ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 3, Issue 5 , May 2016

Chemical Investigation and Biological Activity of Cultivated ArtemisiaJordanica

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ABSTRACT: Artemisia species possess pharmacological properties that are used for medical purposes worldwide. In this paper, the essential oils from the aerial parts of cultivated ArtemisiaJordanica, fromthe southern Jordan desert. A number of analytical methods have been developed for detection and quantification of artemisinin, e.g., thin layer chromatography (TLC) TLC with visible light densitometric detection, high-performance liquid chromatography with UV detection (HPLC–UV), HPLC with electrochemical detection (HPLC–ECD), The main compounds analyzed werepulegone (21.60%) as a major component followed by isomenthone (18.57%), Piperitoneoxid (8.77%), Pulespenone (7.65%), and piperitenone oxide (6.30 %). Additionally, the oils were evaluated for their antibacterial and antifungal activities. The essential oil demonstrated nonselective antifungal activity against plant pathogens. Antibacterial effects assessed by an agar dilution assay demonstrated greater activity of the essential oil against Staphylococcus aureus and Pseudomonas aeruginosa.Itisfound that the active antimalarial compound obtained from the leaves of *Artemisia.*Antioxidant capacities of the plants were also tested. Free radical scavenging activity assay (FRSA): DPPH-radical scavenging activity was tested**.**

I- INTRODUCTION

Artemisia, one of the larger genera in the family Asteraceae and the largest genus in the tribe [1] Anthemideae, comprises from 200 to more than 500 taxa at the specific or sub-specific level. Many *Artemisia*species have a high economic value in several fields [2], as food plants[3] and as antihelminthic and antimalarial inMedicine[4-6]. *Artemisia herba-alba* was known for its therapeutic and medicinal properties, it was used in both traditional and modern medicine [7-8].

Artemisia is centered in and most likely originated from Central Asia [9]. It was well documented in literature that Artemisiaspecies have been used since ancient times for food and medicinal purposes [10]. The genus Artemisiahas been the subject of numerous chemical and biological studies, yielding primarily sesquiterpene lactones [11],diterpenes, coumarins [12], polyacetylenes [13] and flavonoids [14] as the main metabolites.Biological activity of Artemisia Species includes antitumor [15-16], antimalarial [17-18], antibacterial [19, 20], antifungal [21, 22], antimutagenic [22, 23],repellent and antifeedant [24]. Pharmacological studies on A. argyiproved terpinen-4-ol and β-caryophyllene as the antiasthmatic principles of the oil [25]. In Traditional Chinese Medicine, A. argyiis used as raw material and processed into moxa wool [26]. This plant is also identified as “Aeyup” and used as important medicinal material in traditional Korean medicine [27].There are several reports on antioxidant activity of Artemisia oils [28]. There has been a growing interest in research concerning natural antioxidant active compounds, including the plant extracts and essential oils that are less damaging to the mammalian health and environment. Antioxidants retard oxidation and are often added to numerous meat and poultry products to prevent or slow oxidative degradation of fats. Freeradical scavenging, chelating of pro-oxidant metal ions or quenching singlet-oxygen formation mechanisms are involved in antioxidant action of natural antioxidants [28]. The present work is focus on the chemical composition and biological activity of Artemisiaoils from the Southern Jordanian desert.

II . MATERIALS AND METHODS

A. **Plant Materials**

Plant materials were collected from South Jordanian Valley, and cultivated at home

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
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B. Preparation of Extracts

Approximately, 5 g of plant sample was weighedaccurately and macerated with 250 mL of n-hexane at room temperature for 2 days using a laboratory-scale shaker. Then, the n-hexane phases were filtrated and evaporated under vacuum until dryness.The residue was dissolved again in 100 mL of n-hexane and the n-hexane phase was washed in a separatory funnel with 2 % NaOH solution to get rid of the impurity, which is soluble in NaOH. After abandoning the alkali solution present in the lower layer, the upper solution was washed with distilled water several times until it was neutralized.The extract, obtained after distillation under vacuum at 45 ºC in rotary evaporator, was dissolved with 95% ethanol and then filtrated in 250 mL measuring flask. Then, 10 mL of filter liquor was transferredinto a 100 mL measuring flask. 40 mL of 0.2 % NaOH solution was added in the flask, and then, letit react at 50 ºC for 30 min. After that, 0.08 mol/L acetic acid solution was filled up to the mark [27].The procedure described herein was applied to all of the samples.

C-HPLC Analysis

The analytical HPLC system employed consisted of a JASCO high performance liquid chromatography coupled with a diode arraydetector (MD910 JASCO, Tokyo, Japan). The analytical Data were evaluated using a JASCO data processing system (DP-L910/V). The separation was achieved on a Waters Spherisorb5µm ODS2 4.6 ×250mm column (Milford, MA, USA)at ambient temperature. The mobile phase consisted of water with 1%glacial acetic acid (solvent A), water with 6% glacial acetic acid (solvent B), and water-acetonitrile (65:30 v/v) with 5% glacial acetic acid (solvent C). The gradient used was similar to that used for the determination of phenolics in wine [7] with some modifications: The flow rate was 0.5 mL/min and the injection volume was 10 µL. The monitoring wavelength was 233. The identification of each compound was based on a combination of retention time.(A mobile phase consisting of formic acid (% 0.2 v/v): acetonitrile (50:50) by isocratic elution was chosen to achieve maximum separation and sensitivity. Flow rate was 1.0 mL/min. Column temperature was set at 30 °C. The samples were detected at254 nm using photodiode array detector. Results of artemisinin quantities in Artemisia samples were expressed as the mean of three determinations.

D.Antimicrobial activity

Minimum Inhibitory Concentration (MIC) tests: The samples were tested for their antimicrobial testing in vitro by the agar dilution technique. All samples were dissolved in Dimethyl Sulphoxide Solvent (DMSO) for the antimicrobial test and the solutions were sterilized by membrane filtration. Aliquots of samples were diluted with melted typtic Soy agar, tryptone, soytone, sodium chloride and agar to give concentrations of 2000, 1500, 1000, 500, 250, 125, 62.5 and 31.3: g/mL.
The essential oil was tested against microorganisms including *E. coli*,*S. aureus*,and*P.aeruginosa* Bacterial strains were cultured overnight in Nutrient Broth (NB) at 37°C, with the exception of *C.albicans*(30°C).

E. Antioxidant activity

DPPH assay

Hydrogen atoms or electrons donation ability of thecorresponding oils was measured from the bleaching ofpurple colored methanol solution of DPPH. Thisspectrophotometric assay uses stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) as a reagent [8][9]. Fifty microliter of the oil inmethanol was added to 5 ml of a 0.004% methanol solutionof DPPH. After a 30 min incubation period at roomtemperature the absorbance was read against a blank at 517nm. The same procedure was repeated with the ascorbicacid as positive controls. Inhibition free radical DPPH inpercent (I%) was calculated in following way:*I%* = (*A*blank –*A*sample/*A*blank) X 100where *A*blank is the absorbance of the control reaction(containing all reagents except the test compound), and*A*sample is the absorbance of the test compound.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

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III. RESULTS AND ANALYSIS

A.Essential oil analysis

Essential oils extracted from leaves ofArtemisia. Fresh and dry leaves analysis revealed that pulegone (21.60%) as a major component followed by isomenthone (18.57%), Piperitoneoxid (8.77%), Pulespenone (7.65%), and piperitenone oxide (6.30 %). Table (1), and Figure(1 and 2)The constituents of each oil were identified by comparing their retention times (RT) relative to n-alkanes, computer matchingwith the Wiley library, and confirmed by comparing their massspectra with those of authentic samples or with data alreadyavailable in the literature.

Table 1. Chemical composition of the of *Artemisia* Extraction

Compounds	RT (min.)	%
isomenthone	10	18.57
Pulegone	21.5	21.60
Piperitenone oxide	27	8.77
Piperitone oxide	30	6.30
Pulespenone	32	7.65

B- Antimicrobial activity

The antimicrobial activities ofessential oil against microorganisms were examined by thepresence or absence of inhibition zones and zone diameter.Asshown in Table 2.

Table 2.Antimicrobial activity of the essential oil ofArtemisiaJordanica(µg/ml)

Escherichia coli (E. coli)	62.5
Bacillus cereus (B.cereus)	125
Candida albicans	31.5
Staphylococcus aurous	62.5

C-Antioxidant activity

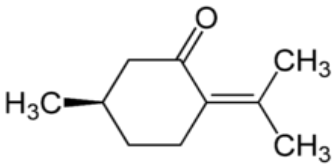
The DPPH radical scavenging method was used toevaluate the antioxidant properties ofArtemisiaJordanicacomparison with those of known natural andsynthetic antioxidants, ascorbic acid. Free radicalscavenging capacities of the tested oil rose with increasingoil concentration and oil concentrations providing 50%inhibition (IC50) as shown in Table 4. According to the results obtained from the study, the highestradical scavenging activity was observed. Thefree radical scavenging activity of the essential oils ofArtemisiaJordanica is more effective than those of *ascorbic acid*.The present study confirmed theantioxidant activity of ArtemisiaJordanicadue toamount of phenolic contents.

Table 3.Antioxidant activity of DPPH antioxidants and extracted oil of ArtemisiaJordanica

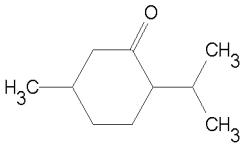
Samples	DPPH IC_{50} (µg/ml)
Ascorbic Acid	45.43
ArtemisiaJordanica	60.41

Figure1- Structure of extracted compounds.

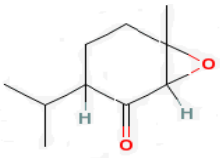
ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 3, Issue 5 , May 2016



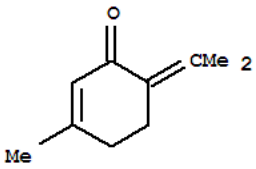
1- pulegone



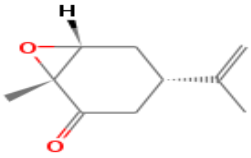
2- Isomenthone



3- Piperitoneoxid



4- Pulespenone



5- - piperitenone oxide

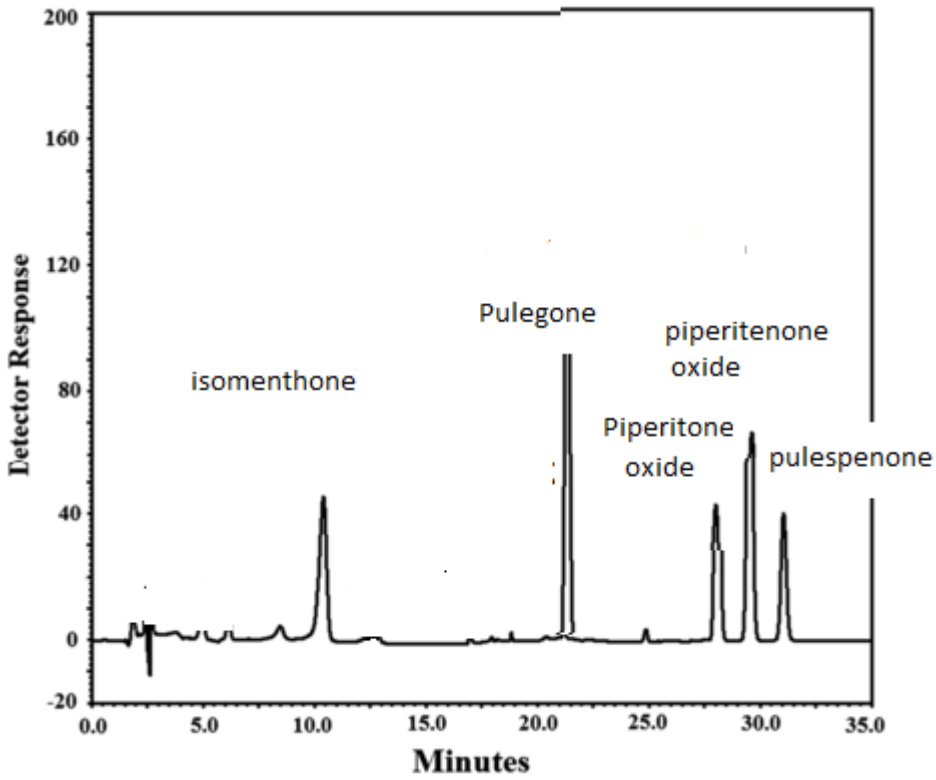


Figure 2. Chromatogram of isolated compounds of Artemisia

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

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IV - CONCLUSION

Despite the prevalence and preference of the modern medical community for single-ingredient drugs, there are examples that illustrate the often ignored benefits of using complex botanical drugs vs. pure ones. In conclusion, the present work was a study into chemistry and biological activity of Jordanian Artemisia from Jordanian desert. Oxygenated mono- and sesquiterpenes. We further proposed a simple method for insuring a controlled dose of artemisinin via in plant a delivery that when combined with the simple methods for stimulating increases of the drug while the crop is in the field, may provide significant relief to the shortage of low cost artemisinin available for use to treat malaria and other neglected diseases in developing countries.

ACNOWLEDGEMENT

The author acknowledges Applied Science Private University, Amman, Jordan, for the fully financial support granted of this research article. Sincere thanks to all my Colleagues at basic science department, for creating inspiring conditions for work. The author acknowledges Mr. MazinGannam at Pharma International Company for HPLC analysis.

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International Journal of Advanced Research in Science, Engineering and Technology

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