Cancer Reports and Reviews

Research Article

ISSN: 2513-9290

Chemical composition and evaluation of cytotoxic activity of *Achillea falcata* essential oil on HeLa and Caco-2 human cancer cell lines

Banan Younos[1], Chadi Soukkarieh[1] and Hossam Murad[2]*

[1]Department of Animal Biology, Faculty of Sciences, Damascus University, Syria

[2]Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), Syria

Abstract

Plants and their products, e.g. essential oils are mostly considered as safe medicine products. In recent years, medicinal plants have used as natural products that contribute to the prevention and treatment of disease.

Medicinal plants play a crucial role in traditional medicine and in the maintenance of human health worldwide. The majority of *Achillea* species include highly bioactive compounds, so they have therapeutic applications. In the present study, the aim was to investigate *in vitro* anti-oxidant and cytotoxic effects of *Achillea falcata* essential oil. The essential oil was extracted from aerial parts using Clevenger method and its components were determined using GC-MS analysis. The anti-oxidant potential of the essential oil was analyzed by DPPH and total phenolic content methods. MTT assay was used to detect cytotoxicity of the essential oil on human cervical cancer cells HeLa and colon cancer cells Caco-2. The results showed that essential oil exhibited a low DPPH scavenging activity, and 32.467 Gallic acid equivalents/g plant oil of total phenolic content. As concentration was increased, the essential oil exhibited a more powerful cytotoxic effect on HeLa and Caco-2 cancer cell lines indicating that *Achillea falcate* essential oil might include anti-cancer compounds triggering the cytotoxicity on HeLa and Caco-2 cancer cells.

Introduction

In the past few years, interest in the antioxidant and radical scavenging properties of medicinal herbs and their extracts has risen enormously. In fact, antioxidants contained in plants seem to be involved in the preservation of human health, e.g. by preventing cancer, slowing down the aging process and reducing the risk of cardiovascular and neurodegenerative diseases. Oxidative damage is a hallmark of all these physio pathological conditions and plants, providing an exogenous source of antioxidants, may aid the natural defence systems of cells [1-3]. Therefore, the assessment of antioxidant and radical scavenging properties of traditionally widely used plants and plant extracts is an important issue in the quest both of new sources of natural antioxidants for functional foods, nutraceuticals [4,5] and of feasible and 'natural' alternatives to synthetic antioxidants in the food industry, since food-preserving compounds are being restricted due to their inherent risk of carcinogenicity [6]. Achillea, one of the most important genera of the Compositae (Asteraceae) family, comprises more than 100 species around the world, mainly distributed in Europe, Asia and North Africa [7], In particular, Achillea falcata has been reported to have beneficial effects on internal hemorrhage, uterine hemorrhoid, stomach ailment, gastritis, and bladder stones [8]. In recent years, some pharmacological properties of this species were scientifically addressed. It has been demonstrated that some *A.falcata* sesquiterpene lactones are cytotoxic agents [9-12], its extracts/essential oil have antimicrobial, antioxidant and antiplatelet properties [8,13-16]. Botanical drugs based on or including A. falcata are regarded as completely safe for human use and non-toxic [8,17]. However, phytochemical, data regarding *A.falcata*, and the studies using the essential oil of A. falcata on cancer cell lines, are quite scarce [18,19]. The aim of this study was to evaluate antioxidant capacity and to investigate the effects of different concentrations of essential oil obtained from *A.falcata* on HeLa and Caco-2 cells and to assess the relationship with their total phenolic content, in the present study both chemical (free radical scavenging activity by DPPH) and MTT assay(cell viability of Hela and Caco-2 cell lines) were used.

Material and methods

Plant material

The aerial parts of the *A.falcata* plants was collected from the Karm Al-Maasara area in Lattakia in July 2016, and it was classified by plant taxonomist Dr. Emad Al-Qadi, Assistant Professor in the Department of Plant Biology at Damascus University. The plant was dried at room temperature during 1-week and then ground with an electric mill. The dried powdered material was stored at + 4°C.

Essential-oil extraction

Essential oil was extracted from the plant samples using a Clevenger-type apparatus where the plant material is subjected to hydro distillation. Conditions of extraction were 100 g of samples, 1:5 plant material/water volume ratio, and a 2.5 hrs distillation. The oil was dehydrated with anhydrous sodium sulphate and immediately stored in airtight glassware in a refrigerator at + 4°C.

Correspondence to: Hossam Murad, Department of Molecular Biology and Biotechnology, Atomic Energy Commission of Syria (AECS), Syria, E-mail: hmurad@aec.org.sy

Received: August 20, 2020; **Accepted:** August 30, 2020; **Published:** September 06, 2020

GC-MS analyses

The essential oil was analyzed using a device chromatograph with a data-handling system. A (Phenyl Methyl Silox HP-5MS% column (0.25 µm × 250 µm × 30 m) was used, with he as carrier gas (1 ml/min). The operating conditions were: injector and detector temperature, 260°C and 280°C, respectively; oven temperature program is started at 60°C, then it goes up 4°C/min to 200°C, subsequently rising at 8°C/min to 260°C, then held isothermally at 260°C for 7.5 min; injection mode, splitless (1 µl 1:1000 n-pentane solution). Linear retention indices were determined in relation to a homologous series of n-alkanes (C8–C22) under the same operating conditions. GC-MS analyses were performed employing the same chromatographic conditions as described above, using Agilent 7890 A.

Antioxidant activity

Evaluation of total phenolic compounds: The amount of total phenolics in the essential oil was determined with the Folin-Ciocalteu method [20], using gallic acid as a standard. Distilled water (450 µL) was combined and vortexed with 50 µL of sample and 250 ml of Folin-Ciocalteu's reagent. Then 2 ml of Na2CO3 (20%) was added, the mixture was vortexed. The absorbance of all samples was measured at 765 nm using a UV/VIS spectrophotomete after incubation at 40°C for 30 min. Quantification was done on the basis of the standard curve of gallic acid (solution of gallic acid 80% MeOH, 0.25–5 µg/mL). The results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry weight. Measurements were taken in triplicate and mean values calculated.

Radical scavenging properties assessment (DPPH assay): Antioxidant capacity was determined with DPPH reagent according to the method described by Mensor *et al.* [21]. The essential oil was diluted with ethanol to four different increasing concentrations between 0.2 and 2 mg/mL. The DPPH concentration in ethanol was 45 µg/ml. 0,3 mL of oil solution (sample) or ethanol (control) was mixed with 1 mL of DPPH and the absorption was recorded after 20 min in the dark, at 515 nm. Vitamin C was used as the referring substances. The following equation was used to calculate concentration of the DPPH radicals:

DPPH scavenging activity (%) = (A0–A1)/A0×100%, where, A0 is the absorbance of the control and A1 is the absorbance of the reaction mixture or standards. The values have been expressed as the mean of three replications.

Cell line and culture condition

HeLa (human cervical cancer cells) were Obtained from the Human cell culture laboratory in Atomic Energy Commission of Syria. MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) was purchased from Sigma Chemical Co. The cells were cultured in RPMI medium supplemented with 10% fetal bovine serum and 100 U/mL penicillin and 100 µg/mL streptomycin. The cells were kept at 37°C in a humidified atmosphere containing 5% CO2. HeLa and Caco-2 cells were treated with different doses (0, 10, 15, 25, and 50 µg/ml) of the essential oil of A. falcate. HeLa cells were treated at two different time intervals (24 and 48 hrs).

Cell viability testing by MTT assay

The HeLa and Caco-2 cells were seeded in different 96-well plates containing 1.5*104 cells/100 µL/well for Hela cells, and 1*104 cells/100 µL/well for Caco-2 cells. The cultured cells were treated with the addition of different doses of the essential oil (0, 5, 15, 25, and 50 µg/ml) of *A.falcata*, and the Hela cultured cells were treated at two different time intervals (24 and 48 hrs). In addition, the DMSO alone was added to another set of cells as the solvent control (DMSO). The cells were then incubated for another 24 hrs prior to the addition of 20 µL of 2 mg/mL solution of MTT into each well. The incubation was continued for another 3 hrs before the media were removed. A mixture of DMSO (150 µL) was added to each well and mixed to ensure dissolving of the formazan crystals before the absorbance at 540 nm was measured. Each experiment was performed in triplicate and the 50% Hela cells inhibitory concentration (IC50) of the oil was calculated. The cell viability ratio was calculated by the following formula:

Inhibitory ratio (%) = (OD/optical density contro –OD treated)/OD control × 100.

Cytotoxicity was expressed as the concentration of oil inhibiting cell growth by 50% (IC50 value).

Statistical study

The experiments were performed in triplicate. Statistical correlation of data was checked for significance by ANOVA and Student's t-test. A p value<0.05 was considered to indicate a statistically significant difference.

Results

Yield and Isolation of the essential oil

The yields of the oils obtained from the aerial parts of *A.falacta* (isolated after hydro distillation for 2.5 hrs from 100 g plant) was 0.367% (w/w), and the density of the essential oil was 0,92 gr/cm^3. The oil was limpid yellow and of agreeable smell.

GC-MS analyses

The GC-MS analysis of the essential oil obtained from the aerial parts of *A.falcata* allowed the identification of 23 compounds (Table 1), representing 92.902% of the oil. The oil was characterized by a high content of oxygen-containing compounds. The main components of the oil were Alpha Thujon (32.082%), trans-2,7-Dimethyl-4,6-octadien-2-ol (11.387%), Artemisia ketone (11.23%), 3-Thujanone (11.18%), Germacrene-D (8.373%).

Antioxidant activity

Evaluation of total phenols: Total phenols content was 32.467(mg GaEs/g plant oil).

DPPH assay: Antioxidant capacity of infusions from *A.falcata* oil measured with DPPH assay are shown in Table 2. The results of our DPPH analysis showed that *A.falcata* oil had low antioxidant activity (IC_{50}>2 mg/mL; at 2 mg/mL the ability to scavenge DPPH was 31.43%.

Cell viability

The anticancer activity of the oil against cancer cells (HeLa and Caco-2) was evaluated by MTT assay. Different concentrations of the oil (0, 10, 15, 25, and 50 µg/ml) were tested against the cancer cells. The cervical cancer cells and colon cancer cells showed substantial dose-dependent susceptibility to the treatment of different concentrations of the oil. The (Table 3) shows Height of cellular death percentage with increasing concentration of oil.

HeLa cells also treated with the oil in different time, at 24 and 48 hrs. The IC_{50} value after 24 and 48 hrs intervals was 13.687 and 10.325 µg/ml respectively (Table 4).

Table 1. Composition of the essential oil from aerial parts of Achillea falcata by GC-MS analyses

#	Compounds	RI	RT	%	Method of identification
1	2-methyl- (CAS)	862	4.7776	0.2094	MS/RI
2	yomogi alcohol	1000	9.5432	3.6262	MS/RI/St
3	Cymene	1019	10.514	0.4286	MS/RI/St
4	1,8-Cineole	1027	10.867	3.0419	MS/RI/St
5	2-Methyl-2,4-hexadiene	1036	11.3626	2.6074	MS/RI
6	Artemisia ketone	1063	12.6999	12.573	MS/RI/St
7	Artemisia alcohol	1092	14.1459	0.6155	MS/RI/St
8	alpha.-Thujon	1114	15.367	35.9224	MS/RI/St
9	Ethyl 3-hydroxy hexanoate	1120	15.836	12.3931	MS/RI
10	L-trans-Pinocarveol	1136	16.664	0.2502	MS/RI
11	Nerol	1148	17.357	0.2573	MS/RI/St
12	2,7-dimethyl-2,6-octadien-4-ol	1174	18.891	1.8998	MS/RI
13	p-Cymen-2-ol	1183	19.4411	0.2153	MS/RI/St
14	Propanal, 2-methyl-3-phenyl-	1236	22.6114	0.2434	MS
15	(3RS,4SR)-2,5-Dimethyl-4-vinyl-5-hexene-1,4-diol	1244	23.1544	1.1808	MS
16	Lavandulol, acetate	1292	26.0939	0.6132	MS/RI/St
17	Carvacrol (CAS)	1303	26.772	0.4239	MS/RI/St
18	1,5,5-Trimethyl-6-methylene-cyclohexene	1332	28.544	0.4007	MS
19	alpha.-Cubebene	1369	30.852	0.2351	MS/RI
20	Jasmone	1396	32.5092	0.2831	MS/RI
21	Methyl eugenol	1405	33.025	0.304	MS/RI
22	Germacrene D	1411	33.432	0.4979	MS/St
23	.beta.-Cubebene	1421	34.029	0.4204	MS
24	GERMACRENE-D	1477	37.734	9.2307	MS/RI/St
25	trans-.beta.-Farnesene	1479	37.498	0.483	MS/RI
26	Bicyclogermacrene	1489	38.103	1.2591	MS/St
27	.beta.-Sesquiphellandrene (CAS)	1522	40.092	3.6455	MS/RI
28	1,5-epoxysalvial-4(14)-ene	1559	42.4477	0.3332	MS/RI
29	Spathulenol	1572	43.2013	1.4255	MS/RI/St
30	3-Phenylpropionic acid, 2-methylbutyl ester	1599	44.9188	0.2686	MS/RI
31	beta.-Eudesmo	1643	47.2473	0.8102	MS/RI/St
32	Methyl jasmonate	1646	47.444	0.2655	MS/RI
33	2,6,11,15-Tetramethyl-hexadeca-2,6,8,10,14-pentaene	1841	56.561	1.0348	MS/St

RI: Retention index identical to bibliography; MS: Identification based on comparison of mass spectra; ST: Standard time retention time identical to authentic compounds.

Table 2. Concentrations of the essential oil from aerial parts of Achillea falcata by DPPH analysis

oil concentration mg/ml	0.2	1	1.5	0.2
DPPH%	11.13	20.495	27.159	31.43
	C Vitamin 39.24			

Table 3. The cellular death ratios of HeLa and Caco-2 cells treated with graded concentrations of essential oil

	The percentages of cellular death	
	HeLa cells	Caco-2 cells
12	21	12914
15	419..	0.902
25	.1904	029210
50	22	0.9.2

Table 4. The cellular death ratios of HeLa cells treated at 24 and 48hrs with essential oil

	The percentages of cellular death	
oil Concentration	At 24hrs	At 48hrs
12	21	02910
15	419..	42
25	.1904	.29.

Statistical study

The cervical cancer and colon cancer cells showed substantial dose-dependent susceptibility to the treatment of different concentrations of the oil, and the Statistical study showed that *A.falcata* oil affects HeLa cells more than Caco-2 cells, The HeLa cells did not showed time-dependent susceptibility to the treatment of the oil at different times.

Discussion

Many of the *Achillea* species are widely used in traditional medicine of several cultures due to many therapeutic properties, such as antioxidant, antispasmodic, anti-inflammatory, antihemorrhoidal, stomachic, emmenagogue and antiseptic [14,15]. Contemporary studies have revealed that many *Achillea* species possess antioxidant and anticancer properties as well [16,17]. In particular, *A.falcata* has been described to have useful effects on internal hemorrhages, stomach ailments, gastritis, and bladder stones [18]. Many other reports have shown anti-proliferative activity of isolated constituents from *A.falcata* [19-21]. Infusion of *A.falcata* has been proved to possess antioxidant activities [22]. Phytochemically, *A. falcata* has been reported to contain a diversity of chemical constituents, most of them being volatile organic compounds present in its essential oil. The various chemical constituents that have been reported in *A. falcata* are monoterpenoids

like cineole, camphor and borneol 92.902%. Flavonoids have also been reported from the plant. Sesquiterpene lactones including 3-β-methoxy-iso-seco-tanapartholide (β-tan) which exhibit potent antitumor properties have also been reported from *A. falcata*. β-tan which was purified from *A.falcata*, differentially inhibited the growth of the epidermal human HaCaT cells at non-cytotoxic concentrations to primary epidermal keratinocytes. However, there are no reports on the Anti-oxidant properties of an *A.falcata* oil, and nor is there any report on the antitumor effect of the oil of *A.falcata* on cervical cancer and colon cancer cells, Therefore, our aim was to evaluate the antioxidant properties of the oil extract of *A.falcata* with demonstrating its effect on HeLa human cervical cancer cells and Caco-2 human colon cancer cells. In our study antioxidant activities for *A.falcata* essential oil was studied by two methods, the first one was evaluation of total phenols which was (32.467 mg GaEs/g), and the second method was by DPPH analysis (IC_{50}>2 mg/mL), and the results of these methods showed that *A.falcata* oil had low antioxidant activity.

The GC–MS analyses of the essential oil obtained from the aerial parts of *A.falcata* allowed the identification of 23 compounds (Table 1), representing 92.902% of the oil. The oil was characterized by a high content of oxygen-containing compounds. The main components of the oil were Alpha Thujon (32.082%), trans-2,7-Dimethyl-4,6-octadien-2-ol (11.387%), Artemisia Ketone (11.23%), 3-Thujanone (11.18%). These compounds were reported in the other studies of the aerial parts of the *A.falcata* oil, but In different concentrations, Where the Alpha Thujon compound was the most abundant in our study, and his concentrate was reached to 35.9224%, followed him Artemisia Ketone compound and his concentrate was 12.573%, and This is different from their concentration in the oils obtained from *A.falcata* extracted from plants grown in Jab. Kneissé (Lebanon), which Alpha Thujon was (3.0%), and Artemisia Ketone (5.2%), [16] and the Ceneole 1,8 compound which is the main compound in the *A.falcata* oil extracted from Antalya (Turkey) (14-24%) [22] which that in our oil are present in lower concentration (3,0419%). These differences may be attributed to the various environmental factors as different geographical, the nature of the soil, Climate difference, or harvest time, In addition to the genetic factor.

Cell viability was assayed after 24 h of treatment for Caco-2 cells,24 and 48 h for Hela cells, using MTT assay, which the method measures the ability of metabolically active cells to convert tetrazolium salt into a blue formazan product, the absorbance of which is recorded at 540 nm using an ELISA microplate reader. Viability results were showed the oil exhibited one dose-dependent growth inhibitory, and don't time-dependent effects on the HeLa cancer cells, and a variation in the effect of oil on the HeLa and Caco-2 cell lines. The IC50 on HeLa cells was 13.687 µg/ml, while the value of IC50 on Caco-2 cells was not reached, even with the highest concentration used in this study which was 50 µg/ml, so that need to additional experiments using higher concentrations, or prolonging the treatment time with this oil to accurately determine this value. In general, may be the different effect of the oil on the different cell lines due to the receptors sensitivity of the cells types to the same oil components, or to the ability of the different oil components to activate different signaling pathway by the cell type.

Conclusion

In conclusion, our study provides the evidence that the oil of *A.falcata* has antioxidant and antitumor properties, whereas, previous studies extracted specific compounds from *A.falcata* essential oil and studied their effect many fields, and the present investigation using the *achillea falcata* oil supported the traditional use of this plant in the Syrian folk medicine.

Acknowledgments

None.

Conflict of interest

Authors declare nothing to disclose.

References

1. Dhalla NS, Temsah RM, Netticadan T (2000) Role of oxidative stress in cardiovascular diseases. *Journal of hypertension* 18: 655-673.
2. Granot E, Kohen R (2004) Oxidative stress in childhood—in health and disease states. *Clinical Nutrition* 23: 3-11. [Crossref]
3. Prior RL, Wu X, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of agricultural and food chemistry* 53: 4290-4302.
4. Shan B (2005) Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *Journal of agricultural and food chemistry* 53: 7749-7759. [Crossref]
5. Kähkönen MP (1999) Antioxidant activity of plant extracts containing phenolic compounds. *Journal of agricultural and food chemistry* 47: 3954-3962.
6. Djeridane A (2006) Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food chemistry* 97: 654-660.
7. Saeidinia S (2005) Composition of the volatile oil of Achillea conferta DC. from Iran.
8. Aburjai T (2007) Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. *Journal of Ethnopharmacology* 110: 294-304.
9. Ghantous A (2009) Structure–activity relationship of seco-tanapartholides isolated from Achillea falcata for inhibition of HaCaT cell growth. *European journal of medicinal chemistry* 44: 3794-3797.
10. Saikali M (2012) Sesquiterpene lactones isolated from indigenous Middle Eastern plants inhibit tumor promoter-induced transformation of JB6 cells. *BMC complementary and alternative medicine* 12: 89.
11. Salla M (2013) Synergistic anticancer activities of the plant-derived sesquiterpene lactones salograviolide A and iso-seco-tanapartholide. *Journal of natural medicines* 67: 468-479.
12. Tohme R (2013) Differential growth inhibitory effects of highly oxygenated guaianolides isolated from the Middle Eastern indigenous plant Achillea falcata in HCT-116 colorectal cancer cells. *Molecules* 18: 8275-8288.
13. Radulović NS (2015) Toxic essential oils. Part IV: The essential oil of Achillea falcata L. As a source of biologically/pharmacologically active trans-sabinyl esters. *Food and Chemical Toxicology* 80: 114-129.
14. Karaalp C, Yurtman AN (2009) Evaluation of antimicrobial properties of Achillea L. flower head extracts. *Pharmaceutical biology* 47: 86-91.
15. Konyalioğlu S (2005) Screening of total flavonoid, phenol contents and Antioxidant capacities of some achillea L. species growing in Turkey. *ACTA Pharmaceutica Sciencia* 46.
16. Senatore F (2005) Composition and antimicrobial activity of the essential oil of Achillea falcata L. (Asteraceae). *Flavour and fragrance journal* 20: 291-294.
17. Alzweiri M (2011) Ethnopharmacological survey of medicinal herbs in Jordan, the Northern Badia region. *Journal of Ethnopharmacology* 137: 27-35.
18. Bruno M (2003) Terpenoids and flavones from Achillea falcata (Asteraceae). *Revista de la Sociedad Química de México* 47: 130-131.
19. Hofer O (1986) 13C NMR and 1H lanthanide induced shifts of naturally occurring alkamides with cyclic amide moieties-amides from achilleafalcata. *Tetrahedron* 42: 2707-2716.
20. Slinkard K, Singleton VL (1977) Total phenol analysis: automation and comparison with manual methods. *American journal of enology and viticulture* 28: 49-55.

21. Mensor LL (2001) Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy research* 15: 127-130. [Crossref]

22. Başer KHC (2016) Essential oils of Achillea species of Turkey. *Natural Volatiles and Essential Oils* 3: 1-14.