxicity towards the normal fibroblasts. The antiproliferative activity was further estimated for the major constituents against T47D cells and the major activity of the oil was mostly due to linalool and linalyl acetate. This is the first time to investigate the essential oil composition of the aerial parts of *L. angustifolia* using SPME. The findings of the present study demonstrated qualitative and quantitative variation in the composition of the essential oil among the used methods.

Keywords: *Lavandula angustifolia*, Lamiaceae, GC-MS, SPME, hydro-distillation, SFE, antiproliferative

Corresponding author: Prof . Dr. Fatma U. Afifi, School of Pharmacy, The University of Jordan, 11942 Amman, Jordan Tel: +962 6 555 5000 Fax: +962 6 533 9649 E-mail:fatueafi@ju.edu.jo

1. Introduction:

Lavandula angustifolia Mill. (syn. *L. officinalis* Chaix *ex* Vill., *L. pyrenaica* DC., *Lavandula vera* DC.) is better known as *L. officinalis*, referring to its medicinal properties. The species name *angustifolia* originates from the Latin meaning "narrow leaf", referred in English as "common lavender", "true lavender", "English lavender" and "garden lavender" and in Arabic as "Khuzama". The genus *Lavandula* has 39 species and belongs to the family Lamiaceae. Its fragrance, colorful flowers and its ability to survive with low water consumption made this species a popular ornamental plant throughout the world, especially in the Mediterranean basin. Moreover, it is fairly tolerant to low temperatures. This strongly aromatic shrub is growing as high as 1 to 2 m tall. The leaves are evergreen, 2–6 centimeters long, and 4–6 millimeters broad. The flowers are pinkish-purple (lavender-colored), produced on spikes 2–8 cm long at the top of slender, leafless stems 10–30 cm long. Traditionally lavender is widely used as a holistic relaxant and said to have carminative, anti-flatulence and anti colic properties (Aburjai and Hudaib, 2005). In addition to its well reported utilization in the cosmetic industry and aromatherapy, several studies deal with its medicinal properties. *L. angustifolia* oil is reported to have antibacterial, antifungal, antimutagenic, antioxidant, analgesic, lipolytic, insect repellent and insecticidal properties (Casella et *al.*, 2002; Shen et *al*., 2005; Benham et *al*., 2006; Mkola et *al*., 2007; Hui et *al*., 2010; Danh et *al*., 2013; Hamad et *al*., 2013; Kayedi et *al*., 2013). More recent studies deal with the benefits of Lavender oil in anxiety, stress and depression as a neuroprotective agent and in the treatment of neurological disorders such as Alzheimer's disease (Tanida et *al*., 2006; Parvin et *al*., 2011; de Souse et *al*., 2015; Effati-Daryani et *al*., 2015).

The essential oil compositions of lavender grown in different countries have been studied mainly using hydro-distillation. In continuation of our interest in the composition and antiproliferative activity of aromatic medicinal plants used in traditional medicine in Jordan the present study was designed to evaluate the composition of the volatile oil of *L. angustifolia* using two extraction methods, namely hydro-distillation and supercritical fluid extraction (SFE). Additionally solid phase micro-extraction (SPME) was applied to determine

the volatile emitted from the fresh flowers. Biologically, *in vitro* antiproliferative effect of its volatile oil and some of the components of the volatile oil (linalool and linalyl acetate) were screened against human breast adenocarcinoma cell line (MCF-7) and human breast ductal carcinoma cell lines (T47D and ZR-75-1). The fibroblasts CCD-1064Sk were used as a control of normal, non cancerous cells.

2. Materials and methods

2.1. Plant Material

Flowers of *L. angustifolia* were collected from Amman, near the campus of The University of Jordan, (31º 57'N, 35º 56' E, 700 meter altitude) during mid summer 2015. The taxonomic identity of the plant was confirmed using descriptive references and by comparison of the plant material with those of the known identity in the Herbarium of the Department of Biological Sciences, School of Science, The University of Jordan (Feinbrun-Dothan, 1978). A voucher specimen (LAM-LA1/FMJ) has been deposited in the Department Pharmaceutical Sciences, School of Pharmacy, The University of Jordan, Amman, Jordan. On the day of collection, one part of the collected flowers was submitted to SPME analysis. Remaining flowers were air dried at room temperature (RT) in the shade until constant weight, and subsequently assayed for essential oil composition using hydro-distillation and SFE methods.

2.2. Hydro-distillation and solid phase micro-extraction (SPME) of the volatile constituents from L. angustifolia GC-MS and GC-FID analysis

Both experiments were performed under the same conditions as described by Afifi et *al*. (Afifi et *al*., 2015). For the hydro-distillation using Clevenger apparatus 150 g flowers were used, which produced 0.8% w/v yield. Supercritical fluid extraction gave higher yield (3.3% w/v). The extractions and analytical experiments were repeated twice. 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 analysis was carried out at 240 ºC for 60 s. 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**.** The extractions and analytical experiments were

repeated twice. Conditions applied for the qualitative and quantitative analysis were described earlier (Afifi et *al*., 2015). Identification of the 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 (Adams, 2001).

2.3. Supercritical fluid extraction (SFE)

The extraction experiments were carried out in a laboratory SFE plant whose P&I diagram has been previously described (Murga et *al*., 2003) In the SFE experiment, 10-15 g of plant material was loaded in the extractor (100 mL capacity). Two syringe pumps (ISCO 260 DM), that work alternatively, provide an uninterrupted flow of CO_2 (Carburos metálicos, liquid CO_2 P99.9%) compressed up to the desired operating pressure, 40.0 ± 0.2 MPa. The pressurized solvent was preheated to the desired extraction temperature (40 ºC) before entering the extractor. The extractor was held in an oven whose temperature is controlled within the accuracy of ±0.5 ºC. The carbon dioxide flow was set to 1.5 ± 0.2 g/min. Depressurized CO2 was quantified with a totalizer flow meter. Extraction yield was determined gravimetrically by weighing the extract at the end of the extraction run.

2.4. In vitro assay for cytotoxic activity

2.4.1. Cell culture

The cell lines under investigation were human breast adenocarcinoma cell lines (MCF-7 ATCC Nr: HTB 22), human breast ductal carcinoma cell lines (T47D, ATCC Nr: HTB 133, and ZR-75-1, ATCC Nr: CRL-1500). Fibroblasts CCD-1064Sk (ATCC® CRL-2076TM) was used as a control of normal, non cancerous cell line. The properties of the cell lines under investigation as well as their culture conditions were as those described earlier (Abu-Dahab et *al*., 2012). The tissue culture media (RPMI 1640) used was supplemented with 10% heated foetal bovine serum, 1% of 2 mM l-glutamine, 50 IU/ml penicillin and 50 µg/ml streptomycin.

2.4.2. Antiproliferative assay

Cells were detached, centrifuged and viability determined by trypan blue exclusion and it exceeded 90% as counted in a hemocytometer. The cell suspension was diluted afterwards

to give the optimal seeding density and 100 µL of the cell suspension was plated in a 96 well plate and incubated at 37 °C in a humidified atmosphere containing 5% CO_2. After 24 hours the cells were treated with the extracts. The crude extracts as well as the volatile oils (initially dissolved in DMSO), were diluted with the medium and passed through a 0.2 µm filter. The IC_{50} of the active extracts was determined through a dose response curve using 8 concentrations (ranging from 0.1 to 100 µg/mL). Incubation for 72 hours followed and at the end of the exposure time, cell growth was analyzed using the sulphorhodamine B (SRB) assay as described earlier (Al-Kalaldeh et al. 2010). Two replicate plates were used to determine the cytotoxicity of each test substance. As positive control Doxorubicin (Ebewe Pharma GMBH Nfg. KG, Austria) was used.

3. Results and discussion

3.1. Volatile oil determination

The hydro-distillation of the ground air dried areal parts of *L. angustifolia* afforded a colorless oil (0.8 %, 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 1. GC/MS analysis of the hydro-distilled oil resulted in the identification of 47 components representing 97.81 % of the total oil content. Oxygenated monoterpenes had the highest contribution to the hydro-distilled essential oil content (84.97 %). The principal components of this fraction were linalool (35.65 %), camphor (13.15 %) 1,8 cineol (11.86 %) and borneol (5.52 %). Monoterpene hydrocarbons accounted for 7.39 % of the total oil with α-pinene (1.03 %) as the main monoterpene hydrocarbon. The total amount of the sesquiterpenes was only for 1.42 % of the total oil content. Hydro-distillation is the most usual method for the determination of the essential oil content of the aromatic plants, described in several pharmacopoeias. Nevertheless, this traditional method can lead to degradation of thermo-labile compounds and partial hydrolysis of water sensible components. The findings of the present study were compared to some of the related studies. In fact, a comparison of the reported volatile components extracted by hydro-distillation from *L. angustifolia* exhibited differences in the occurrence of major components which might be dictated by the environmental conditions, soil characteristics, time of harvest, and methods of drying. For example, linalool was detected in plants grown in Australia (52.59 %), Iraq (24.63

%), Poland (24.6 %), India (28.06 %), Japan (13.00%) and in Iran (0.71 %) with great differences and was not always the major oxygenated monoterpene (Rezazadeh et *al*., 2008; Verma et *al*., 2010; Danh et *al*., 2013; Hamad et *al*., 2013; Smigielski et *al*., 2013; Kamiie et *al*., 2014). Fluctuations in the concentration of linalool (18.74% up to 46.8%) was observed by Zagorcheva et *al*. (2013) using volatile oils obtained from *L. angustifolia* cultivars grown in different regions of Bulgaria. In another recent study, Tayarani-Najaran et *al*. (2014) did not detect any linalool in plants collected in Iran. Again, linalool was absent or in trace amounts in *L. angustifolia* plants collected and studied in China and Romania (Hui at *al*., 2010; Jianu et *al*., 2013). One has to keep in mind also the occurrence of hybrids in this widely cultivated species.

SFE is considered as an alternative method for the production of flavours and fragrances in the food industry preventing thermal degradation, hydrolysis and solvent contamination of genuine compounds in the plants. The advantages of SFE over hydro-distillation are described by several researchers especially using *Salvia* species (Marongiu et *al*., 2001; Alekovski and Sovova 2007; Yamini et *al*., 2008; Micic et *al*., 2011). In supercritical fluid extraction supercritical carbon dioxide acts as an organic solvent and the extraction conditions can also be adapted by variation of temperature and pressure (Ricter and Schellenberg, 2007). The higher extraction yield, obtained in the SFE method, in comparison to hydro-distillation is within the range with those reported in the literature (Rezazadeh et al., 2008; Da Porto et al., 2009). On the other hand, Danh and his co-workers (2013) obtained a much higher yield while performing SFE, since the yield in this type of extraction is greatly influenced by the operating conditions, such as time, pressure, temperature and particle size as well as by the factors (environmental and genetic) influencing the content of the essential oil in the plant material (Danh et *al*., 2013). In the present study linalool (33.63 %) was found as the major oxygenated monoterpene in the SFE method, in addition to the big variety of acetates, which were not detected in the hydro-distilled oil. Compared to the hydro-distilled oil linalyl acetate was found in much higher concentration in the SFE oil (3.76 % versus 8.73 %) indicating hydrolysis of the latter compound into linalool, which was detected in hydro-distilled oil in higher concentration than in the oil obtained by SFC. Other significant constituents were camphor (10.33 %), borneol (7.47 %), 1,8 cineol (6.34 %) and β-farnesene (5.11 %).

Analysis of the SPME obtained volatile oil of *L. angustifolia* resulted in the identification of 38 compounds amounting to 99.76 % of the total oil content. The emitted vapour from the fresh flowers, adsorbed by the SPME fibre was rich in hydrocarbon monoterpenes with high volatility appearing with low retention times. The monoterpene hydrocarbons were detected in the SPME oil about four times more (30.27 % versus 7.39%) in their concentration compared to the hydro-distilled oil. α-pinene, camphene and limonene, as the major hydrocarbon monoterpenes, were found in concentrations of (5.51%, 4.15% and 5.12%, respectively). Nevertheless, the oxygenated monoterpenes linalool (22.10%), 1,8 cineol (19.71%) and camphor (10.68%) remained as the major constituents. This is the first report of applying SPME as a simple, not exhaustive, solvent-free extraction technique to *L. angustifolia* oil, hence no comparison of the findings to other studies could be made. Moreover, the current investigation revealed that higher amounts of oxygenated monoterpenes are found in the hydro-distilled (84.97%) oil as compared to the oil obtained by SPME (64.46 %), indicating that hydro-distillation of *L. angustifolia* may cause oxidation of some compounds.

3.2. Antiproliferative activity of ethanolic extract of L. angustifolia

Cancerous cell lines that represent breast cancer as well as and human fibroblasts (non-malignant control) were incubated with various concentrations of the ethanolic mixture of *L. angustifolia* for 72 hours. The extract showed significant reduction in cell proliferation for all cancer cell lines under investigation (Figure 1) and IC_{50} values were calculated (Table 2). At the same time, no or minimum effect on the proliferation of normal cells was seen, which indicates selective toxicity of the extract towards cancer cell lines. The doxorubicin was used as a positive control and an IC_{50} of 0.16 µM for MCF7 cells and 0.2 µM for T47D cells was calculated compared to untreated controls.

The antiproliferative activity of the ethanol extract of *L. angustifolia* resulted in quite similar IC_{50} values for all cell lines, although the properties of the cells and their surface receptors show a difference, which might indicate a similar mechanism for all cell lines under investigation. This comes in contradiction with what has been previously reported by Wamidh and Mahasneh (2010), where the ethanol extract was evaluated and showed no antiproliferative activity against MCF7 and poor reduction in proliferation against Hep-2 cells. The plant material was collected from Amman (altitude 700 meter) and Ajloun (altitude

1200 meter), but the exact time of collection was not mentioned. This might be one of the factors influencing the difference in the outcome of both studies. It is obvious that to compare the outcomes of the biological activities using natural products, it is necessary to describe exactly the environmental conditions, time of collections, and method of drying, even though the same solvent was used for the extraction to observe the biological activity using the same cell line. It is also to underline the importance of the passage numbers of used cell lines, to allow an accurate comparisons.

The antiproliferative activity of different solvent extracts of *L. angustifolia* has been previously investigated by Tayarani-Najaran et *al.* (2014) and the ethanolic extract for the plant extract grown natively in Iran showed promising antiproliferative activity, nevertheless, with a higher IC_{50} value (82.86 µg/mL). In the same study, it could not be concluded whether the antiproliferative activity was due to apoptosis or not. In another study, the aqueous extract of *L. angustifolia* was prepared and tested on two cell lines (MKN45 and SW742) and a Hodgkin's lymphoma cell line derived from the patients' blood samples. Here, the extract showed potent antiproliferative activity that started at 1.0 µg/mL for the cell lines, nevertheless, the activity against the primary extracted lymphoma cells was minimal (Dalilan et *al*., 2013).

The antiproliferative activity was further evaluated for the volatile oils extracted by SFE and hydro-distillation and their major components using T47D cells. A difference in the biological response was seen, where the SFE fraction showed lower IC_{50} value that its hydro-distilled counterpart (Table 2). The major volatile oil constituents were first screened at a concentration of 50 µm/mL and for those that showed antiproliferative activity of less than 50%, further IC_{50} estimation was done. Table 2 shows the results of those major components and their antiproliferative activity. It is clear that most of the biological activity of the volatile oil is due to the presence of linalool and linalyl acetate, where the values for the inhibition of 50% of the cells were quite close or even less than those of the crude oil.

Over the past decade, substantial scientific evidence has been accumulated and it suggested that the major constituent of the volatile oil of *L. angustifolia* has several biological activities including antiproliferative action. The antiproliferative effects of the *L. angustifolia* essential oil and its main components, linalool and linalyl acetate, on human prostate cancer cells were investigated. It was demonstrated that lavender essential oil was effective in inhibiting tumor

growth of human prostate cancer xenografts in nude mice. More interestingly, linalool, but not linalyl acetate, mainly contributed to this effect. This antitumor effect was associated with apoptosis induction and cell proliferation inhibition (Zhao et *al*., 2016). In another study, linalool and *p*-coumaric acid as biologically active compounds were isolated from the *Plantaginaceae* and their biological activity against a panel of cell lines was estimated (Chang and Shen, 2014). By using WST-1 analysis, it was shown that linalool and *p*-coumaric acid has good inhibitory effects against breast, colorectal and liver cancer cells. Cell cycle analysis here confirmed that the inhibition of proliferation was through induction of apoptosis. We propose that the antiproliferative activity demonstrated in the present study for the different breast cancer cell lines could be in agreement with the earlier proposed mechanisms, namely apoptosis (Chang and Shen 2014; Zhao et *al*., 2016).

Conclusions

Results presented in this study indicated that for investigating the composition of genuine essential oils SFE is more useful than hydro-distillation. On the other hand SPME, a comparatively new method, is a very simple, inexpensive and efficient, solvent-free sample preparation method that minimizes sample size and sample preparation time which makes this method suitable for routine analysis of plant samples. The innovative part in the present study is the use of the SPME for the first time in the determination of volatile oil composition of *L. angustifolia*, grown in Jordan, and using a panel of different breast cancer cell lines. With respect to biological activity, all tested samples exhibited promising antiproliferative activity against MCF7, T47D and ZR-75-1 cells, while the response towards normal fibroblasts indicated the safety of the extract. Further mechanistic study is recommended for the active volatile oil component linalool and linalyl acetate.

Table 1. Comparison of the essential oil composition of the dry leaves of *Lavandula angustifolia* using hydro-distillation, SFE and SPME

RI Lit[a].	RI Exp[b].	Compound	LA 1 %	LA 2 %	LA 3
927	925	Tricyclene	0.25	0.69	-
930	926	α-Thujene	1.43	0.28	-
939	935	α-Pinene	5.51	1.03	0.22
954	951	Camphene	4.15	0.83	0.35
975	974	Sabinene	0.95	0.15	-
979	982	β- Pinene	2.31	0.72	0.45
991	988	Octanol-3	0.78	0.29	-
995	992	Myrecene	2.20	0.51	0.15
995	998	Butylbutanoate	0.61	0.22	-
1003	1006	Phellandrene	0.27	0.46	0.16
1002	1005	Caren Δ^2	0.59	-	-
1005	1009	Hexenylacetate	0.79	-	-
1017	1019	α-Terpinene	0.17	0.18	-
1025	1022	*p*-Cymene	0.29	0.06	0.21
1026	1027	*o*-Cymene	1.02	0.61	-
1029	1031	*d,l* Limonene	5.12	1.28	0.43
1031	1035	1,8 Cineol	19.71	11.86	6.34
1037	1038	*cis* Ocimene	3.84	0.34	-
1040	1041	Lavender lactone	0.24	-	0.15
1050	1047	β-Ocimene (E)	0.53	-	0.14
1060	1061	γ-Terpinene	0.55	0.34	-
1073	1073	Trans Linalool oxide	2.47	2.60	2.02
1089	1087	α-Terpinolene	0.97	0.25	-
1087	1090	*cis* Linalool oxide	1.95	1.77	1.56
1097	1098	Linalool	22.10	35.65	33.63
1126	1130	Campholenal	-	0.17	-
1132	1131	Ocimene<*allo*>	0.12		-
1140	1144	Napinone	-	0.44	-
1152	1149	Hexylisobutanoate	0.41	-	-
1146	1150	Camphor	10.68	13.15	10.33
1150	1148	Camphene hydrate	-	0.34	0.31
1169	1168	Borneol	1.22	5.52	7.47
1177	1180	Terpinene-4-ol	2.78	-	-
1181	1179	Lavandulol	0.13	0.79	1.02
1189	1188	α-Terpineol	-	6.85	4.87
1194	1193	Hexenyl butanoate <2E->	1.62	2.26	1.46
1198	1201	Verbanol	-	-	0.10
1199	1203	γ-Terpineol	-	0.91	0.70
1205	1208	Verbanone	-	-	0.14
1217	1220	*trans* Carveol	-	0.10	0.10
1230	1234	Nerol	-	0.12	0.11
1239	1234	Isobornyl formate	0.34	0.08	0.42
1244	1243	Hexylisovalarate	0.13	0.49	0.59

1247	1245	Hexenyl isovalarate	-	0.22	0.28
1257	1255	Linalyl acetate	2.61	3.76	8.73
1267	1262	Geranial	-	0.14	-
1282	1285	Nerylformate	0.23	-	-
1283	1283	*cis* Verbenylacetate	-	-	0.26
1286	1288	Isobornylacetate	-	-	1.60
1289	1289	Bornylacetate	-	0.09	0.22
1291	1293	*p*-Cymene-7-ol	-	-	0.12
1299	1301	Carvacrol	-	-	0.14
1333	1334	Hexyltiglate	-	0.28	0.30
1342	1339	*trans* Carvylacetate	-	-	0.45
1349	1352	α-Terpinylacetate	-	-	0.36
1362	1363	Nerylacetate	-	0.10	0.16
1381	1383	Geranyl acetate	-	0.20	-
1384	1390	Hexylhexanoate	-	0.27	0.30
1391	1391	Sesquithujene<7-epi->	-	-	0.23
1391	1394	β-Elemene	-	-	0.20
1413	1410	*cis* α-Bergamotene	-	-	0.30
1419	1422	Caryophyllene (E)	0.14	0.18	1.26
1435	1437	*trans* α-Bergamotene	-	-	0.10
1455	1457	β-Farnesene (E)	0.55	0.98	5.11
1473	1475	α-Terpinylisobutanoate	-	-	0.25
1485	1487	Germacrene D	-	0.10	0.95
1515	1510	Geranylisobutanoate	-	0.33	1.79
1583	1582	Caryophyllene oxide	-	0.16	0.87
1593	1596	Viridiflorol	-	-	0.12
1595	1597	Carotol	-	-	0.13
1654	1657	α-Cadinol	-	-	0.33
	Terpenoids				
	Monoterpenes		94.73	92.36	83.42
	Monoterpene hydrocarbons		30.27	7.39	2.11
	Oxygenated monoterpenes		64.46	84.97	81.31
	Sesquiterpenes		0.69	1.42	9.60
	Sesquiterpene hydrocarbons		0.69	1.26	1.68
	Oxygenated sesquiterpenes		-	0.16	7.92
	Miscellaneous		4.34	4.03	2.93
	Total identified		99.76	97.81	95.95

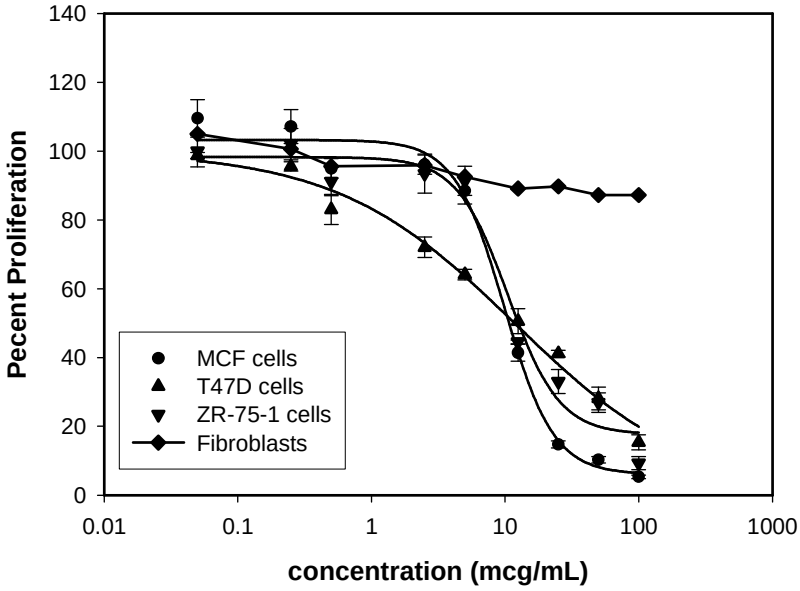
LA 1:*Lavandula angustifolia* fresh, SPME LA 2: *Lavandula angustifolia* dry, hydrodistilled
LA 3: *Lavandula angustifolia* dry, SFE
[a]RI Lit., Reported Retention Index [30]; [b]RI Exp., Retention index relative to (C_8-C_{20}) *n*-alkanes;
[c]The percentage composition based on the GC peak areas

Table 2. IC_{50} values of the volatile oils and their major constitutes extracted from *L. angustifolia*. Results present the average and standard deviation of 4 determinations.

Tested compounds	**IC_{50} value (µg/mL)± St.dev.**
Crude oil (hydro-distillation)	70.07 ± 16.9
Crude oil (SME)	28.97 ± 1.63
Linalool	11.09 ± 2.62
Linalool acetate	25.06 ± 4.08
Camphor	58.12 ± 33.59
Borneol	Not active *
Borneol acetate	Not active *
Cineol	Not active *
Lime	Not active *

*refers to no antiproliferative activity in the concentration range tested.

Figure 1. Antiproliferative activity of ethanolic extract of *L. angustifolia* against MCF7, T47D, ZR-75-1 and fibroblasts. Results present the average and standard deviation of at least 3 replicates.



Competing interests

The authors declare that they have no competing interests.

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