Plant Foods Hum Nutr (2017) 72:96–102
DOI 10.1007/s11130-016-0596-8

ORIGINAL PAPER

Biological Activities of Asteraceae (*Achillea millefolium* and *Calendula officinalis*) and Lamiaceae (*Melissa officinalis* and *Origanum majorana*) Plant Extracts

Mónica R. García-Risco[1] · Lamia Mouhid[2] · Lilia Salas-Pérez[3] · Alexis López-Padilla[1] · Susana Santoyo[1] · Laura Jaime[1] · Ana Ramírez de Molina[2] · Guillermo Reglero[1,2] · Tiziana Fornari[1]

Published online: 18 January 2017

Abstract Asteraceae (*Achillea millefolium* and *Calendula officinalis*) and Lamiaceae (*Melissa officinalis* and *Origanum majorana*) extracts were obtained by applying two sequential extraction processes: supercritical fluid extraction with carbon dioxide, followed by ultrasonic assisted extraction using green solvents (ethanol and ethanol:water 50:50). The extracts were analyzed in terms of the total content of phenolic compounds and the content of flavonoids; the volatile oil composition of supercritical extracts was analyzed by gas chromatography and the antioxidant capacity and cell toxicity was determined. Lamiaceae plant extracts presented higher content of phenolics (and flavonoids) than Asteraceae extracts. Regardless of the species studied, the supercritical extracts presented the lowest antioxidant activity and the ethanol:water extracts offered the largest, following the order *Origanum majorana* > *Melissa officinalis* ≈ *Achillea millefolium* > *Calendula officinalis*. However, concerning the effect on cell toxicity, Asteraceae (especially *Achillea millefolium*) supercritical extracts were significantly more efficient despite being the less active as an antioxidant agent. These results indicate that the effect on cell viability is not related to the antioxidant activity of the extracts.

Keywords *Origanum majorana* · *Melissa officinalis* · *Achillea millefolium* · *Calendula officinalis* · Antioxidant · Antiproliferative

Abbreviations

ABTS	2 2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
DMSO	Dimethyl sulfoxide
DPPH	2,2-Diphenyl-1-picrylhydrazyl
GC-MS	Gas chromatography–mass spectrometry
MTT	3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide
SFE	Supercritical fluid extraction
UAE	Ultrasonic assisted extraction

Introduction

In recent decades, the interest in the use of plants in medicine research has leaned towards finding therapies that replace synthetic drugs by herbal derived products. Plant-type products are used to flavor beverages and food goods, but also as antioxidants with a strong ability to stabilize vegetable fats [1]. Particularly, flavonoids and other phenolic compounds, wildly present in plant extracts [2] possess a chemical structure that has shown a high capacity as free radical scavengers (antioxidants) and therefore, to act against free radical damage [3].

It is well known that oxidative stress and free radicals are involved in triggering cell damage and in carcinogenesis

Mónica R. García-Risco, Lamia Mouhid and Lilia Salas-Pérez contributed equally to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s11130-016-0596-8) contains supplementary material, which is available to authorized users.

✉ Tiziana Fornari
tiziana.fornari@uam.es

[1] Institute of Food Science Research (CIAL), Madrid, Spain

[2] Madrid Institute for Advanced Studies on Food (IMDEA-Food), Madrid, Spain

[3] Polytechnic University of Gomez Palacio (UPGOP), Durango, Mexico

process [4]. In this sense, antioxidants were initially proposed as an alternative to struggle against cancer. However, clinical trials in humans and recent *in vivo* experiments determine a lack of success and even tumorigenic promotion [5], in part because they do not induce oxidative damage reduction *in vivo* [6]. In this regard, it is still a challenge to elucidate if the anticancer potential of plant extracts is related to their effect on free radicals.

Plant extracts are part of the current trend towards functional foods and therapies, and a source of natural biomolecules proposed against tumoral cells. In the present work is reported the study and comparison of the antitumor effect of extracts derived from different family plants: two from Asteraceae (*Achillea millefolium* and *Calendula officinalis)* and two plants from Lamiaceae (*Melissa officinalis* and *Origanum majorana*), obtained by using supercritical fluid extractions (SFE) and ultrasonic assisted extractions (UAE).

SFE uses supercritical carbon dioxide ($SCCO_2$) and extracts the lipophilic fraction of the plant; while UAE with ethanol and/or water results in the extraction of polar compounds such as flavonoids and phenols. Thus, these two techniques may be complemented, by first applying SFE to extract the plant volatile oils, followed by a UAE step to recover the polar compounds from the residual plant matrix. This two-step extraction scheme was applied to produce extracts from *Achillea millefolium* L. (yarrow), *Calendula officinalis* (marigold), *Melissa officinalis* (balm) and *Origanum majorana* (marjoram). The extracts were analyzed in terms of their antioxidant capacity and their antiproliferative effect, in order to determine whether these two biological activities are related, or by contrast, to identify within these herbal species and extracts the most active as an antioxidant or an anticancer agent.

Materials and Methods

A more detailed description of chemicals and sample preparation can be found as supplementary material.

Extractions The SFE (see Table 1) assays were carried out in duplicate using a pilot-plant supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L cylinder extraction cell. Extraction pressure and temperature were 140 bar and 40 °C, respectively. CO_2 flow was 70 g/min and extraction time 180 min. The oleoresin resulting in the separator was collected using ethanol. Then, ethanol was removed using a rotavapor at low temperature (30 °C). The residual vegetal material obtained after the SFE step was re-extracted using an ultrasound probe (Branson Digital Sonifier, Branson Ultrasonics, model 250; Danbury, USA). The UAE assays were carried out using green polar solvents (ethanol or ethanol:water 50:50). It was demonstrated [7] that UAE with low ethanol:water ratios is more efficient than UAE with ethanol to the recovery of phenolic compounds. In this work, both ethanol and ethanol:water (50:50) solvents were used in the extractions in order to find the best conditions to obtain the maximum polar components.

Chemical Characterization To determine the chemical composition, the supercritical extracts were analyzed by GC-MS (Agilent Technologies 7890A). A more detailed description of the GC-MS method can be found as supplementary material.

Regarding the total phenolic content (TPC), the determination was carried out following Folin-Ciocalteu method, and results were expressed as mg gallic acid equivalents in 1 g of extract (mg GAE/g extract). Meanwhile the total flavonoid content (TFC) was determined as described by Zhishen et al. [8], and results were expressed as mg quercetin equivalents in 1 g of extract (mg QE/g extract).

Antioxidant Activity Scavenging activity was determined by a spectrophotometric method based on the reduction of an ethanol solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH), as described previously [9]. The DPPH concentration in the reaction medium was calculated from a calibration curve determined by linear regression ($y = 0.0303\times - .0349$; $R^2 = 0.9955$). Furthermore, the $ABTS^{\bullet+}$ assay, as described by Re et al. [10], was also used to measure the antioxidant activity and results were expressed as TEAC values (mmol TE / g extract).

Antiproliferative Effect The cytotoxicity was studied on pancreatic human tumor-derived cell line MiaPaca-2 and measured by MTT assay, as described in our previous works [9]. Results were expressed through the IC_{50}, which describes the concentration value that produces 50% cell viability inhibition.

Table 1 Supercritical CO_2 extraction of Asteraceae and Lamiaceae plants

Plant part	Yarrow Inflorescences and leaves	Marigold Flowers	Marjoram Leaves	Balm Leaves
Water content (%)	<5	<5	<5	<5
Particle size (μm)	1000	1000	1000	1000
Mass loaded for SFE (g)	383	500	550	713
CO_2/plant ratio (kg/kg)	33	25	23	18

Statistical Analysis The significant differences between extractions and plant extracts were determined by using the GaphPad Prism 6.0 m Software (San Diego, CA, USA).

Results and Discussion

The extraction approach was designed in order to obtain first a lipophilic fraction by CO_2-SFE, which is expected to contain the plant volatile oil. Then, the residual vegetal material was extracted by UAE using polar solvents (ethanol or ethanol:water 50:50) to obtain a second fraction containing the characteristic polar compounds of the plant according to the species. The SFE and UAE extraction yields of both Asteraceae and Lamiaceae plants are given in Table 2. UAE yields were considerably higher than SFE yields for all plants studied, and the yields obtained with ethanol:water were higher than those obtained with pure ethanol. Significant differences ($p < 0.05$) between SFE and UAE (ethanol:water 50:50) yields for marigold and balm were found after carrying out an ANOVA two-way analysis. In general, despite the method or the solvent used, the higher extractions yields were obtained with marigold, followed by marjoram, balm and yarrow.

GC-MS Analysis SFE extracts were analyzed by GC-MS to identify these components, mainly monoterpenes and sesquiterpenes. The results obtained are discussed below.

Marigold. Several sesquiterpenes (γ-muurolen, δ-cadinen, α-muurolol and α-cadinol) were identified in Marigold in different percentages according to sample preparation and SFE parameters [11]. In this work, the sesquiterpenes identified in Marigold SFE extracts are reported in Table 3 and include α- and β-muurolene, α- and β-cadinene, α- cadinol and T cadinol, in agreement with previous works from the literature [12, 13]. Furthermore, also triterpenes, such as α-amyrin (8.67 mg/g) and lupeol (7.39 mg/g) were identified in the SFE extract of marigold (data not shown in Table 3) in accordance with other works which report high content of triterpenes in marigold supercritical CO_2 extracts [14].

Yarrow. Table 4 present the main compounds identified in yarrow SFE extracts. In accordance with the literature, the results obtained in this work indicate that the most important compounds of *Achillea millefolium* volatile oil include monoterpenes such as terpineol and borneol [15], and sesquiterpenes such us germacrene D caryophyllene, bisabolene, α-bisabolol, δ-cadinen [16], spathuleol and cadinol [15].

Table 3 Volatile oil compounds identified by GC-MS in the SFE extract of Marigold

Retention time	Compound	% Area	Type
9795	β-Muurolene	13.86	S (hydrocarbon)
9984	α-Cadinene	23.36	S (hydrocarbon)
10,051	β-Cadinene	21.26	S (hydrocarbon)
10,236	α-Muurolene	9.79	S (hydrocarbon)
11,497	T-Cadinol	13.76	S (bicyclic alcohol)
11,636	α-Cadinol	17.96	S (bicyclic alcohol)

S sesquiterpene, *T* triterpene

Caryophyllene, caryophyllene oxide, β-eudesmol and α-curcumene were identified and quantified in this work in concentrations of 0.14, 2.21, 5.22 and 8.07 mg/g of extract, respectively. Some authors have reported around 4, 7, 16 and 2 mg/g, respectively, to the same compounds [17], in an extract produced by hydrodistillation. Nevertheless, others studies comprising yarrow supercritical extracts have not reported the presence of α-curcumene [18].

Balm. Previous studies indicated that the volatile oil of balm is rich in monoterpenes such as citral [19], citronellal and α-caryophyllene [20], and sesquiterpenes such as caryophyllene oxide [20]. These compounds were identified in the extracts obtained by steam distillation, infusion and by SFE extractions, and were also identified in the SFE extract obtained in this work. Table 5 present the monoterpenes and sesquiterpenes observed in balm SFE extract.

Marjoram. Table 6 shows the marjoram supercritical extract composition. The presence of monoterpenes such as terpineol, and sesquiterpenes like spathulenol or β-caryophyllene as the most abundant compounds, agree with previous works focused on SFE [21]. In addition, 23 isoprenoids were identified in this work as reported in Table 5.

Total Phenolic Compounds (TPC) The assessment of the content of TPC is of great interest due to the recognized capacity of these substances to act as scavenging agents and thus,

Table 2 Extraction yield (mass of extract /mass of plant material)

	Yarrow	Marigold	Marjoram	Balm
SFE	0.79 ± 0.03	2.78 ± 0.21	1.51 ± 0.16	0.65 ± 0.04
UAE (ethanol)	2.82 ± 0.01	6.83 ± 0.49	6.17 ± 0.90	4.35 ± 0.06
UAE (ethanol:water 50:50)	10.82 ± 0.42	29.13 ± 1.41	12.83 ± 0.15	22.99 ± 0.37

Table 4 Volatile oil compounds identified by GC-MS in the SFE extract of yarrow

Retention time	Compound	% Area	Type
4589	Yomogi alcohol	2.56	M (acyclic alcohol)
5115	Eucalyptol	2.23	M (cyclic ether)
5471	Artemisia ketone	2.12	M (acyclic ketone)
5823	Sabinene	2.80	M (bicyclic hydrocarbon)
6078	Linalool	1.31	M (acyclic alcohol)
6224	Thujone	1.84	M (cyclic ketone)
6853	Citronellal	5.63	M (acyclic aldehyde)
7203	Borneol	16.28	M (bicyclic alcohol)
7482	Terpineol	2.56	M (propan-2-ol)
9287	Carvacrol	3.52	M (phenol)
10,411	Verbenol	1.76	M (bicyclic alcohol)
11,234	Caryophyllene	0.95	S (hydrocarbon)
11,356	Nerolidol acetate	0.90	S (acyclic ester)
12,074	α-Curcumene	3.61	S (cyclic hydrocarbon)
13,077	Elemol	1.37	S (propan-2-ol)
13,469	Cubenol	5.43	S (bicyclic alcohol)
13,541	Spathulenol	5.55	S (tricyclic alcohol)
13,637	Caryophillene oxide	4.90	S (oxide)
13,765	Viridiflorol	5.12	S (tricyclic alcohol)
14,300	α-Cadinol	8.22	S (bicyclic alcohol)
14,555	Eudesmol	11.19	S (propan-2-ol)
14,900	Bisabolol	2.73	S (cyclic alcohol)
16,833	Corymbolone	7.44	S (bicyclic keto-alcohol)

M monoterpene, *S* sesquiterpene

they are closely connected with the antioxidant activity of the plant extracts. Main phenolic compounds reported in yarrow were flavonoids, highlighting rutin, vicenin-2, or apigenin and luteolin and their glycosylic derivatives [22, 23]. In addition, caffeic acid derivatives and ascorbic acids were also found in yarrow [24] ultrasonic extracts. Regarding marigold, the other Asteraceae plant investigated in this work, triterpenoids with several hydroxyl substitutions were described previously [18], such as α- and β-amyrin, lupeol and faradiol. Concerning Lamiaceae plants, both Balm and Marjoram were reported to contain phenolic acids and flavonoids, mainly apigenin, luteolin and quercetin [25, 26].

Table 5 Volatile oil compounds identified by GC-MS in the SFE extract of balm

Retention time	Compound	% Area	Type
6078	Linalool	3.70	M (acyclic alcohol)
6853	Citronellal	23.87	M (acyclic aldehyde)
7203	Borneol	4.37	M (bicyclic alcohol)
7482	Terpineol	4.79	M (propan-2-ol)
9125	Thymol	6.13	M (phenol)
9287	Carvacrol	25.44	M (phenol)
11,234	Caryophyllene	4.79	S (hydrocarbon)
13,077	Elemol	2.90	S (propan-2-ol)
13,637	Caryophyllene oxide	17.73	S (oxide)
13,763	Epiglobulol	2.34	S (bicyclic alcohol)
14,555	Eudesmol	3.94	S (propan-ol alcohol)

M monoterpene, *S* sesquiterpene, *D* diterpene, *T* triterpene

Table 6 Volatile oil compounds identified by GC-MS in the SFE extract of marjoram

Retention time	Compound	% Area	Type
5115	Eucalyptol	11.48	M (cyclic ether)
6078	Linalool	9.84	M (acyclic alcohol)
6865	Camphor	0.55	M (ketone)
7203	Borneol	4.16	M (bicyclic alcohol)
7482	Terpineol	0.38	M (propan-2-ol)
9125	Thymol	2.52	M (phenol)
9287	Carvacrol	50.72	M (phenol)
9927	γ- Elemen	0.94	S (hydrocarbon)
10,077	α- Terpineol acetate	1.71	M (ester)
105,075	Neryl acetate	0.34	M (ester)
11,234	Caryophyllene	2.57	S (hydrocarbon)
12,076	Longiborneol	0.46	S (bicyclic alcohol)
12,373	α-Elemen	0.76	S (hydrocarbon)
12,456	α-Himachalen	0.48	S (hydrocarbon)
126,115	γ- Cadinene	0.34	S (hydrocarbon)
12,701	Epiglobulol	1.30	S (tricyclic alcohol)
127,785	Globulol	0.57	S (tricyclic alcohol)
13,541	Ent-spathulenol	3.13	S (tricyclic alcohol)
13,637	Caryophyllene oxide	1.70	S (oxide)
13,778	Ledol	2.02	S (tricyclic alcohol)
14,276	γ- Eudesmol	0.76	S (propan-2-ol)
143,665	β- Guaiene	0.83	S (hydrocarbon)
14,555	β- Eudesmol	2.44	S (propan-2-ol)

M monoterpene, *S* sesquiterpene, *D* diterpene, *T* triterpene

The TPC of plant extracts investigated in this work is show in Fig. 1a. The SFE extracts presented values of 12.6 to 56.2 mg GAE /g extract, compared with those obtained by UAE extractions, with values of 65.0 to 232.7 mg GAE /g extract. Although all SFE extracts exhibit low content of TPC in comparison with UAE extracts, marjoram extract showed considerably higher amounts than the other supercritical extracts. In the case of UAE extracts, despite the solvent used, marjoram resulted statistically with the highest content of TPC, followed by balm, yarrow and marigold (Fig. 1a, Table S1).

Total Flavonoid Compounds (TFC) Content of TFC is shown in Fig. 1b. As in the case of phenolic compounds,

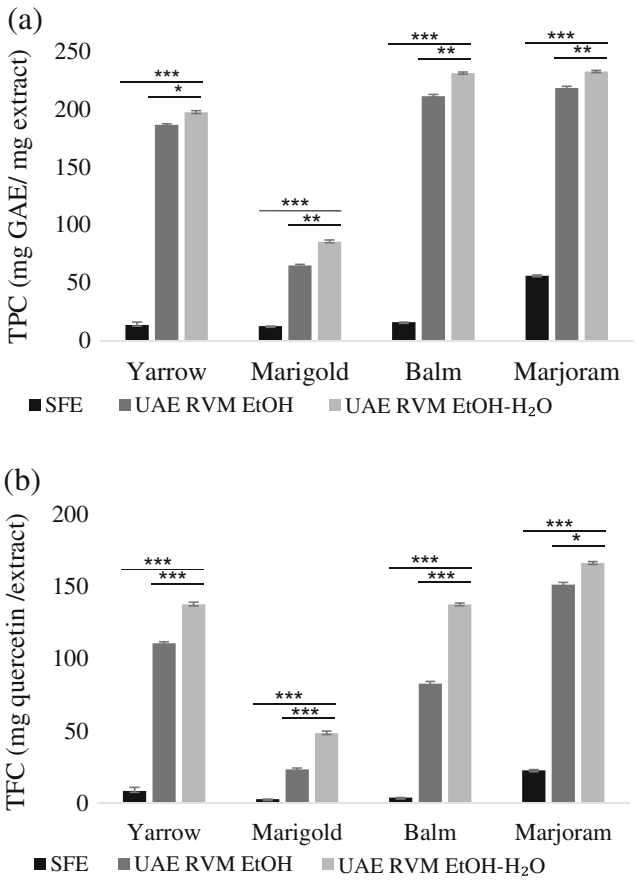

Fig. 1 **a** TPC and **b** TFC of the extracts. Data represent means ± SEM of at least two independent experiments. Asterisks indicate statistical differences between treatments after one-way Anova comparison. *$p < 0.05$, **$p < 0.01$; ***$p < 0.001$

low amounts of flavonoids were determined in the SFE extracts in comparison with UAE extracts. Marjoram extracts were those with the highest significant TFC content, despite the method or the solvent used in the extraction (Fig. 1b, Table S2). In general, TFC follow the same tendency observed in TPC. Furthermore, a lineal connection between TPC and TFC can be established for all the extracts, as illustrated in Fig. S1, although somewhat more scattering relationship is observed in the case of balm extracts.

Antioxidant Activity The antioxidant activity of all extracts produced was determined by to different methods: the DPPH radical method, stating the antioxidant capacity in terms of the EC_{50} value, and the ABTS radical method, by means of the TEAC value. Table 7 shows the results obtained for all plant extracts produced. As can be observed, both methods indicate the same tendency for all plant extracts: UAE ethanol:water extracts presented the highest antioxidant activity (lowest EC_{50} values and highest TEAC values), followed by UAE ethanol extracts. Additionally, for all plants studied, SFE extracts exhibit significantly lower antioxidant activity than UAE extracts. The highest antioxidant activity was found in marjoram extracts, despite the type of extraction or solvent used. Results were rather similar for balm and yarrow, and definitively marigold extracts presented the lowest antioxidant capacity. Particularly, the EC_{50} value resulted for marjoram ethanol:water extract (13.74 μg/ml) is in the same order of magnitude that reported for rosemary extracts, which is a commercial antioxidant for foodstuffs (E392) [9]. As can be observed in Fig. S2(a), high correlation resulted between the EC_{50} values and TPC ($R^2 = 0.985$).

Nevertheless, although the ABTS test show the same tendency regarding the antioxidant capacity of the extracts (marjoram > balm ≈ yarrow > marigold) the TEAC values do not show a linear correlation with TPC Fig. S2(b). The same conclusions can be established regarding the connection between the total content of flavonoid compounds (TFC) and the antioxidant activity of the UAE extracts, although in this case the regression coefficient of the EC_{50} vs. TFC correlation is somewhat lower ($R^2 = 0.858$).

Antiproliferative Activity Cells were treated with different concentrations (from 10 to 100 μg/mL) of each extract during 48 h. Extracts from Lamiaceae family (balm and marjoram) do not demonstrate any effect on the growth of MiaPaca-2 cells: the IC_{50} was higher than 100 μg/mL, showing the absence of a

Table 8 Antiproliferative effect in MiaPaCa-2 cell line: IC_{50} (μg/mL) values after 48 h of exposure (mean ± SEM of three independent experiments)

	SFE	UAE ethanol	UAE ethanol:water
Marjoram	> 100	> 100	> 100
Balm	> 100	> 100	> 100
Yarrow	31.45 ± 8.56	65.04 ± 2.55	> 100
Marigold	39.84 ± 4.57	> 100	> 100

Table 7 Antioxidant activity of extracts: EC_{50} (mg/ml) and TEAC (mmol/g) values

	Yarrow		Marigold		Balm		Marjoram	
	EC_{50}	TEAC	EC_{50}	TEAC	EC_{50}	TEAC	EC_{50}	TEAC
SFE	367.15	0.077	442.40	0.046	318.38	0.033	229.84	0.642
UAE (ethanol)	43.44	0.327	154.77	0.062	38.04	0.238	22.17	0.967
UAE (ethanol:water)	40.27	0.644	123.35	0.331	22.67	0.697	13.74	1550

significant effect on cell proliferation in the range of concentrations tested. By contrast, yarrow and marigold extracts (Asteraceae family) show a clear effect (Table 8). The IC_{50} for yarrow SFE and UAE extracts is in a range between 30 and 65 μg/mL, similar to Rosemary SFE extract tested in other tumor types [27, 28], which can lead to suggest yarrow and marigold extracts as potential antitumorogenic agents.

Conclusions

Lamiaceae plant family seems to be the most appropriate source of antioxidant extracts and the use of polar solvents the best extraction method to produce them. However, Asteraceae family shows the most promising results as source of potential antiproliferative agents and SFE the most efficient method to produce extracts for this purpose. These results support previous studies suggesting a potential anticancer activity of plant extracts independently of their antioxidant activity.

Related to composition, Lamiaceae species are particularly rich in phenolic monoterpenes and sesquiterpenes (around 32% balm and 53% marjoram), with 7–12% of cyclic alcohols. In the contrary, the volatile oil content in Asteraceae plants comprise high amounts of bicyclic and tricyclic alcohols (52% yarrow and 32% marigold) and almost no phenolic alcohols were identified. This result encourage further studies to elucidate a possible effect of bicyclic and tricyclic monoterpene and sesquiterpene alcohols as responsible of the antiproliferative effect of these Asteraceae extracts.

Acknowledgements The authors gratefully acknowledge the financial support from Ministerio de Economía y Competitividad of Spain (project AGL2013-48943-C2) and the Comunidad Autónoma de Madrid (ALIBIRD, project number S2013/ABI-2728).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Ìnanç T, Maskan M (2012) The potential application of plant essential oils/extracts as natural preservatives in oils during processing: a review. J Food Sci Eng 2:1–9. doi:10.1007/s11746-013-2351-8
2. Kähkönen MP, Hopia AI, Vuorela HJ, Rauha J, Pihlaja K, Kujala TS et al (1999) Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47(10):3954–3962. doi:10.1021/jf990146l
3. Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Bio Med 20(7):933–956. doi:10.1016/0891-5849(95)02227-9
4. Dreher D, Junod AF (1996) Role of oxygen free radicals in cancer development. Eur J Cancer 32(1):30–38. doi:10.1016/0959-8049(95)00531-5
5. Sayin VI, Ibrahim MX, Larsson E, Nilsson JA, Lindahl P, Bergo MO (2014) Antioxidants accelerate lung cancer progression in mice. Sci Transl Med 6(221):221ra15. doi:10.1126/scitranslmed.3007653
6. Halliwell B (2012) Free radicals and antioxidants: updating a personal view. Nutr Rev 70(5):257–265. doi:10.1111/j.1753-4887.2012.00476.x
7. Paini M, Casazza AA, Aliakbarian B, Perego P, Binello A, Cravotto G (2016) Influence of ethanol/water ratio in ultrasound and high-pressure/high-temperature phenolic compound extraction from agri-food waste. Int J Food Sci Tech 51(2):349–358. doi:10.1111/ijfs.12956
8. Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem 64(4):555–559. doi:10.1016/S0308-8146(98)00102-2
9. Vicente G, Molina S, González-Vallinas M, García-Risco MR, Fornari T, Reglero G et al (2013) Supercritical rosemary extracts, their antioxidant activity and effect on hepatic tumor progression. J Supercrit Fluid 79:101–108. doi:10.1016/j.supflu.2012.07.006
10. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Bio Med 26(9):1231–1237. doi:10.1016/S0891-5849(98)00315-3
11. Campos LM, Michielin EM, Danielski L, Ferreira SR (2005) Experimental data and modeling the supercritical fluid extraction of marigold (*Calendula officinalis*) oleoresin. Journal Supercrit Fluid 34(2):163–170. doi:10.1016/j.supflu.2004.11.010
12. Wiłkomirski B (1985) Pentacyclic triterpene triols from *Calendula officinalis* flowers. Phytochemistry 24(12):3066–3067. doi:10.1016/0031-9422(85)80062-5
13. Chalchat J, Garry RP, Michet A (1991) Chemical composition of essential oil of *Calendula officinalis* L.(pot marigold). Flavour Frag J 6(3):189–192. doi:10.1002/ffj.2730060306
14. Hamburger M, Adler S, Baumann D, Förg A, Weinreich B (2003) Preparative purification of the major anti-inflammatory triterpenoid esters from marigold (*Calendula officinalis*). Fitoterapia 74(4):328–338. doi:10.1016/S0367-326X(03)00051-0
15. Rahimmalek M, Tabatabaei BES, Etemadi N, Goli SAH, Arzani A, Zeinali H (2009) Essential oil variation among and within six *Achillea* species transferred from different ecological regions in Iran to the field conditions. Ind Crop Prod 29(2):348–355. doi:10.1016/j.indcrop.2008.07.001
16. Rohloff J, Skagen EB, Steen AH, Iversen T (2000) Production of yarrow (*Achillea millefolium* L.) in Norway: essential oil content and quality. J Agric Food Chem 48(12):6205–6209. doi:10.1021/jf000720p
17. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A et al (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium subsp. millefolium* Afan. (Asteraceae). J Ethnopharmacol 87(2):215–220. doi:10.1016/S0378-8741(03)00149-1
18. Bocevska M, Sovová H (2007) Supercritical CO_2 extraction of essential oil from yarrow. J Supercrit Fluids 40(3):360–367. doi:10.1016/j.supflu.2006.07.014
19. Topal U, Sasaki M, Goto M, Otles S (2008) Chemical compositions and antioxidant properties of essential oils from nine species of Turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. Int J Food Sci Nutr 59(7–8):619–634. doi:10.1080/09637480701553816
20. Schnitzler P, Schuhmacher A, Astani A, Reichling J (2008) *Melissa officinalis* oil affects infectivity of enveloped herpesviruses. Phytomedicine 15(9):734–740. doi:10.1016/j.phymed.2008.04.018

21. Vagi E, Simandi B, Suhajda A, Hethelyi E (2005) Essential oil composition and antimicrobial activity of *Origanum majorana* L. extracts obtained with ethyl alcohol and supercritical carbon dioxide. Food Res Int 38(1):51–57. doi:10.1016/j.foodres.2004.07.006
22. Benedek B, Kopp B (2007) *Achillea millefolium* L. sl revisited: recent findings confirm the traditional use. Wien Med Wochenschr 157(13–14):312–314. doi:10.1007/s10354-007-0431-9
23. Benetis R, Radusiene J, Janulis V (2008) Variability of phenolic compounds in flowers of *Achillea millefolium* wild populations in Lithuania. Medicina (Kaunas) 44(10):775–781
24. Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua S et al (2011) Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochim Pol 58(2):203–219
25. Dastmalchi K, Dorman HD, Oinonen PP, Darwis Y, Laakso I, Hiltunen R (2008) Chemical composition and *in vitro* antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. LWT-Food Sci Technol 41(3):391–400. doi:10.1016/j.lwt.2007.03.007
26. Hossain MB, Camphuis G, Aguiló-Aguayo I, Gangopadhyay N, Rai DK (2014) Antioxidant activity guided separation of major polyphenols of marjoram (*Origanum majorana* L.) using flash chromatography and their identification by liquid chromatography coupled with electrospray ionization tandem mass spectrometry. J Sep Sci 37(22):3205–3213. doi:10.1002/jssc.201400597
27. Yesil-Celiktas O, Sevimli C, Bedir E, Vardar-Sukan F (2010) Inhibitory effects of rosemary extracts, carnosic acid and rosmarinic acid on the growth of various human cancer cell lines. Plant Foods Hum Nut 65(2):158–163. doi:10.1007/s11130-010-0166-4
28. González-Vallinas M, Molina S, Vicente G, de la Cueva A, Vargas T, Santoyo S et al (2013) Antitumor effect of 5-fluorouracil is enhanced by rosemary extract in both drug sensitive and resistant colon cancer cells. Pharmacol Res 72:61–68. doi:10.1016/j.phrs.2013.03.010