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*Full Length Research Paper*

# Biological activities of the hydro-alchoholic and aqueous extracts of *Achillea biebersteinii* Afan. (Asteraceae) grown in Jordan

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**Aqueous and hydro-alcoholic extracts of *Achillea biebersteinii* Afan. (Asteraceae) grown in Jordan were screened for their antioxidant, antibacterial and antiplatelet efficacy. Total phenols and flavonoids were determined colorimetrically. The radical scavenging activities were evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS) radical scavenging activity assays. Antimicrobial activities were determined by the disc-diffusion method, and the minimum inhibition concentration and the minimum bactericidal concentration tests. Gram positive bacteria presented sensitivity to the hydro-alcoholic extract in the disk diffusion test. No significant activity was observed against Gram negative bacteria and *Candida albicans*. Hydro-alcoholic extract had a bactericidal activity against *Streptococcus pneumoniae*, *Bacillus cereus, Enterococcus faecalis* and *Klebsiella pneumoniae* rather than inhibitory effect. *In vitro* antiplatelet activity was determined with human whole blood using an electrical impedance method. At concentrations (50, 100, and 200 µg/ml), the extracts showed a non-dose dependent enhancement of platelet aggregation induced by adenosine diphosphate (ADP) (51.82, 52.73 and 51.82%, respectively) and collagen (0.00, 11.76 and 23.53%, respectively). High performance liquid chromatography-mass spectrometry (HPLC-MS) analysis resulted in the identification of 8 phenolic compounds; quercetin 3-β-D-glucoside was the main component.**

**Key words:** *Achillea biebersteinii*, Asteraceae, antioxidant activity, antibacterial activity, antiplatelet activity, Jordan.

## INTRODUCTION

The genus *Achillea* (commonly referred to as yarrow) comprises over 100 species worldwide, which are mainly distributed in the northern hemisphere (Nemeth and Bernath, 2008). The name *Achillea* refers to the Achilles in the literary Trojan War of the Iliad who used yarrow to treat the soldiers' wounds (Benedek and Kopp, 2007).

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Yarrow is widely used in traditional European medicine to treat fevers, hypertension, gastrointestinal disorders and to stop bleeding, and as an antihaemorrhoid (Benedek and Kopp, 2007). According to Persian traditional literature, *Achillea* species have been used in medicine for treatment of hemorrhage, pneumonia, rheumatic pain and in wound healing (Zargari, 1996; Saeidnia et al., 2005). Herbal teas prepared from some *Achillea* species are traditionally used for abdominal pain and flatulence in Turkey (Honda et al., 1996). 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 (Oran and Al-Eisawi, 1998). Recent studies demonstrated that the different *Achillea* species possess antioxidant and antiproliferative capacities (Csupor-Löffler et al., 2009; Vitalini et al., 2011; Thoppil, et al., 2013).

*Achillea biebersteinii*, commonly known as "Qaysoum" in Arabic, is one of the predominant *Achillea* species in the Mediterranean region (Zohary and Feinbrun-Dothan, 1978). It is a perennial, herbaceous and aromatic plant, 20 to 50 cm long, usually growing in patches. Heads are yellow, less than 1 cm in diameter, aggregated in a dense flat topped inflorescence. In Jordan, this plant grows wild in heavy red soils and along the road sides of the capital Amman and of the northern districts of Jordan (Al-Eisawi, 1998). The occurrence of five *Achillea* species has been reported in Jordan; *A. bibersteinii* Afan., *A. santolina* L., *A. fragrantissima* (Forskal) Schulz Bip., *A. falcata* L. and *A. aleppica* DC. The latter species is considered as a rare species of Jordan (Zohary and Feinbrun-Dothan, 1978).
In Turkey, *A. biebersteinii* is promoted as a folk remedy for the treatment of abdominal pain, wounds and stomach ache (Sezik et al., 2001; Baris et al., 2006). Akkol et al. (2011) demonstrated that *A. biebersteinii* displays remarkable wound healing activity. Reports concerning its antimicrobial and antioxidant properties are also available in the literature (Sökmen et al., 2004; Baris et al., 2006). To the best of our knowledge, there are no comprehensive studies performed for evaluation of the effectiveness of *A. biebersteinii* 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, and antimicrobial efficacy of *A. biebersteinii* grown wild in Jordan.

## Materials and methods

### Plant

*A. biebersteinii* was collected from Al-Jubeiha region, in the vicinity of the University of Jordan, Amman, during the period extending from April to May, 2011. The plant was identified by Prof. Barakat E. Abu-Irmaileh at the Department of Plant Protection, Faculty of Agriculture, The University of Jordan. Voucher specimen 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 until constant weight, and subsequently used for extraction.

### 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 (EtOH) for 15 min and kept overnight. Extracts were then filtered and evaporated *in vacuo* to give a crude residue of 22 and 20%, respectively (%, w/w).

### 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 (Alali et al., 2007). 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.

### Evaluation of total flavonoids

The total flavonoid content was determined as described by Nikolova (2011) using rutin as reference compound. 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).

### Radical scavenging properties assessment

The radical scavenging activities of aqueous and hydro-alcoholic extracts of *A. biebersteinii* were evaluated using 2,2'diphenyl-1-pycril hydrazyl (DPPH)- and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)-radical scavenging activity assays which have been widely used to test radical scavenging activity (Litescu et al., 2010). All used reagents were analytical grade (Sigma). All measurements, in triplicate, were performed at 25°C. Trolox ($10^{-3}$ mol $L^{-1}$) and DPPH ($3 \times 10^{-3}$ mol $L^{-1}$) stock solutions were freshly prepared in MeOH and stored under argon atmosphere at 4°C,

protected from light, to avoid the oxidative degradation. The Ultraviolet and visible (UV-VIS) spectrometry procedure was carried out by measuring the changes in the value of the DPPH maximum absorbance at 519 nm. Trolox equivalent scavenging capacity (TESC) was evaluated instead of the determination of $IC_{50}$. This approach was performed in order to compare the extracts efficacy against DPPH radical to that exhibited for ABTS cation radical. For DPPH, the determinations were performed according to the optimum measuring time settled on the base of system stability named 6 min after antioxidant samples additions. Final results were reported as trolox equivalent antioxidant capacity (TEAC) equivalent to the mass of plant material used in extracts preparation. The evaluation of trolox antioxidant capacity equivalent against ABTS cation radical was performed by generating the $ABTS^{\cdot+}$ subsequent ABTS reaction with potassium persulphate. The determination is performed at fixed wavelength 731 ± 2 nm. Final results were expressed as TEAC equivalent to the mass of plant material used in extracts preparation.

**High-performance liquid chromatography-mass spectrometry (HPLC-MS) evaluation of the *A. biberstennii* extracts**

The experiments to develop a method for measuring polyphenols were based on the HPLC method published by Cristea et al. (2009) for the measurement of these compounds. 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 (Cristea et al., 2009).

**Antimicrobial activity**

***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 *Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 15126*.*

***Culture media and inoculum preparation***

Bacterial strains were frequently subcultured and maintained in 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 scale. The yeast (*Candida*) strains were grown overnight at 37°C in 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 scale. Protocols followed were in accordance to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS, 1997, 2003).

**Screening for antimicrobial activities**

***Antimicrobial susceptibility testing***

The agar well diffusion method was used. 50 µl of diluted inoculum ($10^5$ CFU/ml) of test organism was spread 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 were 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.

***Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by broth microdilution method***

Stock solutions of 100 mg/ml were prepared. These were serially diluted to obtain various ranges of concentrations between 5 to 100 mg/ml in 96 well microplates (Nunc). 10 µl of microorganisms were added. After that, the samples were incubated at 35°C for 24 h, in duplicate. On the other hand, sterile water and 80% methanol were used as negative control standards. After the period of incubation, the well containing the least concentration of extract showing no visible sign of growth was considered as the MIC. 20 µl from each well 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 and did not have any effect on the strains used.

***Platelet aggregation***

The antiplatelet activity of the hydro-alcoholic extract of *A. biebersteinii* was determined 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,

**Table 1.** Total phenolic content and total flavonoids of aqueous and hydro-alcoholic extracts of *A. biebersteinii*.

| Parameter | Aqueous extract | Hydro-alcoholic extract |
|---|---|---|
| Total phenolic content (mg GAE/g plant extract)[a] | 27.89±0.21 | 54.61±0.60 |
| Flavonoid content (mg RE/g plant extract)[b] | 1.43±0.02 | 3.79±0.03 |

All data are shown as mean ±SD. All determinations were carried out in duplicate. [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.

**Table 2.** Antioxidant activity of aqueous and hydro-alcoholic extracts of *A. biebersteinii*.

| Parameter (µmol/g) | Aqueous extract | Hydro-alcoholic extract |
|---|---|---|
| DPPH TEAC | 211.20 | 352.77 |
| ABTS TEAC | 352.27 | 598.20 |

The scavenging capacity is expressed in µmol trolox equivalent antioxidant capacity (TEAC) per g plant extract.

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 (Jantan et al., 2006). Aspirin was used as a positive control.

## RESULTS AND DISCUSSION

### Evaluation of total phenolic compounds and total flavonoid

Many plants, including different *Achillea* species contain large amounts of polyphenolic compounds, in particular flavonoids, phenolic acids and tannins, which may effectively exert antioxidant and free radical scavenging activities (Zia-Ul-Haq et al., 2013a, b, c; Zubair et al., 2013; Cai et al., 2006; Giorgi et al., 2009). Therefore, in the present study, total phenolic compounds and total flavonoid content of *A. biebersteinii* aqueous and hydro-alcoholic extracts were determined. As shown in Table 1, total phenol content (expressed as mg gallic acid equivalents/g plant extract) was higher for hydro-alcoholic extract (54.61 ± 0.60) compared to aqueous extract (27.89 ± 0.21). Flavonoids, as one of the most diverse and widespread group of natural compounds, are probably the most important natural phenolics (Rendeiro et al., 2012). These compounds possess a broad spectrum of biological activities including antioxidant and radical scavenging properties. The content of flavonoids (mg/g plant extract), in rutin equivalents was higher for hydro-alcoholic extract (3.79 ± 0.03) compared to aqueous extract (1.43 ± 0.02).

### Antioxidant and radical scavenging activities

The radical scavenging activities of both extracts of *A. biebersteinii* were evaluated using the methods of DPPH and ABTS radical scavenging activity assays, which have been widely used to test radical scavenging activity. The scavenging capacity is expressed in TEAC. As shown in Table 2, both extracts showed obvious DPPH and ABTS radical scavenging activity. Hydro-alcoholic extract exhibited more efficient radical scavenging activity compared to the aqueous extract in both methods. In the tested extracts, the value of TEAC against $ABTS^{\cdot+}$ are higher with respect to TEAC obtained against DPPH. This is expected since both DPPH and ABTS are free radicals that react on the base of a combined mechanism equally as hydrogen atom transfer (HAT) and electron transfer (ET) but while DPPH does not react with flavonoids without substituted OH in B-ring or with aromatic acids with single OH group, ABTS does not discriminate between OH phenolics providing a response related with total groups able to quench a radical reaction. Consequently, it could be said that DPPH scavenging highly depends on the degree of electron's delocalisation; for the DPPH approach (protocol) the radical scavenging/antioxidant effect increases if the partial phenolic ionisation occurred in the system of analysis (Litwinienko and Ingold, 2004).

In order to provide the evidence of results matching

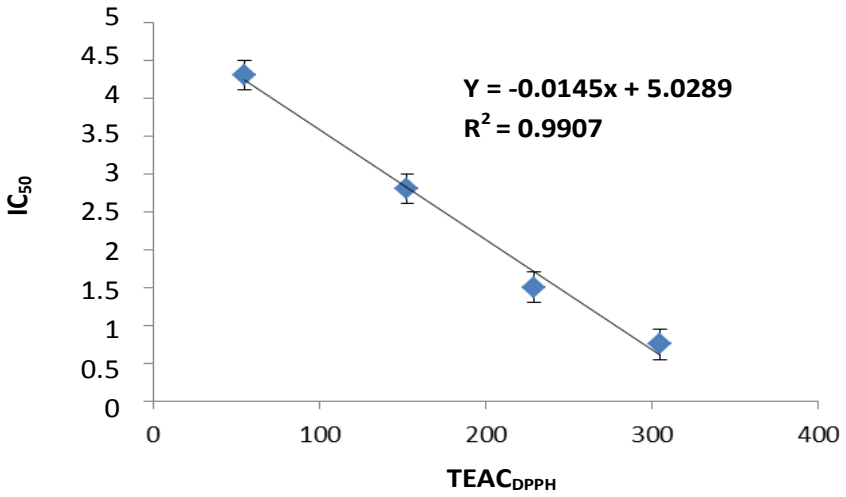


**Figure 1.** Correlation between DPPH method and $IC_{50}$ method.

**Table 3.** HPLC-MS pattern and polyphenolic content of aqueous and hydro-alcoholic extracts of *A. biebersteinii*.

| Active compound | Aqueous extract (µg/g) | Hydro-alcoholic extract (µg/g) |
|---|---|---|
| Caffeic acid | 0.78 | 0.52 |
| Ferulic acid | 86.24 | 72.18 |
| Luteolin | 3.40 | 11.04 |
| Quercetin | 2.44 | 4.44 |
| Myricetin | 0.26 | 0.32 |
| Kaempferol | 0.10 | 0.30 |
| Rutin | 0.92 | 1.32 |
| Quercetin 3-β-D-glucoside | 415.36 | 774.06 |

between our carried out DPPH method and $IC_{50}$ method, the fitting of the values for the same concentration of the extracts was performed (Figure 1). Obviously, the higher concentration (in mg/ml) of extract exhibited the highest radical scavenging activity against DPPH by both methods, either TEAC or $IC_{50}$, the linearity between the two methods being very good ($R^2 = 0.9907$).

Phytochemically, hydrodistilled volatile oil of the aerial parts was the most studied fraction of *A. biebersteinii*; only few reports have been previously published about the chemical constituents and/or the biological activities of *A. biebersteinii* aqueous and ethanol/methanol extracts. In a previous study from Jordan, a good correlation was found to exist between the antioxidant activity and the total phenolic contents of both MeOH and aqueous extracts prepared from a wild growing *A. biebersteinii* (Tawaha et al., 2007). A recent study from Iran showed the impact of phenolic and flavonoid compounds in the total antioxidant activity of the *A. biebersteinii* MeOH extract (Bashi et al., 2012). Other studies in Turkey reported antioxidant activity of *A. biebersteinii* using different assay methods (Konyalioglu and Karamenderes, 2005; Sökmen et al., 2004; Baris et al., 2006). Our results showed an agreement with previous studies, demonstrating the association of phenolic and flavonoid compounds in the total antioxidant activity of *A. biebersteinii*.

## High-performance liquid chromatography-mass spectrometry (HPLC-MS) of the extracts

Eight compounds, dominated by quercetin 3-β-D-glucoside, were identified (Table 3, Figures 2 and 3). Chemical composition of the essential oil of *A. biebersteinii* using gas chromatography-mass spectrometry (GC-MS) analysis was reported (Sökmen et al., 2004), but to the best of our knowledge, this is the first investigation of the aqueous and hydro-alcohol ethanol extracts.

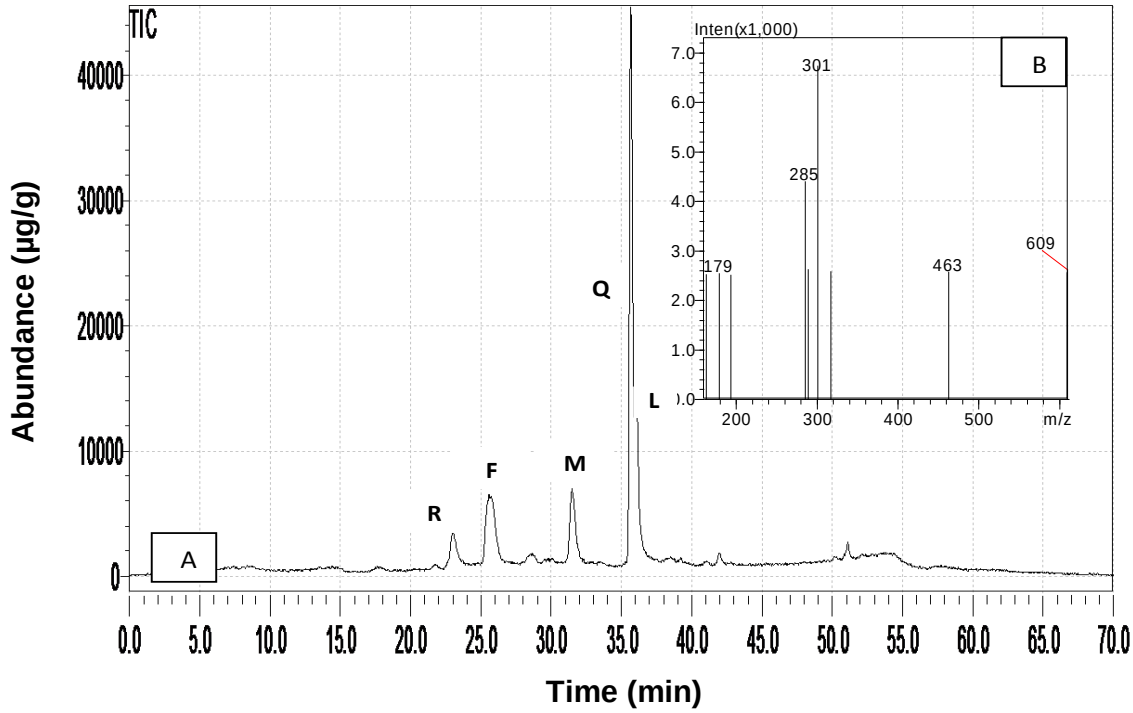


**Figure 2.** (A) HPLC-MS chromatogram of *A. biebersteinii* hydro-alcoholic extract (R (rutin); F (ferrulic acid); M (myricetin); Q (quercetin glucoside); L (luteolin)); (B) mass spectra total ion current (TIC) of the corresponding sample.

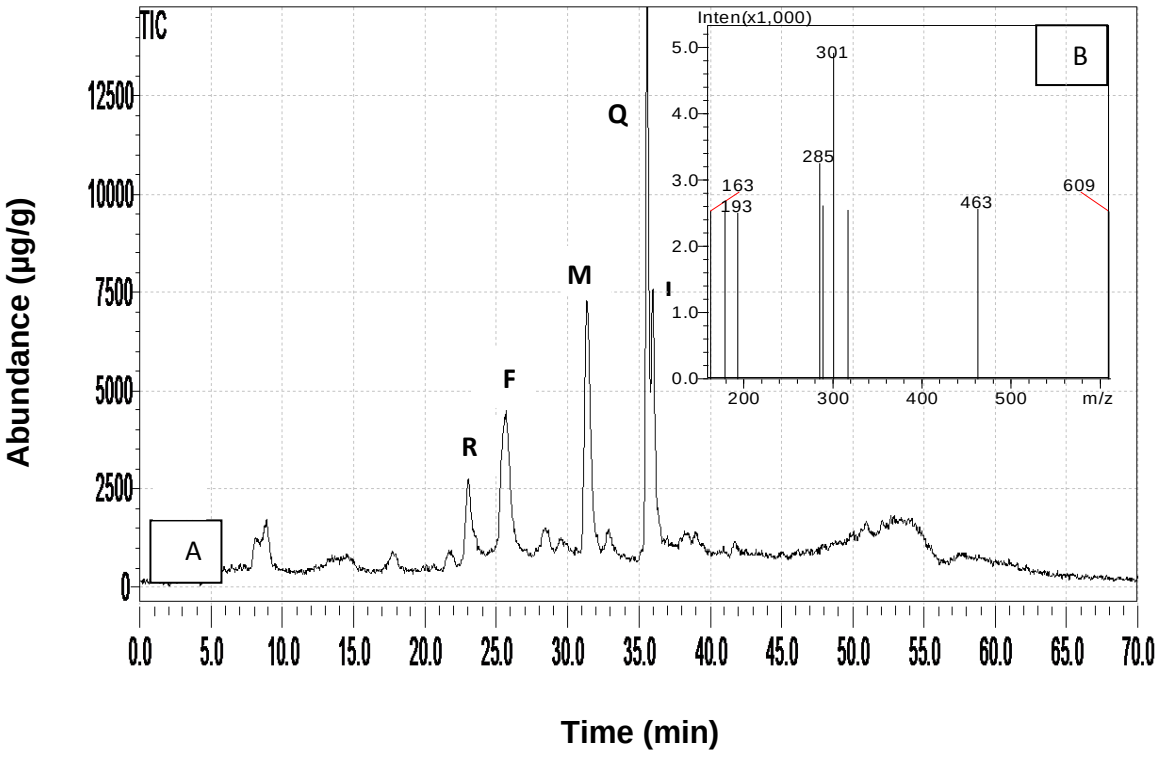


**Figure** 3. A. HPLC-MS chromatogram of A. biebersteinii aqueous extract. (R (rutin); F (ferrulic acid); M (myricetin); Q (quercetin glucoside); L (luteolin)); B. Mass spectra of corresponding sample.

**Table 4.** Antimicrobial activity of hydro-alcoholic and aqueous extracts of *A. biebersteinii.*

| Microorganisms | | GM** 10 µg | Aqueous extract | Hydro-alcoholic extract | GM | Aqueous extract | Hydro-alcoholic extract | Aqueous extract | Hydro-alcoholic extract | GM |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Antibacterial activity by Kirby Bauer disk diffusion (cm) | | | Minimal Inhibitory Concentration MIC (mg/ml)** | | | Minimal Bactericidal Concentration MBC (mg/ml) | | |
| *Gram positive pathogenic bacteria** | *S. aureus ATCC 33863* | 1.8 | 2.2 | 2.2 | 0.005 | 25 | 6.25 | 50 | 6.25 | 0.01 |
| | *Clinical Strain MRSA 755* | R* | 2 | 2.2 | 4 | 25 | 3.12 | 25 | 6.52 | >4 |
| | *S. pneumoniae ATCC 49619* | 1.5 | R | 3 | 0.005 | R | 1.56 | R | 1.56 | 0.01 |
| | *B. cereus ATCC 14579* | R | R | 2.2 | 4 | R | 1.56 | R | 6.25 | >4 |
| | *E. faecalis ATCC 29212* | R | R | 1.4 | 4 | R | 12.5 | R | 12.5 | >4 |
| *Gram negative pathogenic bacteria*** | *K. pneumoniae ATCC 13883* | R | 1.2 | 1.1 | 4 | 25 | 3.12 | 25 | 3.12 | >4 |
| | *S. sonnei ATCC 9290* | 1.9 | R | R | 0.001 | R | R | R | R | 0.01 |
| | *P. aeroginosa ATCC 10145* | 2.0 | R | R | 0.001 | R | R | R | R | 0.01 |
| | *E. coli ATCC 35218* | 2.0 | R | R | 0.001 | R | R | R | R | 0.01 |
| | *S. typhimurum ATCC 14028* | 2.2 | R | R | 0.001 | R | R | R | R | 0.01 |

*R: Resistant. **GM: Gentamicin.

## Antimicrobial activity

Antimicrobial activities of *A. biebersteinii* aqueous and hydro-alcoholic extracts were determined by the disc-diffusion method, and MIC and MBC were determined using pathogenic bacteria and yeasts. All Gram positive bacteria except *E. faecalis* in the disk diffusion test presented sensitivity to hydro-alcoholic extract (Table 4). The tested extracts did not show any significant activity against Gram negative bacteria and Candida species (results not shown). This could be partially explained by the different structures of cell envelopes. The efficiency of the extracts with antimicrobial activity depends generally on cellular enzymes in the cytoplasm and the cell wall. The 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 and more pores in their cell envelope. So 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 (Al-Saimary et al., 2007). MIC range was (1.56 to 6.25) against both Gram positive and Gram negative bacteria. Results showed that the hydro-alcoholic extract had bactericidal activity against *S. pneumoniae*, *B. cereus, E. faecalis* and *K. pneumoniae* rather than inhibitory effect.

Previous data regarding antibacterial and/or antifungal activities of *A. biebersteinii* is limited to only two reports. While Baris et al. (2006) reported that MeOH extract did not exert any antimicrobial activity, Bashi et al. (2012) showed that Gram-positive bacterial strains were more susceptible to *A. biebersteinii* MeOH extracts compared to Gram-negative bacterial strains and fungi. Both studies did not test the effect of the aqueous extract.

## Platelet aggregation

The hydro-alcoholic extract of *A. bieberstienii* was tested for its antiplatelet activity on human whole blood *in vitro*. In the absence of the extract in the whole blood, ADP and collagen showed 100% platelet aggregation. At lower concentration (25 µg/ml), hydro-alcoholic extract did not show any inhibition of platelet aggregation induced by ADP and collagen. At higher concentrations (50, 100, and 200 µg/ml) this extract showed a none-dose-dependent enhancement of platelet aggregation induced by ADP (51.82, 52.73 and 51.82%) while collagen induced aggregation was enhanced less effectively but dose dependently (0.00, 11.76 and 23.53), indicating its mechanism of action is

**Table 5.** Percentage enhancement of hydro-alcoholic extract of *A. biebersteinii* on platelet aggregation of human whole blood induced by ADP (10 µM) and collagen (2 µg/ml).

| Extract (µg/ml) | ADP | ADP | Collagen |
|---|---|---|---|
| | Aqueous extract | Hydro-alcoholic extract | |
| 25 | 8.33±5.89 | 9.09±6.43 | 00.00±00.00 |
| 50 | 36.67±2.36 | 51.82±8.36 | 00.00±00.00 |
| 100 | 55.00±3.54 | 52.73±5.14 | 11.76±4.16 |
| 200 | 56.67±16.50 | 53. 64±18.64 | 23.53±4.16 |

Values are presented as mean ± SE. Experiments were done in duplicates.

independent of thromboxane pathway (Table 5). Despite the fact that *Achillea* species have been used in traditional medicine of several cultures to stop bleeding, this study is the first to report the direct effect of *A. bieberstienii* on enhancement of platelet aggregation. Although some active compounds detected by HPLC-MS have been reported to possess antiplatelet aggregation activity; such activity was detected at very high concentrations (Ostertag et al., 2011).

It has been reported that the presence of a sugar (glucose) moiety inhibited this biological activity of flavonoids (Hostetler et al., 2012). To the best of our knowledge, quercetin 3-β-D-glucoside, the most abundant flavonoid glucoside in the tested extracts, has no reported platelet activity. Thus, further studies needed to correlate the chemical constituents of the hydro-alcoholic extract and the platelet aggregation activity of *A. biberstennii*.

## Conclusion

The present investigation with the aqueous and hydro-alcoholic extracts of *A. biberstennii* supported the traditional use of this plant in the Jordanian folk medicine as a haemostatic agent and as an antimicrobial active representative of the genus *Achillea*.

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## ABBREVIATIONS

**ABTS,** 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); **DPPH,** 2,2'diphenyl-1-pycril hydrazyl; **ESI,** electrospray ionization; **EtOH,** ethanol; **GAE,** gallic acid equivalents; **HPLC-MS,** high-performance liquid chromatography-mass spectrometry; **LCMS-2010 detector,** liquid chromatography mass spectrometer; **MeOH,** methanol; **NCCLS,** National Committee for Clinical Laboratory Standards; **TEAC,** trolox equivalent antioxidant capacity; **TESC,** trolox equivalent scavenging capacity; **Trolox,** 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid.

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