

Antibacterial and anti-inflammatory activity of sesquiterpene lactones compounds extracted from *Artemisia Herba Alba* plant

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الفعالية المضادة للالتهابات والمضادة للبكتيريا لمركبات السسكوايترينين المستخلصة من نبات الشيح البلدي

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المستخلص

يعد الشيح نبات واسع الانتشار في العراق والنوع البلدي منه والمسمى *Artemisia herba alba* يستخدم كمضاد للطفيليات والبكتيريا وله فعالية ضد الالتهابات ,وتعود هذه الفعالية الى مركبات لاكتوناتالسسكوايترينين Sesquiterpene lactones والتي هي الناتج الاساسي لزيت الشيح . وقد تم في هذه الدراسة استخلاص هذه المركبات من نبات الشيح البلدي واختبار فعالية خمسة تراكيز نصفية منها (200,100,50,25,12.5) ملغم/ملم , تجاه خمسة انواع من الجراثيم التي عزلت من حالات مرضية , كان تأثير التركيز الاعلى 200ملغم/ملم معنوياً تجاه العزلة *Ps.aeruginosa* ,بينما كان غير معنوي تجاه الجراثيم *K.pneumoniae* ,*S.pneumoniae* ,*S.pyogenes* وذلك مقارنة بتأثير المضاد الحيوي القياسي المستخدم على نمو الجراثيم اعلاه. وعند دراسة تأثير مركبات لاكتوناتالسسكوايترينين المضاد للالتهابات المحدثة بالكاراجينان في اقدام الفئران المختبرية , وجد أنها ذات فعالية معنوية ($P<0.05$) في خفض الالتهاب الناتج عن حقن مادة الكاراجينان ولكنها ليست بتلك الكفاءة التي ابداهها استخدام المضاد الحيوي الاسبرين في خفض التهاب اخمص القدم الخلفية للفئران المختبرية الناتج من حقن مادة الكاراجينان .

Abstract

The *Artemisia Herba Alba* plant, is widespread in Iraq and its municipal kind called *Artemisia herba alba* is used as anti-parasites and anti-bacterial agent, it also has effective against different kinds of infections, this activity returns to Sesquiterpene lactone compounds, which are the main product of the *Artemisia Herba Alba* oil. In this study, these compounds were extracted from the *Artemisia Herba Alb* plant. The effectiveness of five one-half concentrations (200,100,50,25,12.5) mg / ml, against five species of pathogenic bacterial isolates from urinary tract infections (UTI) cases were tested , The activity of Sesquiterpene lactone compounds was significant ($P<0.05$) for highest concentration of 200 mg /ml against *P. aeruginosa*, while the activity was not significantly against *K. pneumoniae* ,*S. pneumoniae* ,*S. pyogenes* when it is compared with standard antibiotic which is used against these pathogenic bacteria. In this study the anti-inflammatory activity of Sesquiterpene lactone compounds which induced by injection of carrageenan at the feet of laboratory mice,

we found that these compounds was significantly ($P < 0.05$) more active in reducing the inflammation caused by the injection of carrageenan but not those efficiency shown by the use of anti-inflammatory aspirin to reduce inflammation to the laboratory mice resulting from the injection of carrageenan.

Introduction

Many of the researchers in Iraq had been concerned with plant extracts and study their activity on microorganisms and other live animals, including the pharmaceutical effects of on animals. As interesting the World Health Organization in herbal medicine has started since 1978, and this organization has adopted two conditions in medicinal herbs ; the efficacy and safety. (Jawad, 1999).

The municipal plant *Artemisia Herba alba* which is called in Arabic language; Shih. It Has been used in the field of traditional medicine of many cultures since ancient times, was used traditional Moroccan medicine for the treatment of hypertension and diabetes (Al Zeggwagh et al., 2008), In French language it called; Armoise Blanche and it wide spread in north Africa, Egypt and the Middle East (Salehand et al., 2006; Seddieket al., 2007; Mobarak et al., 2008; Mohamed et al., 2010).

The infusions of *Artemisia Herba alba* teas added to it a soap was used since ancient times through the anus to clean the intestines for its antiseptic properties (Alsnava, 2004) , also it considered as antipyretic, jet expectorant, anthelmintic and it is useful for wounds (Majeed et al., 1988). It also has other important uses; treatment of bacterial infections, anti-colic, and to stoop the of hemorrhage (Laid et al., 2008). There are many kinds of this plant is commonly used for the treatment of various diseases such as hepatitis, cancer, infections and injuries caused by bacteria, fungi and viruses (Mohamed et al., 2010). The plant has demonstrated the effectiveness of an antioxidant (Djeridane et al., 2006). The aqueous extracts of this plant has high efficiency as an antidote against different venom bites (Mohamedet al., 2010).

The Sesquiterpene lactones compounds can also give some neurological effects for its high affinity to gamma amino butric acid (GABA) receptor (Salah and Jager, 2005). The plant has high value pharmaceutical in several areas: as a food source (Benmansur et al., 1990; Fenardji et al., 1994; Benmansour and Taleb-Bendiab; 1998), an anti-Coccidian (Naidoo et al., 2008; Cacho del et al., 2010) and anti-malarial (WHO, 2005; Ene et al., 2009). This plant also consider as important element in The European pharmacopoeia from early of nineteenth century (Mohamed et al., 2010). The anti-parasitic activity of this plant return to its contain of Sesquiterpene lactones compounds and santonin (Rachkovskaia ,1978 ; Akhtar et al., 1982).

Through a survey of medicinal plants in the Sinai desert found that *Artemisia* effects removing stomach disorders (Friedman et al., 1986). In addition to its other known benefits in lowering blood sugar (hypoglycemic effect) and it useful in the treatment of diabetes (Al-Waili, 1988; Marrif et al., 1995).

The Sesquiterpene lactones compounds considered among the most important and best compounds which can be obtained from *Artemisia* which give its medical and pharmaceutical important, where there are various types of Sesquiterpene lactones compounds especially in the upper parts of the plant (leaves) (Mohamed et al., 2010). These compounds are colorless, bad taste and from side of the pharmacological effectiveness they are; anti-tumor, anti-tumers and cytotoxic activity (Rodrigues et al., 1976).

The santonin compound (kind of consider article also Alsantonin Santonine (kind of Sesquiterpene lactones compounds) which isolated from *Artemisia herba alba* as a little crystalline substance, soluble in water and takes yellow color when exposed to light, when increasing its exposure to light, it convert to brown resinous substance (Qutb, 1981).

When bad taste Sesquiterpene lactones compounds given orally, it reversely increases the gastric secretion, especially HCl acid secretion so the plant used to strengthen the appetite and treatment of disorders of the stomach , the liver and bile disorder. (Alsnava, 2004)



Figure (1):(Mohamed *et al.*, 2010) Municipal plant *Artemisia herba-alba*

The current study aiming the following targets

- 1- Isolate and diagnose five types of pathogenic bacterial.
- 2- Extraction and isolate for Sesquiterpene lactones compounds from Municipal *Artemisia herba alba* plant.
- 3- To study the antibacterial activity of Sesquiterpene lactones compounds against five types of isolated pathogenic bacteria.
- 4- Study the anti-inflammatory properties of Sesquiterpene lactones compounds extracted from *Artemisia herba alba*.

Materials and methods

Bacterial isolates

The bacterial isolates were obtained from patients suffering from urinary tract infections (UTI) coming to the general Tikrit Hospital, they were diagnosed following microbial species depending on the morphological qualities after dye prescriptions colonies and biochemical activities

Pseudomonas aeruginosa

Sreptococcus pneumoniae

Sreptococcus pyogenes

Klebsiella pneumoniae

Staphlococcus aureus

Extraction of sesquiterpene lactones compounds

About 100 g of *Artemisia herba alba* plant leaves were grinded well, then the extraction of leaves powder by using 500 ml of chloroform for 90 minutes and this process repeated for three times with shaking. Extract then filtrated and vaporization by a rotary evaporator under pressure in 35° until it dried, thereby the rest was taken brownish green color, which dissolved in 50 ml of ethanol (95%), and added to it the same amount of lead acetate, then let the mixture at room temperature for 30 minutes. The mixture was filtrated and then the extract was concentrated with a rotary evaporator to half of its volume, then extracted for three times using 100 ml of chloroform in each time, then pull water from the chloroform extract by adding anhydrous sodium sulfate. After filtration the extract, it drying in a rotary evaporator where it gave the rest of the oily structure-yellow Sesquiterpene lactones compounds (4.2g) (Benmansour, 1990).

Study of antibacterial activity of *Artemisia herba alba* extract on pathogenic bacteria

Serial of one-half concentrations from Plant extract (1205, 25, 50, 100, and 200) mg/ml. Distilled water was used in preparation of these concentrations. The antibacterial activity of extract against isolated bacteria was tested with agar well diffusion method. The Muller Hinton culture media was inoculated by using cotton swab (using spreading method technique) with bacterial broth (1.5×10^8 cell/ml), after that wells in 6 mm. in diameter in cultured media had been done (6 wells for every petri dish and four petri dish for every bacterial strain), 0.2 ml of each concentration of extract was dropped in five well, the sixth was filled with sterile distilled water (control well). Active antibiotic disc was used against each bacterial spp. (Cifotaxime

for *Ps.aeruginos* and Ampicillin for rest of pathogenic bacteria in the study). Culture media were incubated in 37° for 24 hours. The antibacterial activity was determined by measuring zone of inhibition area (Kavanagh, 1972; Grooveand Randall, 1995).

Study anti-inflammatory activity of Sesquiterpene lactones compounds extracted from *Artemisia herba alba* plant leaves

The method of Winter et al. (1962) was used to assess efficiency of anti-inflammatory for the studied samples by observing their ability to inhibit edema resulting from injection carrageenan in toe back (Khan et al., 2009; Arfan et al., 2010); tested samples and control samples was given orally for three groups of mice (10 mice per group), after that measured thickness of right toe rear using Vernier in times: 0 and 1 and 3 hours after injection carrageenan. Animals divided randomly to three groups, first group were given the extract of sesquiterpene lactones compounds in dose 80 mg / kg body weight and the second group was given aspirin in dose of 200 mg / kg of body weight, while the third group was given distilled water (control group). The dose volume in all animals was 0.2 ml / mouse (extract diluted with distilled water) and prepared to form suspensions by adding 0.5 g of Arabic gum per 5 ml of dilution water. After one hours of injection drugs, animals in toe right rear were injected with 0.05 ml of carrageenan which induced inflammations, after half an hour of injection carrageenan, the thickness of injected toe was calculated. The increase in thickness were calculated in the three group. The minimized effect of Sesquiterpene lactones compounds extract in occurrence of edema and ascites induced by carrageenan considering as an anti-inflammation activity.

Results

1- through the study of antibacterial activity of Sesquiterpene lactones compound extract from *Artemisia herba alba* on pathogenic germs we reached that concentration 200 mg/ml was the best concentrations in inhibition of microbial growth when it compared with the standard antibiotic Cefotaxime against *P. aeruginosa* bacteria, while the activity of Sesquiterpene lactones compounds in inhibition against bacteria: *K. pneumoniae*, *S. pneumoniae*, *S. pyogenes* and *S. aureus* at concentration of 200 mg/ml, and in other concentrations was lower than f the second standard antibiotic Ampicillin (Table 1).

Table (1): The averages of inhibition zones for different concentrations of sesquiterpene lactones compound extracted from *Artemisia herba alba* (mg/ml) measured in mm

Bacteria Spp.	Concentrations of Sesquiterpene lactones compound extracted from <i>Artemisia herba alba</i> (mg/ml)					Standard Antibiotics
	200	100	50	25	12.5	
	Zones of Inhibition Measured in mm.					
<i>Ps.aeruginosa</i>	20 ± 3	15 ± 2	8 ± 1	7 ± 1	0 ± 0	15 ± 2
<i>K.pneumoniae</i>	19 ± 2	8 ± 1	0 ± 0	0 ± 0	0 ± 0	21 ± 3
<i>S.pneumoniae</i>	18 ± 2	15 ± 2	11 ± 2	8 ± 1	2 ± 1	23 ± 3
<i>S.pyogenes</i>	19 ± 3	18 ± 3	15 ± 2	13 ± 2	5 ± 1	30 ± 4
<i>Staph.aureus</i>	21 ± 3	18 ± 3	17 ± 2	11 ± 2	7 ± 1	27 ± 3

- Standard antibiotic that used against *Ps.aeruginosa* was Cefataxime while Ampicillin used for other bacteria spp.

2-This study showed that Sesquiterpene lactones compound had a significant effective ($p < 0.05$) in reducing inflammation which represented by edema (swollen toe) that induced by carrageenan injection when it compared with aspirin and control (distilled water) groups (Table 2).

Table (2): The activity of Sesquiterpene lactones compounds extracted from *Artemisia herba alba* (mg/ml) in reducing of inflammation induced by carrageenan injection.

Groups	Thickness of increasing in right toe using Vernier (mm.)		Thickness of increasing in right toe using Vernier (mm.) after given extract, aspirin, and distilled water
	Before carrageenan injection	After 3 hours of carrageenan injection	
Control group	2.12 ± 0.46	3.86 ± 0.48	1.74 ± 0.42
Aspirin group 200 mg/ml	2.08 ± 0.52	3.55 ± 0.64	1.47 ± 0.52
Sesquiterpene lactones compounds group 80 mg/ml	2.09 ± 0.48	3.17 ± 0.58	1.08 ± 0.48

- (p<0.05) in compared with control group.

Discussion

Many studies had confirmed that Sesquiterpenes lactones are the main component of effective in many kinds of medicinal plants which are used for the treatment of inflammatory diseases, and in recent years the anti-cancer properties of many Sesquiterpenes lactones compounds attracted special attention for these compound (Ren et al., 2006).

The main reason for the effectiveness of the Sesquiterpenes lactones extracted from *Artemisia herba alba* return to its contain of effective compounds such as alphasantonin, beatasantonin and lipophilic flavonoid and thus the ability to stimulate the immune system and its defenses against bacterial growth by stimulating T-cell (Akhtar,1982), also a new studies have shown that Sesquiterpenes lactones compounds have a good effective a biological against germs. The results of this study also agree with researchers Cho (2002), who used these compounds which extracted from *Artemisia princeps* species and they found that it have clearly inhibition activity against intestinal pathogens like *Staph.aureus* and *Clostridia* while it not shown any side effects toward the existing normal bacteria which found naturally in the intestines and which confirms positive and effective role of these compounds when used as anti-microbial and thus is effective selectively. Results is agree with Zafra and Blazquenez (2006) who found that the Sesquiterpenes lactones compounds have an effective toward induced inflammation in laboratory animals.

Inflammation, is a series of dynamic processes that arise due to various factors, the changes that can arise from these factors include changes in the supply of blood and increased permeability of blood vessels accompanied by secretion inflammatory circles such as: Prostaglandins (PGs), Cyclooxygenases (COXs) and Lipoxygenases. There is a large number of herb that can be used in treat inflammation with different causes (Wiat , 2006). The genus *Asteraceae* consider one of the richest sources that can be depend on it get the inflammation inhibitors, especially Lipoxygenases where the Sesquiterpenes lactones compounds are of the most important of these factors (Tornhamre et al., 2001).The Lipoxygenases are compounds with numerous of activities, where activities can contribute to the completion of many reactions, including reactions of metabolism the toxic substances (Khan et al., 2009) . Lipoxygenases participate in metabolic presses of fatty acids and its secondary metabolites which is the factor responsible for inflammatory response in body (Rioux and Castonguay, 1988), so many of the inhibitory substance for Lipoxygenase compounds can be used as a source of anti-inflammatory drugs.

Carrageenan is best compound can use for induce inflammation for the purposes of the study anti-inflammatory effectiveness of various pharmaceutical substances on laboratory animals (Arfan et al., 2010). That the initial inflammatory symptoms arise as a result liberalization of inflammatory mediators, when symptoms edema that appeared later is the result of the presence lipoxygenases and prostaglandins and other inflammatory mediators like proteases and lysosomes (Khan et al.,2009) and this study result is agree with Araruna and Carlos (2010).

Aspirin is one of Non-Steroidal Anti-inflammatory Drugs (NSAIDs) and its mechanism of action depends on inhibition of cyclooxygenase and thus inhibits the synthesis of prostaglandins (Brunton et al., 2008).From it was mentioned in above can be said that the results showed superior activity for Sesquiterpenes lactones compounds due to being behave anti-inflammatory with multiple mechanisms of action in compared with aspirin. In another study conducted by Perez Gutierrez (2009) about the effectiveness of antibacterial compounds of Sesquiterpenes lactones extracted from *Vaucheria sessilis* , he found that this compounds have broad spectrum of antibacterial activity against gram positive and negative bacteria.Many of studies had been conducted about the antibacterial activities of Sesquiterpenes lactones compounds which extracted from different plants against gram positive bacterial and in less against gram negative. Sesquiterpenes lactones compounds like vernodaline, vernolide and Dihydroveranolide had activity against *Staphylococcus aureus* and *Bacillus subtilis* (gram positive) and against *Escherichia coli* and *Klebsiella pneumonia* (gram negative) (Chaturvwdlin, 2011), also Yashphe et al. (1979) found that *Artemisia herba alba* antibacterial activity against gram positive and negative bacteria like; *Streptococcus hemolyticus* , *Staphylococcus aureus* ,*Escherichia coli*, *Shigella sonnei* , *Salmonella typhosa*.

In another study found that *Artemisia herba alba* oils were effective against gram negative bacteria such as *Escherichia coli* and the *Pseudomonas aeruginosa* and on

gram positive such as *Staphylococcus aureus* (Sherif et al., 1987). As mentioned Charchari et al.,(1996) that *Artemisia herba alba* has a limited effect on gram negative bacteria, like family of enterobacteria, also extracts *Artemisia herba alba* have some of inhibitory effects in some of resistant strains of bacteria, especially *Staphylococcus aureus* (Darwish et al.,2002).The side group of the carbonyl conjugated with Sesquiterpenes lactones compounds has a strong activity which should be taken into consideration because the antibacterial mechanism of these compounds due to be have saturated ketone groups and non-ketone group (Djeddi et al., 2008).Cowan (1999) pointed in his review of the most important medicinal plants and its important compounds that possess anti-microbial characters that Sesquiterpenes lactones compounds one of the most important and highly active of the terpenes compounds which have antibacterial activity depend on its ability in destroyed of bacterial cell wall by its contain of lipophilic.

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