

THE ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ETHANOLIC EXTRACT FROM *ARTEMISIA HERBA ALBA* GROWN WESTERN IRAQ

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ABSTRACT:

Nowadays, medicinal plants used in folk medicine are increasingly studied and used on pharmaceutical, food and nutraceutical fields, wormwood *Artemisia herba -alba* is one of traditional medical characterized by scientists of preservation its wealth in different composition which led to the definition of several chemotypes the main objectives of this work were to evaluate the antioxidant activity of aerial part of wormwood, the plant had been refractive index of the order of 0.911 and 1.66. The qualitative and quantitative phytochemicals detected were alkaloids, tannis, saponin, flavonoids, phenolic compounds and carbohydrates in moderate amount (12.8, 9.42, 10.65, 6.8 and 11.5) respectively. Antibacterial activities of ethanolic extract the weeds was determined against clinical isolates of *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia Coli*, *Pseudomonas aeruginosa*, the results revealed a potential antibacterial activity against pathogenic bacteria, The activity of ethanolic extract had been highest an Minimal inhibitory concentration of 50 mg ml⁻¹ against *Staphylococcus aureus* had 4.7 cfu ml⁻¹, and lowest in *E.coli* had 2.8 cfu ml⁻¹. The results it could be extracted that wormwood possessed an interesting effects and had been promising use as a natural source of antioxidant and antibacterial agents.

الفعالية المضادة للاكسدة والبكتريا للمستخلص الكحولي للشيح *Artemisia herba-alba* النامي غرب العراق

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الكلمات المفتاحية: الشيح ، ومضادات الأكسدة ، ومضاد للبكتيريا ، مستخلص الايثانول

المستخلص:

في الوقت الحاضر ، تتم دراسة النباتات الطبية المستخدمة في الطب الشعبي بشكل متزايد واستخدامها في مجالات الصيدلة والغذاء والمغذيات ، والأرتميشيا *erba-alba* هي واحدة من الطب التقليدي الذي يتميز به علماء الحفاظ على ثروتها في تركيبة مختلفة مما أدى إلى تعريف العديد من القوالب الكيميائية التي كانت الأهداف الرئيسية لهذا العمل هي تقييم النشاط المضاد للاكسدة في الجزء الجوي من الشيح ، وكان النبات قد انخفض معامل الانكسار من 0.911 و 1.66. كانت المواد الكيميائية النباتية النوعية والكمية المكتشفة هي القلويات ، التانيس ، السابونين ، الفلافونويد ، المركبات الفينولية والكربوهيدرات في كمية معتدلة (12.8 ، 9.42 ، 10.65 ، 6.8 و 11.5) على التوالي. تم تحديد الأنشطة المضادة للجراثيم من استخراج الإيثانول الأعشاب ضد العقاقير السريرية من المكورات العنقودية الذهبية ، *Proteus* الشائع ، *Escherichia* كولي ، *Pseudomonas aeruginosa* ، كشفت النتائج وجود نشاط مضاد للجراثيم محتمل ضد البكتيريا المسببة للأمراض ، وكان نشاط المستخلص الإيثانولي أعلى تركيز مثبط الحد الأدنى من 50 ملغ مغلف العنقوديات المذهبة كان 4.7 cfu ml⁻¹ ، وأدنى في *E.coli* كان 2.8 cfu ml⁻¹. النتائج التي يمكن استخلاصها أن الشيح تمتلك آثار مثيرة للاهتمام وكان الاستخدام واعد كمصدر طبيعي للعوامل المضادة للاكسدة ومضاد للبكتيريا.

INTRODUCTION:

Iraq is possessed various aromatic wild flora that is used as herbals. Over the past few years, several research groups have focused on the chemical characterization and the biological activities of natural products (Hirasa and Takemasa, 1998; Tenore *et al.*, 2011; Marangoni and Maura, 2011). The plant and other substances of natural origin have being used throughout the world for human and animal health care from time immemorial, wormwood From the family *Asteraceae*. Growing in arid and semi-arid climates. It is characteristic in deserts of the Middle East And North Africa and steppes which commonly called (Chih)(Mighri *et al.*, 2010; Zouari *et al.*, 2010) wormwood one of folkloric medicins traditionally been used in the treatment of diabetes, bronchitis, diarrhea, neuralgias and hypertension (Awad *et al.*, 2012). The plant is reported to possess hypoglycemic (Khlifi *et al.*, 2013), anticancer (Jaouadi *et al.*, 2014), antiangiogenic (Nia *et al.*, 2015), insectisidal (Zeggwagh *et al.*, 2014), hypotensive and diuretic (Abu-Darwish *et al.*, 2015), anti-inflammatory (Khalaf *et al.*, 2015), large number of medicinal plants species have highest antioxidant activity, including *Ocimum sanctum*, *Piper cubeba* Linn., *Allium sativum* Linn., *C. sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plant species (Alans *et al.*, 2005) thus, this study was applied to determine some chemical composition the antioxidant and antibacterial activity of the ethanolic extract of leaves of wormwood.

MATERIALS AND METHODS

Plant material

A plant is a greenish-silver perennial dwarf shrub, perennial shrub that forms clumps of 30 to 70 cm, white and woolly, stem numerous and tomentose. The leaves

are short, usually pubescent with silvery flower heads with 2-5 sessile or sub – sessile flowers. These latter are hermaphroditic, while the fruit is an achene. The receptacle is naked and the corolla is inserted very obliquely on the ovary" (Benjilali *et al.*, 1980; Dahmani-Hamzaoui and Baaliouamer, 2005)

The plant aerial part of wormwood collected from desert of western Iraq during flowering period in February 2012, After war the samples were dried under shade paper towel in lab and then homogenized into fine powder using a mortar and pestle then stored in air tight bottles and were used for all the extraction processes (Sofowore, 2002).

Source of organisms

The microbial strains are standard which were obtained from Microbiology lab of children and women hospital of Erbil. The studied bacterial strains are *Proteus vulgaris*, *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The cultures were grown on Tryptone soya broth 37 °C for 24 hours in the test tube in an incubator.

Phytochemical analysis

The presence of some chemical compounds in the pulverized samples of plant were determined using standard methods such as (tanins, flavonoids, saponin, carbohydrate, resin, alkaloids and phenolic compounds (Evans, 2002; Omoregie *et al.*, 2010).

The biological activities

Ethanolic extract was prepared by macerated 200g of pulverized plant in ethanol alcohol (90%), and complete the extraction by Soxhlet apparatus, the ethanol concentrate was evaporated to dryness in hot air. the extract were filtered. The supernatant was then collected and

kept in dark sample bottles until used (Imelouane *et al.*, 2011).

Minimum inhibitory concentration (MIC)

The lowest concentration of alcohol extract which observation a visible colony of bacterial growth after incubation at 37°C for 24 h.(Abu-Darwish *et al.*, 2015). Antibacterial activities of alcohol extract of wormwood was used against *aureus*, *subtilis*, *fluorescens* and *Escherichia coli*. The technique used in our study is the whole assay as described in (Ayoola *et al.*, 2008). Which is believed to test sensitivity of microbial strains to extract on a nutrient broth media then it was compared with antibiotic (Sparfloxacin, Erhthromycin and Flouconazole) had standard active concentration (2.5 mg ml⁻¹)

Determination of Antioxidant Activity:

The Antioxidant activity by DPPH (2,2-Diphenyl-1-picrylhydrazyl) test (Re *et al.*, 1999), the antioxidant capacity of the ethanolic extract of wormwood was tested by the method which uses the DPPH, like a relatively stable free radical. In this test, purple color of DPPH is reduced in to yellow compound, the diphenyl picrylhydrazine whose intensity of the color is inversely proportional to the reducing capacity of antioxidants present in extract. The reaction is occurred in a flask had 2 ml containing of 0.4 ml of DPPH solubilized in ethanol. For the test, the sample was prepared by dissolution in absolute ethanol (500 µg ml⁻¹). From this solution, different dilutions are made to have various concentrations about microgram per ml (30, 60, 125, 250 and 500 µg ml⁻¹). A white control is also carried out with absolute ethanol only. For each concentration, the test was replicated three times. The samples are then left in

dark for 25 minutes, and discoloration compared to the negative control containing only the solution of DPPH is measured at 517 nm. The antioxidant activity is estimated by following equation (Shukla *et al.*,2015):

$$AA \% = ([Abs \text{ control} - Abs \text{ test}] / Abs \text{ control}) \times 100$$

AA : antioxidant activity, Abs : absorbance at 517 nm

Determination of Ferric Reducing Antioxidant Power (FRAP Assay)

The antioxidant capacity was determined following the procedure described in (Akrouit *et al.*,2010), one milliliter of wormwood extract was added to 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of hexacyanoferrate of potassium [K₃Fe(CN)₆] (10 g l⁻¹), and warmed at 50°C for 20 min in water bath Then added 2.5ml of trichloroacetic acid (THA) (100 g ml⁻¹) to the mixture and centrifuged at 3000 rps. for ten minutes. lastly, 2,5ml of the supernatant were mixed with distilled water (2,5 ml) and 0.5 ml of ferric chloride (1g l⁻¹). The absorbance was measured in a spectrophotometer at 700 nm. A control was prepared with vitamin C (Ascorbic acid) and utilized as positive control (Re,1999).

RESULTS AND DISCUSSION:

Table(1) shows the results of quantitative screening of ethanolic extract of the aerial parts . It showed the moderate presence of bio-principle such as alkaloids ,tannins, flavonoids, Saponins, phenolic and carbohydrate. They are transformers which modify the body's reactions to carcinogens, viruses, and allergen. They show anticancer, anti-inflammatory, antimicrobial and anti-allergic activity (Martino *et al.*, 2013) and may be useful in therapeutic roles (Benjilali,1980). Alkaloids are organic compounds that

contain nitrogen, and are physiologically active with sedative and analgesic properties. They are used for relieving pains, anxiety and depression, Saponins are used in veterinary vaccines as adjuvant helping to enhance immune response. Further more, thus used as mild detergents and used commercially as well as for research (Bouzidi, 2016). More over they used in intracellular histo-chemistry staining to allow antibody access to intracellular proteins (Dob and Benabdelkader, 2006). Active materials such as tannins which isolated from (shih) exhibited inhibitory effect on some positive and negative gram bacteria (Koekkoek and Zanten, 2016), the tannins were resulting in the inhibition of all protein synthesis, thus possessing both antimicrobial and antioxidant activities, alkaloids and phenolic compounds have been extensively used as antiseptic and disinfection materials, this chemical constituents were served as supplements to be potential source of useful pharmaceutical drugs (Margaritelis, 2016).

Table 1 Phytochemical screening of crude ethanolic extract of *A.herba alba*

Amount %	Phytochemical constituents
Tannins	12.18
Flavonoids	10.65
Saponins	9.42
Carbohydrates	11.5
Resin	4.6
Alkaloids	13.6
Phenolic compounds	6.8

Table (2) showed the antibacterial effects of ethanolic extract of aerial plant parts against the pathogenic bacteria, the concentration 50 mg ml⁻¹ had more potent against the tested bacteria, this could be because the active compounds are more soluble in polar solvent (ethanol) this was also notice by (Takruri *et al.*, 2008; Abu-Darwish *et al.*, 2015; Lakehal *et al.*, 2016).

The common use of plant materials as a curative agent against diseases remain an important part of Iraqi people and over 25% of the population in west depends directly on it for health care the phytochemical screening of the ethanolic extract showed a moderate presence of bio-principle like alkaloids, tannins, saponin, carbohydrate, flavonoids. this was in agreement with the work of (Janačković *et al.*, 2015) and (Zohra *et al.*, 2015), in leaves of wild plant grows in northern badia of Jordan have rich of vitamins and proximate composition such as (wormwood) which important source of some micronutrient and antimicrobial (Akrouit *et al.*, 2010).

Table 2: Antibacterial activity of ethanolic extract. (Minimum Inhibitory Concentration (MIC) measured (cfu ml⁻¹))

Tested bacteria	MIC(mg ml ⁻¹)			Antibiotic		
	25	30	50	Sp	Er	Fl
<i>Staphylococcus aureus</i>	2.4	3.4	4.7	3.9	2.7	0.2
<i>Proteus vulgaris</i>	2.2	3.5	3.2	2.9	3.2	0
<i>Escherichia Coli</i>	2.8	1.2	1.5	2.2	3.6	1.4
<i>Pseudomonas aeruginosa</i>	2.9	3.7	4.3	4.6	4.1	3.3

Sp=Sparfloxacin, Er= Erthromycin, Fl= Flouconazole, LSD = 1.31

Antioxidant activity:

The antioxidant activity of ethanolic extract of *A. herba alba* was tested in by comparing it to the activity antioxidants like ascorbic acid by the two in vitro analysis; inhibition of DPPH agent and the ferric reducing antioxidant power (FRAP) The result in (Figure 1) revealed a best linearity to the ethanol extract of wormwood to ascorbic acid. In this method, showed that ($r^2 = 0.855$) than the positive control ($r^2 = 0.6530$). This may indicate that the reducing effect of the of wormwood is more correlated with its

concentration, its effect is direct and does not exhibit phase affected by the mass effect, However, natural chemical compound, and has been submitted as antioxidant potential [(Bouzidi *et al.*, 2016; Margaritelis, 2016). Oxidative injury now appears as the fundamental mechanism causing number of human neurologic and other disorders such as autoimmune pathologies, inflammation, viral infections and digestive system disorders including gastrointestinal inflammation and ulcer and the Significant antibacterial and antioxidant activity may be due to the useful effect of these compounds. Natural compounds are best for the treatment because of their low toxic, and with out

side effects which lead the patient to serious complications. All results are reported in Figure 1, 2 showed that wormwood was able to reduce the stable free radical DPPH $2.60\mu\text{g ml}^{-1}$, while the ethanolic extract of wormwood used consists of several natural active substances must have this antioxidant capacity. If we consider that it is the camphor which has antioxidant power in we used contents essential oil, vitamins, different chemical materials and it is the majority compound. This result still encourages us to give more important natural substances in the field of additives (Margaritelis, 2016, Bouzidi *et al.*, 2016).

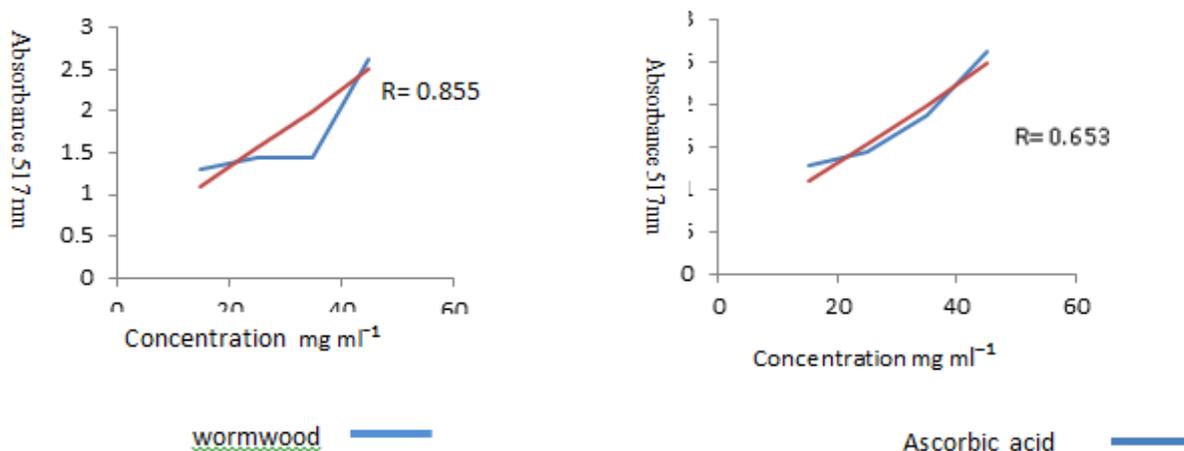


Figure1: ferric reducing antioxidant power (FRAP Assay) left ascorbic acid, right ethanol extract

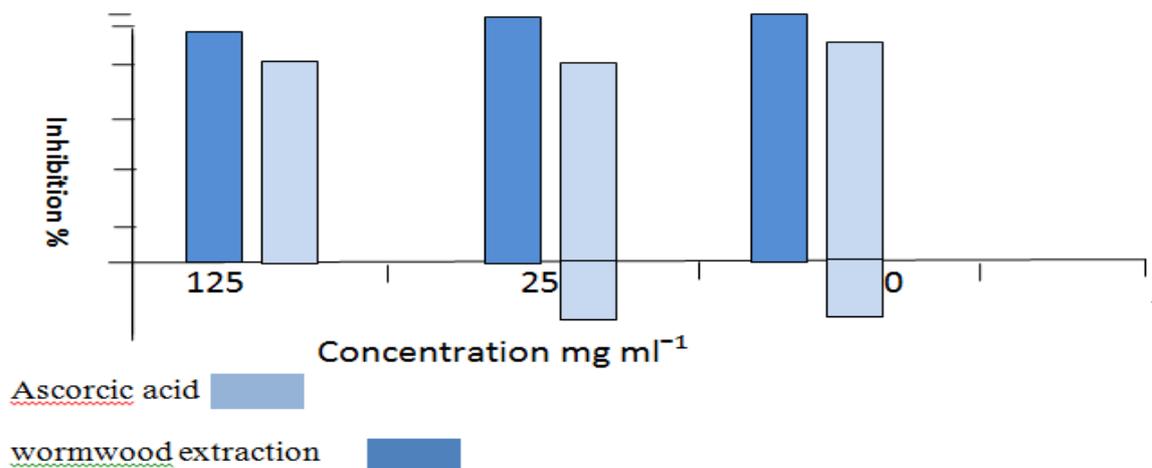


Figure 2:Test DPPH histogram, expressed as percentage inhibition the antioxidant activity.

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