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Biological Activities of the Hydro-alcoholic and Aqueous Extracts of *Achillea falcata* L. *(Asteraceae)* Grown in Jordan

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Authors' contributions

This work was carried out in collaboration between all authors. Authors HMH and FUA wrote the manuscript and designed the study. All authors contributed the study. Authors FUA and HIAJ performed the photochemical investigations. Author HMH performed the Biological evaluations. Author SAM performed the antimicrobial evaluation and author SCL performed Chromatographic investigation.

Original Research Article

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ABSTRACT

Aim: This study aimed to screen the aqueous and hydro-alcoholic extracts of *Achillea falcata* L. *(Asteraceae)* grown in Jordan for their antioxidant, antibacterial, antiplatelet and anti-proliferative efficacy.
Study Design: HPLC-MS evaluation of the aqueous and hydro-alcoholic extracts and *in vitro* investigations.
Place and Duration of Study: Faculties of Pharmacy and Science, The University of Jordan and Centre of Misanalysis, National Institute for Biological Sciences, between August 2012 and June 2013.
Methodology: Total phenols and flavonoids were determined colorimetrically. The radical

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scavenging activities were evaluated using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity assay. Antimicrobial activities were determined by the disc-diffusion method, and the minimum inhibition concentration and the minimum bactericidal concentration tests. *In vitro* antiplatelet activity was tested on human whole blood using an electrical impedance method. Anti-proliferative activity was investigated using the MTT assay. High performance liquid chromatography-mass spectrometry (HPLC-MS) evaluation was performed.

Results: Hydro-alcoholic extract had a bactericidal activity against *Streptococcus pneumoniae*, *Bacillus cereus* and *Klebsiella pneumoniae* rather than inhibitory effect. No significant activity was observed against gram negative bacteria and *Candida albicans*. *In vitro* antiplatelet activity was tested on human whole blood using an electrical impedance method. At concentrations (50, 100, and 200 µg/ml), hydro-alcoholic extract did not show effect on platelet aggregation. Extracts did not possess cytotoxic activity against the MCF-7 cells at concentrations up to 200 µg/ml. HPLC-MS analysis resulted in the identification of 8 phenolic compounds in the hydro-alcoholic extract and 6 compounds in the aqueous extract; quercetin 3-β-D-glucoside was the main component for both extracts.

Conclusion: The present investigation supported the traditional use *A. falcata* in the Jordanian folk medicine as a depurative agent and as an antimicrobial active representative of the genus *Achillea*.

Keywords: Achillea falcate; Asteraceae; Antioxidant activity; Antiplatelet activity; Cytotoxicity; Jordan.

ABBREVIATIONS

(ABTS) *: 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)*
ESI) *: electro spray ionization*
(GAE) *: Gallic acid equivalents*
(HPLC-MS) *: High-performance liquid chromatography-mass spectrometry*
(LCMS-2010 detector) *: liquid chromatograph mass spectrometer*
(NCCLS) *: National Committee for Clinical Laboratory Standards*
(TEAC) *: Trolox equivalent antioxidant capacity*
(Trolox) *: 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid*

1. INTRODUCTION

The genus *Achillea* (commonly referred to as yarrow) comprises over 150 species worldwide, which are mainly distributed in the northern hemisphere, most indigenous to Europe and the Middle East [1,2]. *Achillea* species are widely used in traditional medicine of several cultures due to numerous pharmacological properties, such as anti-inflammatory, antioxidant, antispasmodic, antihemorrhoidal, stomachic, antiseptic and emmenagogue [3,4,5,6,7,8]. Recent studies demonstrated that the different *Achillea* species possess antioxidant and antiproliferative capacities [9,10,11]. In particular*, A. falcata* has been reported to have beneficial effects on internal hemorrhages, stomach ailments, gastritis, and bladder stones [12]. Other reports showed anti-proliferative activity of isolated constituents from *A. falcata* [13,14,15,16]. Infusion of *A. falcata* has been proved to possess antioxidant activities [17].

In Jordanian folk medicine, *Achillea* species are used as herbal remedies against fever, common cold, for digestive complaints, as haemostatic and topically for slow-healing wounds and skin inflammations [12]. *A. falcata*, is one of the most commonly reported plants to be used in the traditional medicine in the Ajloun Heights region of Jordan; recommended to be used as carminative, depurative, stomachic and antispasmodic [18]. *A. falcata* with its sulfurus yellow fllowers reaches a height of 30-50 cm and easily distinguished by its erect stem, narrow finally falcate or deflexed leaves [2]. In isolation of chemical constituents from the *Achillea* species grown in Lebanon, Turkey and Iran; emphasis was given to the chemical composition of the volatile fraction. This fraction contained invariably monoterpenoids, represented by cineol, camphor and borneol [4,8,19]. Some researchers evaluated also the presence of flavonoids [17,18].

To the best of our knowledge, there are no comprehensive studies performed for biological evaluation of the effectiveness of *A. falcata* in Jordanian folk medicine, despite its widespread use -internally and externally-, in form of an infusion. Hence, the aim of this study was to evaluate the antioxidant, antiplatelet, antimicrobial and antiproliferative efficacy of *A. falcata* grown wild in Jordan.

2. MATERIAL AND METHODS

2.1 Plant Material

A. falcata was collected from Al Jubeiha region, in the vicinity of the city of Amman, during the period extending from April to May, 2012. The plant was identified by Prof. Barakat E. Abu-Irmaileh at the Department of Plant Protection, Faculty of Agriculture, University of Jordan. Voucher specimen (AST003FMJ) has been deposited in the Department of Pharmaceutical Sciences, Faculty of Pharmacy, The University of Jordan. Flowering aerial parts were air dried at room temperature (RT) in the shade for one week until constant weight, and subsequently assayed for extraction.

2.2 Preparation of Extracts

The extracts were prepared by refluxing each 10 g of the dried coarsely powdered plant material with 100 ml distilled water or 70% ethanol for 15 min and keeping the extract overnight. Extracts were then filtered and evaporated in vacuo to give a crude residue in the following yields (%, w/w): 24% and 19% respectively.

2.3 Evaluation of Total Phenolic Compounds

Stock solutions of 50 mg/ml (80% methanol (MeOH) or H_2O) were prepared. Total phenolic content was estimated using the Folin-Ciocalteu colorimetric modified method using gallic acid as a standard [20]. Briefly, 50 µl aliquots from each of the replicates were mixed with 450 µl of distilled water and 2.5 ml of 0.2 N Folin-Ciocalteu reagent. After 5 min, 2 ml of saturated sodium carbonate (Na_2CO_3; 75 g L^{-1}) was added. The absorbance of the resulting blue solution was measured at 765 nm after incubation at 30°C for 1.5 h with intermittent shaking. Quantitative measurements were performed based on a six point standard calibration curve of 20, 100, 200, 300, 400, 500 mg L^{-1} of gallic acid in 80% MeOH. The total phenolic content is expressed as gallic acid equivalents (GAE) in milligrams per gram dry material.

2.4 Evaluation of Total Flavonoids

The total flavonoid content was determined as described by Nikolova using rutin as reference compound [21]. One milliliter of the plant extract (10 mg/ml in 70% ethanol or H_2O) was mixed with 1 ml aluminum trichloride ($AlCl_3$) in EtOH (20 g/L) and diluted with EtOH to 25 ml. The absorption at 415 nm was registered after 40 min at RT. Blank samples were prepared from 1 ml plant extract and one drop of acetic acid, and diluted to 25 ml. The absorption of rutin solutions was measured under the same conditions. Standard rutin solutions were prepared from 0.05 g rutin. All determinations were carried out in duplicate. The amount of flavonoids in plant extracts, expressed as rutin equivalents (RE), was calculated by the following formula:

$$X = (A \times m_0 \times 10) / (A_0 \times m)$$

Where: X is the flavonoid content, mg/g plant extract in RE; A is the absorption of plant extract solution; A_0 is the absorption of standard rutin solution; m is the weight of plant extract (g), and m_0 is the weight of rutin in the solution (g).

2.5 Radical Scavenging Properties Assessment

The radical scavenging activities of aqueous and hydro-alcoholic extracts of *A. falcata* were evaluated using 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-radical scavenging activity assay which has been widely used to test radical scavenging activity [22]. All used reagents were analytical grade (Sigma). All measurements, in triplicate, were performed at 25°C. Trolox (10^{-3} mol L^{-1}) stock solution was freshly prepared in MeOH and stored under argon atmosphere at 4°C, protected from light, to avoid the oxidative degradation. The evaluation of trolox antioxidant capacity equivalent against ABTS cation radical was performed by generating the $ABTS^{\cdot+}$ subsequent ABTS reaction with potassium persulphate as described by Litescu et al. (2010) [22]. The determination is performed at fixed wavelength 731 ± 2 nm. Results were reported as trolox equivalent antioxidant capacity (TEAC) equivalent to the mass of plant material used in extracts preparation.

2.6 High-performance Liquid Chromatography-mass Spectrometry (HPLC-MS) Evaluation of the *A. falcata* Extracts

The experiments to develop a method for measuring polyphenols were based on the HPLC method published by Cristea et al. for the measurement of these compounds [23]. The HPLC-DAD-MS measurements were performed using a complete HPLC SHIMADZU system, Nucleosil 100-3.5 C18 column, KROMASIL, 100× 2.1 mm. The system was coupled to a MS detector, LCMS-2010 detector (liquid chromatograph mass spectrometer), equipped with an electrospray ionization (ESI) interface. The HPLC-column was equilibrated for 1 h before injections were started. The mobile phase was a gradient prepared from formic acid in water (pH = 3, solvent A) and formic acid in acetonitrile (pH = 3, solvent B): 0.01 to 20.00 min, 5 to 30% solvent B; 20.00 to 40 min, 30% solvent B; 40.01 to 50.00 min, 30 to 50% solvent B; 50.01 to 52.00 min, 50 to 5% solvent B; 52.01 to 70.00, 5% solvent B. The flow rate was: 0.01 to 5.00 min, 0.1 ml/min; 5.01 to 15 min, 0.2 ml/min; 15.01 to 35 min, 0.1 ml/min; 35.01 to 60 min, 0.2 ml/min; 60 to 70 min, 0.1 ml/min. The mobile phase was sonicated in order to eliminate the dissolved air and then subjected to filtration using a PTFE 0.2 µm membrane. The samples were filtrated before injection using syringe driven filter unit 0.2 µm (Macherey-Nagel). The analyses were performed at 20°C for the period of

70 min. Then the column was washed over a period of 15 min with mobile phase using the flow rate 0.1 ml/min. After completion of series of analyses, the HPLC system was cleaned with water and MeOH for 1 h. ESI source and negative ionisation mode was used. Nitrogen was used as the nebulising and drying gas. The SCAN (m/z 50 to 800) mode was used for identification of hesperidin and the SIM mode was used when a search for some particular ions should be done.

2.7. Antimicrobial Activity

2.7.1 Microorganisms

Gram positive bacteria used in this study were: *Staphylococcus aureus* ATCC 3386, clinical strain methicillin resistant *Staphylococcus aureus (MRSA) 755, Streptococcus pneumoniae* ATCC 49619*, Bacillus cereus* ATCC 14579 and *Enterococcus faecalis* ATCC 29212 while the Gram negative bacteria were: *Klebsiella pneumoniae* ATCC 1388*, Shigella sonnei* ATCC 9290*, Pseudomonas aeroginosa* ATCC 1014*, Escherichia coli* ATCC 35218 and *Salmonella typhimurum* ATCC 14028. Two *Candida* species were used, namely *C. albicans* ATCC 10231 and *C. glabrata* ATCC 15126.

2.7.2 Culture media and inoculum preparation

Bacterial strains were frequently subcultured and maintained on nutrient agar plates (NA). For antimicrobial assay, microbial cultures freshly grown at 37°C were appropriately diluted in sterile normal saline and adjusted to 0.5 McFarland standards. The yeast strains were grown overnight at 37°C on Sabouraud dextrose agar plates (Oxoid), and inocula for the assays were prepared by diluting the cultures in 0.85% NaCl solution and adjusted to 0.5 McFarland standard. Protocols followed were in accordance to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [24, 25].

2.7.3 Antimicrobial susceptibility testing

For bacterial cultures, the agar well diffusion method was used by spreading 50 µl of diluted inoculum (10^5 CFU/ml) of test organism on Muller Hinton agar plates (Oxoid) according to NCCLS guidelines. However, yeast suspensions were diluted to obtain 10^4 CFU/ml and were spread on Sabouraud dextrose agar plates. Wells of 6 mm diameter were punched into the agar medium and filled with 50 µl (100mg /ml) of plant extract and solvent blanks. The plates were incubated for 18 h at 37°C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Gentamicin (10 µg) (Oxoid) and fluconazole (25 µg) discs were used as positive control.

2.7.4 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by broth micro dilution method

Stock solutions of 100 mg/ml were prepared. These were serially diluted in 96-well plates (Nunc) and the final concentrations ranged from 100 to 5 mg/ml when reconstituted with bacterial and yeast suspension The wells were inoculated with $5x10^5$ cfu/mL of the test bacterial strain according to NCCLS M7-A5 and with 1 x 10^5 cfu/mL candida strain according to NCCLS M-27. The micro plates were incubated for 24 h at 35^oC in duplicates. One of the 12 columns served as growth control (bacterial suspension or yeast without plant extract), on the other hand, sterile water and 80% methanol were used as negative control. Gentamicin and flucanazole solutions (Sigma) were used as positive control for bacteria and

Candida species, respectively. Readings were obtained by visualizing the lowest concentration without visible growth and was defined as MIC.

For MBC determination, 20 µl from each well from MIC essay plates that showed complete inhibition 100% after 24 h of incubation were subcultured on blood agar and incubated at 35°C for 24 h. MBC was determined as the lowest concentration that showed either no growth or fewer than three colonies to obtain an approximately 99 to 99.5% killing activity. All experiments were carried out in triplicates. Stock solution for the EtOH extract was prepared using 80% MeOH. Methanol (80%) was used as a control which did not have any effect on the strains used.

2.8 Platelet Aggregation

The antiplatelet activity of the hydro-alcoholic extract of *A. falcata* was studied in human whole blood *in vitro.* Blood was collected from healthy volunteers who had not taken any medication, including aspirin, within the last two weeks. Blood was withdrawn using vacutainer containing 3.8% sodium citrate (9:1 v/v). The blood sample was diluted with normal saline in the ratio of 1:1. Extract was dissolved in dimethyl sulfoxide (DMSO) and diluted with normal saline to obtain concentration of 6.25 mg/ml. Extract (4,8,16, and 30 µl) was added to a cuvette containing the diluted whole blood and the mixture was allowed to incubate at 37°C for 4 min prior to the addition of ADP (10 µM) or collagen (2 µg/ml). ADP and collagen were products of Chrono- Log Corp. The total volume of the mixture was 1 ml. The final concentrations of extract in the mixture were (25, 50, 100, and 200 µg/ml). The platelet aggregation was measured by the whole blood Chrono-log 700 lumi-aggregometer using an electrical impedance method. The mean platelet aggregation in whole blood was measured as a change in impedance over 6 min after the addition of the inducers by comparison with that of a control group impedance. The final concentration of DMSO in the whole blood was 0.5% to eliminate the effect of the solvent on the aggregation [26]. Aspirin was used as a positive control.

2.9 Cytotoxicity Assay

The MCF-7 cell line was subjected to cytotoxicity assay. For adherent cells (PLF), 1 x 104 cells were seeded in each well. Colorimetric Cell Titer 96 non-Radioactive Cell Proliferation Assay (Promega, Madison, USA) was used to detect cells proliferation in each well as per manufacturer's instructions. Briefly, 15µl of dye solution were added on each well. The cells were incubated at 37ºC and 5% CO_2 for 4 h. Then, 100µl of the solubilisation solution were added to solublize formazan precipitate. The absorbance was recorded at 570nm using a colorimetric plate reader (Sunrise- Basic TECAN, Austria). Cellular proliferation was expressed as a percentage of cell viability of MCF-7 cells relative to untreated controls.

3. RESULTS AND DISCUSSION

3.1 Evaluation of Total Phenolic Compounds and Total Flavonoid Content

Different *Achillea* species had been reported to contain large amounts of polyphenolic compounds, in particular flavonoids, phenolic acids and tannins, which may effectively exert antioxidant and free radical scavenging activities [26,27, 28]. Therefore, in the present study, total phenolic compounds and total flavonoid content of *A. falcata* aqueous and hydro-alcoholic extracts were determined. As shown in Table 1, total phenols content

(expressed as mg gallic acid equivalents/g plant extract) was higher for hydro-alcoholic extract (28.13 ± 0.56) compared to aqueous extract (25.64 ± 0.21). Flavonoids content of (mg/ g plant extract), in rutin equivalents was also higher for hydro-alcoholic extract (1.32 ± 0.02) compared to aqueous extract (0.93 ± 0.01).

Table 1. Total phenolic content, total flavonoids and antioxidant activity of aqueous and hydro-alcoholic extracts of *A. falcata*

Parameter	Aqueous Extract	Hydro-alcoholic extract
Total phenolic content (mg GAE/g plant extract)*[a]	25.64 ± 0.21	28.13 ± 0.56
Flavonoid content (mg RE /g plant extract)*[b]	0.93 ± 0.01	1.32 ± 0.02
Antioxidant activity (ABTS TEAC µmol/g)[c]	224.88	227.82

**Mean ±SD (n=2). [a]Data are expressed as mg of gallic acid equivalents (GAE) per g plant extract. [b]Data are expressed as mg of rutin equivalents (RE) per g plant extract. [c]The scavenging capacity is expressed in µmol Trolox equivalent antioxidant capacity (TEAC) per g plant extract.*

3.2 Antioxidant and Radical Scavenging Activities

The radical scavenging activities of both extracts of *A. falcata* were evaluated using the ABTS radical scavenging activity assay. The scavenging capacity is expressed in Trolox equivalent antioxidant capacity, (TEAC). Hydro-alcoholic extract exhibited a more efficient radical scavenging activity (227.82 µmol/g) compared to the aqueous extract (224.88µmol/g). Only few reports have been previously published about the chemical constituents and/or the biological activities of *A. falcata* aqueous and ethanol/methanol extracts. The infusions of *Achillea* species were tested on antioxidant enzyme systems of erythrocytes and *A. falcata* L. was the most effective species among the tested against CAT (catalase), GPx (glutathione peroxidase) and SOD (superoxide dismutase) enzyme systems of erythrocytes [17].Recently Polatoglu et al (2013) tested DPPH scavenging activity of *A. teretifolia A. vermicularis* and *A. biberstennii* and observed highest activity with *A. teretifolia* oil [29]. This antioxidant capacity was attributed to the flavonoid and phenol contents. Our results showed an agreement with previous studies demonstrating the association of phenolic and flavonoid compounds in the total antioxidant activity of *A. falcata* [17].

3.3 High-performance Liquid Chromatography-mass Spectrometry (HPLC-MS) of the Extracts

Eight compounds were identified in the hydro-alcoholic extract compared to six compounds identified in the aqueous extract (Table 2). Quercetin 3-β-D-glucoside was dominant in both, hydro-alcoholic (174.4 µg/g) and aqueous (83.58 µg/g) extracts, followed by ferulic acid, (39.24 µg/g) and (33.12 µg/g) for the hydro-alcoholic and aqueous extracts, respectively. Although the chemical composition of the essential oil of *A. falcata* growing in Lebanon using GC-MS analysis has been reported, to the best of our knowledge, this is the first investigation on aqueous and hydro-alcoholic extract [19].

Table 2. HPLC-MS pattern and polyphenolic content of aqueous and hydro-alcoholic extracts of *A. falcata*

Active compound	Aqueous Extract µg/g	Hydro-alcoholic extract
Caffeic acid	*-*	*0.46*
Ferulic acid	*33.12*	*39.24*
Ellagic acid	*-*	*0.78*
Luteolin	*0.30*	*1.56*
Quercetin	*0.24*	*1.24*
Myricetin	*0.16*	*0.32*
Rutin	*0.54*	*0.58*
Quercetin 3-β-D-glucoside	*83.58*	*174.4*

3.4 Antimicrobial Activity

Antimicrobial activities of *A. falcata* aqueous and hydro-alcoholic extracts were determined by the agar well diffusion method and MIC and MBC by microdilution method against pathogenic gram positive and gram negative bacteria and yeast. All gram positive bacteria in the agar well diffusion test showed sensitivity to hydro-alcoholic extract though not for aqueous extract (Table 3). However, the tested extracts failed to show any significant activity for gram negative bacteria and both strains of *Candida*. This might be explained by the different structures of cell envelopes and the cellular enzymatic activities of the gram negative bacteria and the yeasts.

Another explanation could be due to the fact that active ingredients may also bind to bacterial adhesins (receptors) on the cell surface. Furthermore, the cell membrane of gram positive bacteria contains mucopolysaccharides, proteins and less phospholipids. However, gram negative bacteria have more phospholipids in their cell envelope. Therefore, the permeability of the antimicrobial agents is highly efficient in gram positive bacteria depending on the reaction with the protein layer found as mucoplysaccharides and peptidoglycans [30].

Results also showed that the hydro-alcoholic extract had a bactericidal activity against *S. pneumoniae*, *B. cereus,* and *K. pneumoniae* rather than inhibitory effect. Our results are supported by the only one report available for methanol extract of *A. falcata* grown in Turkey exhibited inhibitory activity mainly against gram-positive bacteria [31]. Neither aqueous nor ethanol extracts have been tested prior to our study for antimicrobial activity.

3.5 Platelet Aggregation

In Jordanian folk medicine, *Achillea* species are used to stop bleeding [12]. In support of the traditional use of the genus *Achillea* in the Jordanian folk medicine as a haemostatic *A. bieberstienii* has been reported to enhance platelet aggregation [24] but to the best of our knowledge no similar scientific reports were found on *A. falcata* in the literature.

Table 3. Antimicrobial activity of ethanol and aqueous extracts of *A. falcate*

Microorganisms*		Gentamicin 10µg	Aqueous Extract	Hydro-alcoholic Extract t	Gentamicin	Aqueous Extract	Hydro-alcoholic Extract	Gentamicin	Aqueous Extract	Hydro-alcoholic Extract
		Antibacterial activity by Agar Well Diffusion (cm)			Minimal Inhibitory Concentration MIC (mg/ml)**			Minimal Bactericidal Concentration MBC (mg/ml)		
Gram Positive Bacteria*	S. aureus	1.8	2	2	0.005	50	6.25	0.01	50	25
	MRSA	R**	R	1.6	4	R	12.5	>4	R	25
	S. pneumoniae	1.5	R	2	0.005	R	3.12	0.01	R	3.12
	B. cereus	R	R	2	4	R	1.56	>4	R	1.56
	E. faecalis	R	R	1.2	4	R	12.5	>4	R	25
Gram Negative Pathogenic Bacteria*	K. pneumoniae	R	R	2.2	4	R	12.5	>4	R	12.5
	S.sonnei	1.9	R	R	0.001	R	R	0.01	R	R
	P aeroginosa	2.0	R	R	0.001	R	R	0.01	R	R
	E. coli	2.0	R	R	0.001	R	R	0.01	R	R
	S. typhimurum	2.2	R	R	0.001	R	R	0.01	R	R

** Antimicrobial activity against Candida results are not shown in the table.*

***R: Resistant.*

The hydro-alcoholic extract of *A. falcata* was tested for its antiplatelet activity on human whole blood *in vitro*. No effect on platelet aggregation was noticed at concentrations of (25, 50, 100, and 200 µg/ml). This observation reveals the fact that plant species used in the traditional medicine should be identified properly before recommending them for therapeutic purposes [32]. All *Achillea* species referred to locally in Jordan as "Qaysoum" and recommended for a big variety of diseases despite differences they exhibited in scientific evaluation.

3.6. Cytotoxicity Effect

There are some reports about the anti-proliferative activity of the isolated constituents from A. *falcata* [13,14,15,16]. The MTT assay was used as a relative measure of cell viability to study the anti-proliferative activity of the aqueous and hydro-alcoholic *A. falcata* extracts against the MCF-7 cells. At concentrations up to 200 µg/ml, extracts did not possess cytotoxic activity.

4. CONCLUSION

The present investigation using the aqueous and hydro-alcoholic extracts of *A. falcata* supported the traditional use of this plant in the Jordanian folk medicine as a depurative agent and as an antimicrobial active representative of the genus *Achillea*, provided the exact identification of the species recommended.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nemeth E, Bernath J. Biological activities of yarrow species (*Achillea* spp.). Curr Pharm Des. 2008;14:3151-67.
2. Feinbrun-Dothan N. Flora Palaestina, The Israel Academy of Sciences and Humanities, Jerusalem, Israel. 1978;(3):342
3. Benedek B, Kopp B. *Achillea millefolium* L. s.l. revisited: recent findings confirm the traditional use. Wien Med Wochenschr. 2007;157:312-14.
4. Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sökmen A, Akpulat A. Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. *millefolium* Afan. *(Asteraceae).* J Ethnopharmacol. 2003;87:215-20.

5. Yeşilada E, Hond G, Sezik E, Tabata M, Goto K, Ikeshiro Y. Traditional medicine in Turkey.IV.Folk medicine in the Mediterranean subdivision. J Ethnopharmacol. 1993;39:31-8.
6. Honda G, Yesilada E, Tabata M, Sezik E, Fujita T. Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kutahya, Denizli, Mugla, Aydin provinces. J Ethnopharmacol. 1996;53:75-87.
7. Sezik E, Yeşilada E, Honda GE. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. J Ethnopharmacol. 2001;75:95-115.
8. Saeidnia S, Gohari A, Yassa N, Shafiee A. Composition of the volatile oil of *Achillea conferta* DC, from Iran. Daru. 2005;13:34-6.
9. Csupor-Löffler B, Hajdú Z, Zupkó I, Réthy B, Falkay G, Forgo P, et al. Antiproliferative effect of flavonoids and sesquiterpenoids from *Achillea millefolium* s.l. on cultured human tumour cell lines. Phytother Res. 2009;23:672-6.
10. Vitalini S, Beretta G, Iriti M, Orsenigo S, Basilico N, Dall'Acqua S, et al. Phenolic compounds from *Achillea millefolium* L. and their bioactivity. Acta Biochim Pol. 2011;58:203-9.
11. Thoppil RJ, Harlev E, Mandal A, Nevo E, Bishayee A. Antitumor activities of extracts from selected desert plants against HepG2 human hepatocellular carcinoma cells. Pharm Biol. 2013;51:668-74.
12. Oran SA, Al-Eisawi DM. Check-list of medicinal plants in Jordan. Dirasat. 1998;25:84-112.
13. Ghantous A, Nasser N, Saab I, Darwiche N, Saliba NA. Structure-activity relationship of seco-tanapartholides isolated from *Achillea falcata* for inhibition of HaCaT cell growth. Eur J Med Chem. 2009;44:3794-7.
14. Tohme R, Al Aaraj L, Ghaddar T, Gali-Muhtasib H, Saliba NA, Darwiche N. Differential Growth Inhibitory Effects of Highly Oxygenated Guaianolides Isolated from the Middle Eastern Indigenous Plant *Achillea falcata* in HCT-116 Colorectal Cancer Cells. Molecules. 2013;18: 8275-88.
15. Salla M, Fakhoury I, Saliba N, Darwiche N, Gali-Muhtasib H. Synergistic anticancer activities of the plant-derived sesquiterpene lactones salograviolide A and iso-seco-tanapartholide. J Nat Med. 2013;67:468-79.
16. Saikali M, Ghantous A, Halawi R, Talhouk SN, Saliba NA, Darwiche N. Sesquiterpene lactones isolated from indigenous Middle Eastern plants inhibit tumor promoter-induced transformation of JB6 cells. BMC Complement Altern Med. 2012;12:89. DOI.10.1186/1472-6882-12-89.
17. Konyalioglu S, Karamenderes C. The protective effects of *Achillea* L. species native in Turkey against H_2O_2-induced oxidative damage in human erythrocytes and leucocytes. J Ethnopharmacol. 2005;102:221-7.
18. Aburjai T, Hudaib M, Tayyem R, Yousef M, Qishawi M. Ethnopharmacological survey of medicinal herbs in Jordan, the Ajloun Heights region. J Ethnopharmacol. 2007;110:294-304.
19. Senatore F, Napolitano F, Arnold NA, Bruno M, Herz W. Composition and antimicrobial activity of the essential oil of *Achillea falcata* L. *(Asteraceae).* Flavour Fragr J. 2005;20:291-4.
20. Alali FQ, Tawaha K, El-Elimat T, Syouf M, El-Fayad M, Abulaila K, et al. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. Nat Prod Res. 2007;21:1121-31.
21. Nikolova M. Screening of radical scavenging activity and polyphenol content of Bulgarian plant species. Pharmacognosy Res. 2011;3:256-9.
22. Litescu SC, Eremia S, Radu GL. Methods for the determination of antioxidant capacity in food and raw materials. Adv Exp Med Biol. 2010;698:241-9.

23. Cristea V, Deliu C, Oltean B, Butiuc-Keul A, Brummer A, Albu C, et al. Soilless cultures for pharmaceutical use and biodiversity conservation. Acta Hort. 2009;843:157-64.
24. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved standard – Eight edition. NCCLS document M7-A6. NCCLS, Wayne, Pennsylvania, USA; 2003.
25. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard M27-A. NCCLS, Wayne, PA, USA. 1997.
26. Hammad HM, Albu C, Matar SA, Litescu SC, Al Jaber HI, Abualraghib AS, et al. Biological activities of the hydro-alcoholic and aqueous extracts of *Achillea biebersteinii* Afan. (Asteraceae) grown in Jordan. Afr J Pharm Pharmacol. 2013;7:1686-94.
27. Cai YZ, Sun M, Xing J, Luo Q, Corke H. Structure-radical scavenging activity relationships of phenolic compounds from traditional Chinese medicinal plants. Life Sci. 2006;78:2872-88.
28. Giorgi A, Bombelli R, Luini A, Speranza G, Cosentino M, Lecchini S, et al. Antioxidant and cytoprotective properties of infusions from leaves and inflorescences of *Achillea collina* Becker ex Rchb. Phytother Res. 2009;23:540-5.
29. Polatoğlu K, Karakoç ÖC, Gören N. Phytotoxic, DPPH scavenging, insecticidal activities and essential oil composition of *Achillea vermicularis*, *A. teretifolia* and proposed chemotypes of *A. biebersteinii* (*Asteraceae*). Ind Crop Prod. 2013;51:35-45.
30. Al-Saimary IE, Bakr SS, Khudaier BY, Abass YK. Efficiency of antibacterial agents extracted from *Thymus vulgaris* L. *(Lamiaceae).* Internet J Nutri Wellness. 2007;4:DOI. 10.5580/269.
31. Karaalp C, Yurtman AN, Yavasoglu NUK. Evaluation of Antimicrobial Properties of *Achillea* L. Flower Head Extracts. Pharm Biol, 2009;47(1),86-91
32. Afifi FU, Abu-Irmaileh B. Herbal medicine in Jordan with special emphasis on less commonly used medicinal herbs. J Ethnopharmacol. 2000;72:101-10.

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