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HPLC Analysis of Chemical Composition of Selected Jordanian Medicinal Plants and their Bioactive Properties

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ABSTRACT

Three medicinal plants grown wild in Jordan, namely *Achillea santolina* L, *Achillea fragrantisimma*, *Asteriscus graveolens* (Forssk.) Less, were extracted with ethyl acetate by continuous shaking at room temperature for three days. The antibacterial activity of the crude extract was evaluated. The extracts were analyzed for their phenolic and flavonoids content by HPLC-PDA. The HPLC analysis of the plant extracts revealed the presence of flavonoids and phenolic compounds in the three plant extracts. Results revealed a strong antibacterial activity of *A. graveolens* against three bacterial strains (*B. subtilis, E. coli,* and *S.aureus*). while *A. fragrantissima* inhibited the growth of *B. subtilis*. Bioactivities were attributed mainly to the immense content of phenol-based compounds in plants.

INTRODUCTION

Huge developments in the pharmaceutical industries and chemistry are taking place nowadays, due to new findings in herbal medicine. Plants were used as the important sources of most drugs for eliminating pain and treating diseases[1]. In fact, using naturally derived bioactive products had led to overcome the side effects and new resistant pathogens of the most known synthetic drugs. As

many of natural products haven't been identified until now, it is important to find new bioactive compounds that can be used for treating various ailments. Plants have been considered as natural sources of potentially safe biologically active metabolites[2, 3].The variety of secondary metabolites in plants could be used as pure secondary metabolites or in a synergy form to have good bioactivities in several biological systems[4]. Actually, modern medicine depends on numerous therapeutic agents that were isolated from medicinal plants such as Colchicines, digitoxin, quinine, aspirin, taxol, morphine and atropine[5,6].

In Jordan, there are a total of 2500 plant species, belonging to 700 genera; 485 species from 99 different plant families are used as medicinal plants in traditional medicine[7-9]. However, usage of these plant as folk medicine without any awareness of their side effects or dose toxicity, impose drawback in their medicinal interest[10]. Moreover, many studies were carried out on Jordanian folk medicinal plants, mainly on their bioactivity such as antimicrobial and antioxidant activities[11-13]; in vitro screening of phytochemistry[14,15] and ethnobotany[16,17,6]. Hence, this study aimed to evaluate the bioactivity and to analyze the chemical compositions by HPLC-PDA of three selected Jordanian medicinal plants that are used as a folk medicine in treating various diseases Table 1.

Table 1: List of selected Jordanian medicinal plants and their usage

Scientific name	Traditional use/medical use	Reference
Achillea santolina L. (Labiatae)	Carminative, depurative, stomachaches, antispasmodic and anti-diabetes,	13
Achillea fragrantisimma (Labiatae)	Carminative, depurative, stomachaches, antispasmodic, anti-diabetes, anti-tumor and rheumatic pain	2
Asteriscus graveolens (Forssk.) Less. (Asteraceae)	Anti-diabetic, anti-inflammatory, for gastric and bowel diseases, diuretic, hypotensive and depurative	18

EXPERIMENTAL

Plant materials collection and extraction

All plant samples were collected from different localities in the southern part of Al-Karak governorate-Jordan. They were collected on the basis of traditional practices by herbalists and healers and were identified according to Al-Eisawi[19] and taxonomically authenticated by Dr. Ferryal Al-khreisat, Biology Department, Mutah University. Plants aerial parts were dried in shade until constant weight and pulverized. 100 g of powdered plant materials were soaked in 1L of ethyl acetate with continuous shaking (150 rpm, Forma Orbital Shaker, Thermo electron cooperation, USA) at room temperature for 3 days. The filtrates were concentrated *m* at 45°C using rotary evaporator (Buchi R-215, Switzerland) and the resulting residue was dissolved in methanol to a final concentration of 0.1 g/ml.

In vitro antibacterial activity determination

The antibacterial activity of plants crude extracts was determined by agar diffusion test and the minimum inhibitory concentration (MIC) was determined by serial dilution assay according to the Clinical and Laboratory standards Institute guidelines[20]. *Staphylococcus aureus* ATCC 43300, *Escherichia coli* ATCC 25922, *Micrococcus luteus* ATCC 10240,and *Bacillus subtilis* ATCC 6633, seeded on LB agar plates (0.5% tryptone, 0.5% yeast extract, 1% NaCl, 1.8% agar) were used as a test bacterial strains.

In disk diffusion assay 0.5, 1 and 1.5 mg/ disc of plant crude extract were used, while 2 mg/ ml of the plant extract and10µg/ml positive control (penicillin G) were used as initial concentrations in serial dilution assay.

HPLC system and chromatographic conditions

The HPLC is a Waters Alliance (e2695 separations module), equipped with 2998 Photo diode Array detector (PDA). Data acquisition and control were carried out using Empower 3 chromatography data software (Waters, Germany). The HPLC analytical experiments of the crude extracts of the three aerial samples were run on ODS column of Waters (XBridge, 4.6 ID x 150 mm, 5 µm)

with guard column of Xbridge ODS, 20 mm x 4.6mm ID, 5 µm. The mobile phase is a mixture of acetic acid in water (0.5%) (Solvent A) and acetonitrile (solvent B) ran in a linear gradient mode. 100% (solvent A) descended to 70% (solvent A) in 40 minutes. Then to 40% (solvent A) in 20 min.and finally to 10% (solvent A) in 2 min. and stayed there for 6 min. and then back to the initial conditions in 2 minutes. The HPLC system was equilibrated for 7 min. with the initial acidic water mobile phase (solvent A) before injecting next sample. All the samples were filtered with a 0.45 µm PTFE filter. The PDA wavelengths range was from 210-500 nm. The flow rate was 1 ml/min. Injection volume was 20 µl and the column temperature was set at 25°C.

RESULTS AND DISCUSSIONS

HPLC-PDA profiles of the extracts

Figure 1 shows the chromatogram of the crude extract of Achillea santolina L at 340 nm. This wavelength was selected because the major 6 peaks showed a maximum absorption at this wavelength. The eluted compounds were detected in the range of 47-58 min. indicating nonpolar compounds. At 258 nm, a new major peak eluted at 42.8 min. was appeared as well as another 3 minor peaks at 28.0, 41.1, and 42.1 min. (Fig. 2). The eluted compounds were detected in the range of 28-57 min. indicating polar and nonpolar compounds combination. The

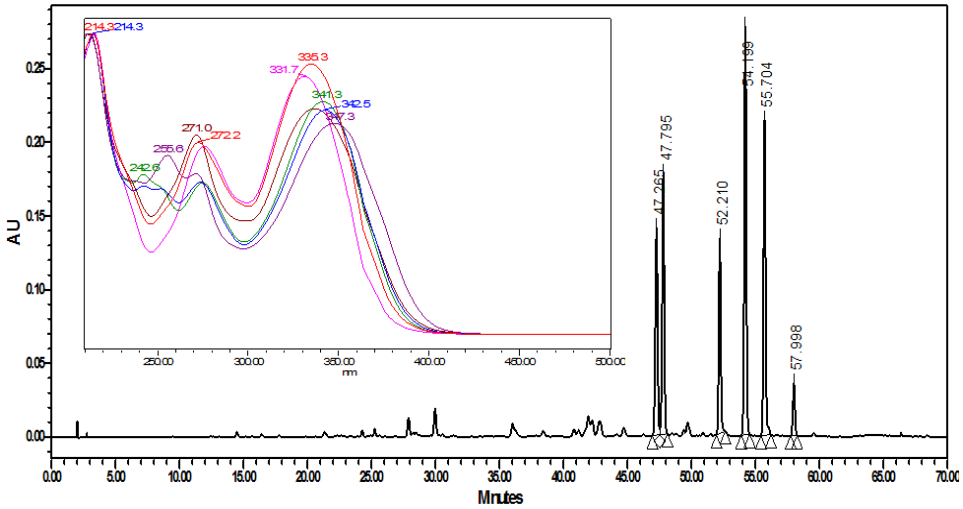

Fig. 1. HPLC-PDA chromatogram of crude extract of *Achillea santolina* L at 340 nm, their overlaid UV-Vis spectra (left corner of the chromatogram)

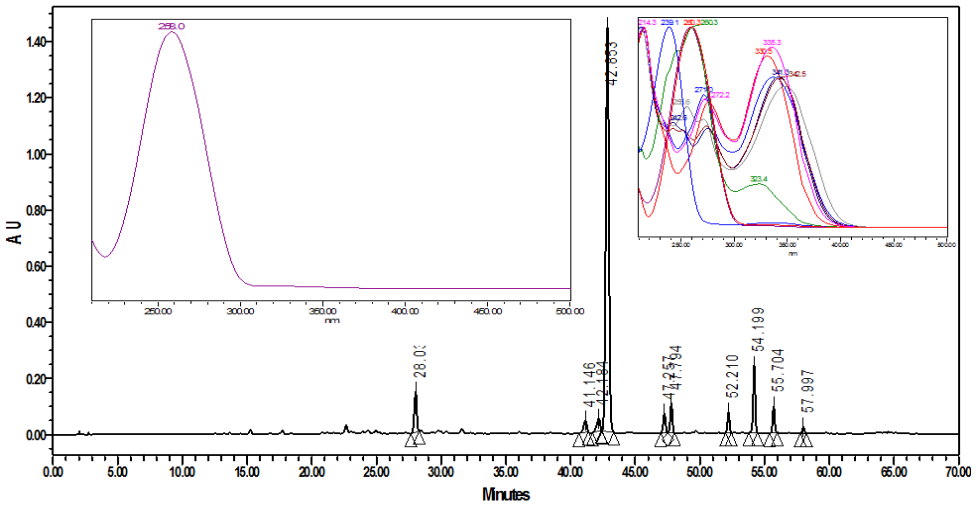

Fig. 2. HPLC-PDA chromatogram of crude extract of *Achillea santolina* L at 258 nm, their overlaid UV-Vis spectra (right corner of the chromatogram), and the UV-Vis spectrum of the major peak eluted at 42.8 minutes (left side to the major peak)

UV-Vis ranges of these compounds were in the range of 210-350 nm indicating flavonoids abundance[21]. These compounds are not part of the standards injected as per their retention and UV-Vis spectra tell.

As shown in Fig. 2, about 10 minor compounds were seen, of which only one compound showed major dominance indicating flavonoids abundance[21].

Figure 3 shows the chromatogram of the crude extract of Achillea fragrantisimma at 350 nm with main peak eluted at 44.8 min. and other 11 minor compounds showed a maximum absorption at this wavelength. The eluted compounds were detected in the range of 21-49 min. indicating polar and nonpolar compounds combination. The UV-Vis ranges of these compounds were in the range of 210-350 nm.

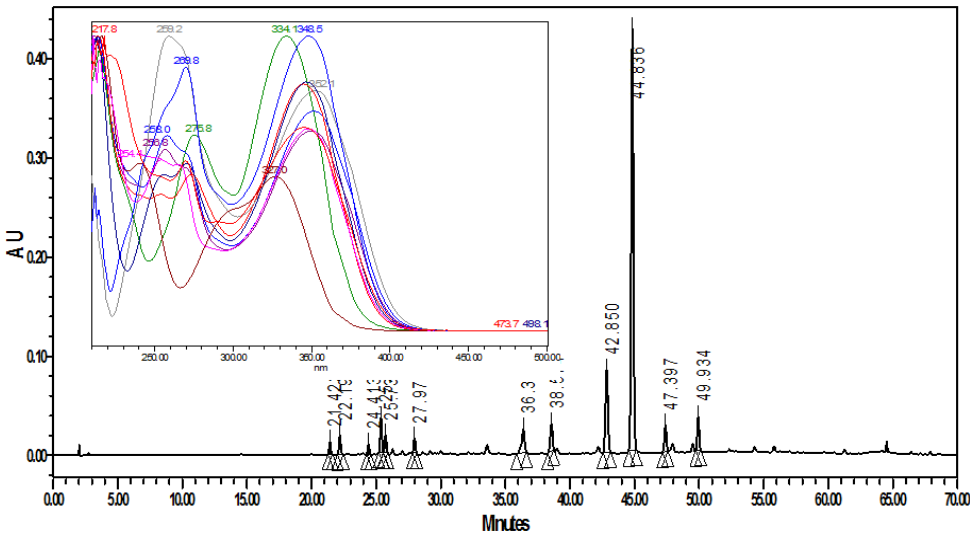

Fig. 3. HPLC-PDA chromatogram of crude extract of *Achillea fragrantisimma* at 350 nm, their overlaid UV-Vis spectra (left corner of the chromatogram)

Figure 4 shows the chromatogram of the crude extract of Asteriscus graveolens (Forssk) Less (Asteraceae) at 350 nm with two main peaks (eluted at 33.4 and 38.9 min.) and other six peaks showed a maximum absorption at this wavelength. The eluted compounds were detected in the range of 14-55 min. indicating polar and nonpolar compounds combination. The UV-Vis ranges of these compounds were in the range of 210-350 nm.

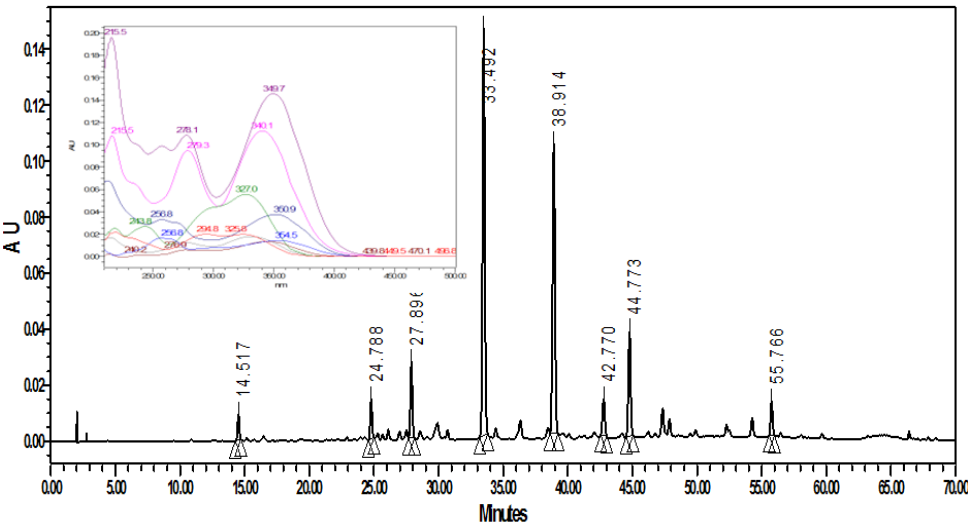

Fig. 4. HPLC-PDA chromatogram of crude extract of *Asteriscus graveolensat* 350 nm, their overlaid UV-Vis spectra (left corner of the chromatogram)

Antibacterial activity of the plant extracts

The tested crude extracts, except that of A. santolina showed weak to moderate antibacterial activity against the Gram positive B. subtilis (Table 2). The inhibition zones caused by applied extracts were 10-15 mm at 1.5 mg/disc. Interestingly, A. graveolens inhibited the growth of all tested bacterial strains with MIC 0.5-1 mg/ml. Meanwhile; A. fragrantissima inhibited the growth of B. subtilis at MIC 0.25 mg/ml (Table 3).

Numerous studies described the various pharmacological effects of Achillea extracts in management of several diseases. Among its species, A. fragrantissima and A. santolina were reported to contain metabolites with various bioactivities including antibacterial potentials[22]. These activates were attributed to broad ranges of secondary metabolites such as flavonoids, phenolic acids, cumarines, terpenoids and sterols.

In accordance to our finding, the antibacterial effect of hydro-alcoholic and aqueous extracts of A. fragrantissma was reported against Gram positive bacteria with pronounced resistance among *Gram negative* ones[23] however, its phenolic extract showed weak effect against Pseudomonas mirabilis with MIC 25 mg/ml[24]. Moreover, its essential oil extract exerted a bactericidal effect on both *Gram positive* and negative bacteria as well as on the yeast Candida albicans[25, 26].

Although *A. santolina* crude extract was devoid of antibacterial potential, however, it was reported previously that it was active against some species of *Gram positive* and *Gram negative* bacteria with MIC 40-60 mg/ml[27] indicating that it might be active at concentrations at least 40 fold than that applied in this study. Furthermore, the activity of *A. graveolens* against both *Gram positive* and *Gram negative* tested bacterial strains reported herein coincided with documented activity of the same plant species collected from Algeria[28]. Therein, the ethyl acetate crude extract of *A. graveolens* revealed weak to moderate activity against *Gram positive* bacterial and was inactive to *Gram negative* bacteria with MIC 312-625 µg/ml and 1 mg/ml, respectively.

Chemical analysis of ethyl acetate crude extracts of these three plants revealed presence of phenolic compounds and flavonoids in addition to mixtures of nonpolar and polar metabolites. Presence of these metabolites in association of tannins and terpenoids confer antimicrobial and antioxidant activities for such extracts. Intriguingly, flavonoids are able to form complexes with biological molecules and microbial cell wall and may deteriorate their role, thereby causing microbial growth inhibition[29, 22, 28].

Table 2: Antibacterial activity of plants crude extract against susceptible bacterial strains in agar diffusion test

Plant name	Inhibition zone (mm ± SD[a]) 1/1.5 (mg/disc)		
	B. subtilis	*E. coli*	*S. aureus*
A. santolina	NA[b]	NA	NA
A. fragrantissima	13.7 ± 1.5/14.7 ± 0.6	NA	NA
A. graveolens	8.7 ± 1.2/ 10.7 ± 0.6	7.7 ± 0.6/ 11.7 ± 0.6	9.3 ± 0.6/ 11.3 ± 1.2

a) Standard deviation, b) NA: Inactive n

Table 3: Minimal inhibitory concentration of plants crude extract against susceptible bacteria

Plant name	MIC (mg/ml)		
	B. subtilis	E. coli	S. aureus
A. fragrantissima	0.25c	> 2	> 2
A. graveolens	0.5s	1s	0.5c
Penicillin G	0.00016c	> 0.01	0.00032c

c: bacteriocidal /s: bacteriostatic

CONCLUSION

The extract from *A. graveolens* exhibited a significant *in-vitro* antibacterial activity of all tested bacterial strains with MIC 0.5-1 mg/ml that can be an important source of natural antibacterial. This bioactivity is quite significant and could present alternative treatments for many infections. The extracts of the three plants are rich with phenolic and flavonoids.

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