

Antimicrobial Activity of *Achillea falcata* Extracts Against some causative agents

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Abstract

The antibacterial and antifungal effects of aqueous and alcoholic extracts of *Achillea falcata* L. against different bacterial species (*Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Bacillus cereus*, *Bacillus subtilis* and fungal species (*Candida albicans*, *Aspergillus flavus* and *Aspergillus niger*) were studied. The results revealed that no activity of aqueous extract against both of G^{+Ve}, G^{-Ve} bacteria and fungi except weak activity against *E. coli* (8 mm of inhibition zone), while alcoholic extract showed varied activities against *E. coli*, *K. pneumonia*, *Pro. Vulgaris*, *E. faecalis*, *S. aureus*, *B. cereus*, *C. albicans* and *A. flavus* (24,25,12,12,22,10, 14,8) mm inhibition zones respectively except *P. aeruginosa*, *S.typhi*, *S. epidermidis*, *S. pyogenes* and *B. subtilis*. These results revealed that the alcoholic extract showed significant activity (at $P \leq 0.05$) comparable with aqueous extract. The minimum inhibitory concentration (MIC) values of alcoholic plant extract for bacteria were ranged between 110-250 $\mu\text{g/ml}$, while it was 250 $\mu\text{g/ml}$ and 1000 $\mu\text{g/ml}$ for both *C. albicans* and *A.flavus* respectively.

Key words: Gram positive and negative bacteria, Fungi, and *Achillea falcata* Extracts.

Introduction

The major global public health problem is increasing drug resistance in pathogenic microorganisms as well as the appearance of the desirable side effects of antibiotics and the emergence of previously uncommon infections . The search for new antibacterial compounds with improved activity to replace the antibiotics that have become inactive is a necessity. Traditional healers use many plants in all civilizations and cultures , hence plants have always played a key role in health care systems worldwide (1,2). Today research confirms

that herb boost the immune system by stimulating the production of fighting white blood cells (3). They have also tested for antiulcerogenic, antihelminthic, Hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal (4). The genus *Achillea* (Asteraceae) was named after the mythological Greek warrior. Achilles who used *Achillea* species for healing wounded-soldiers during the Trojan war. The genus *Achillea* comprises of ~85 species, most of which are endemic to Europe and the Middle East (5, 6). Herbalists praise *Achillea* as a medicinal herb that has been used in popular medicine for its antihemorrhagic, healing and analgesic properties (7). The aerial parts of *A. millefolium* L., a well-known species among the members of *Achillea*, are commonly used in European traditional medicine for the treatment of gastrointestinal disorders and hepatobiliary complaints, as well as for wound healing and skin inflammations (8), the phytochemical studies revealed that these may be due to presence of different constituents such as flavonoids (aglycones and glycosides), sesquiterpene lactones and essential oils (9).

Materials and Methods

Plant materials

The native *A. falcata* were collected during the flowering period in April to May 2011 from Blodan , Syria and identified by Dr. Abdul Hussein Al -Khayat , from Department of Biology, College of Education /Salahaddin University, Erbil - Iraq.

Preparation of plant extracts

Alcoholic and aqueous extracts were prepared by taking 100g of dried and finely ground aerial parts in 500ml (ethanol 80%, distilled water) respectively, boiled for 2h, and filtrated twice , then the extracts were dried under reduced pressure at 40°C. The extracts were weighed and stored at +4°C for further experiments (10).

Inoculums Preparation

Stock Inoculums suspensions were prepared as described by (11).

Tested Microorganisms

Tested microorganisms were included: *E. coli* , *K. pneumonia*, *Pro. vulgaris*, *P. aeruginosa*, *S. typhi*, *E. faecalis*, *S. aureus*, *S. pyogenes*, *S. epidermidis*, *S. pyogenes*, *B. cereus*, *B. subtilis*, *C. albicans* *A. flavus*, and *A. niger* . All microorganisms were clinically isolated and identified depending on morphological, cultural, and biochemical tests. Then each bacterial isolates were inoculated in Nutrient broth and incubated overnight at 37°C. These cultures were used as the initial Inoculums preparation of bacteria. While *C. albicans* was inoculated on the surface of Sabaroud dextrose agar (SDA), incubated overnight at 35°C. Three to five isolated colonies of similar colony morphology were

taken from this culture, and subcultured again on SDA for 48 hrs. These plates were used as the initial inoculum preparation. On the other hand, Spore formations of the tested isolate *A. flavus* were induced by growing the isolates on potato dextrose agar (PDA) slants at 35°C, two slants were prepared. At the end of incubation period 5ml of sterile saline containing 0.5% tween 80 was added to each tube and the surface was rubbed with a sterile loop to dislodge conidia from the hyphal mat. The collected spore suspension was vortexed for 15 min to 20s. The suspension then filtered once through sterile gauze to remove hyphae (12).

Inoculum quantification

Inoculum quantification were performed to determine the variable number of colony forming units (CFU) per milliliter (13). The plates were inoculated with 0.1 ml of bacterial and fungal suspension then incubated at 37°C for bacteria and 28°C for fungi were observed for counting of colonies (12). The colony forming units (CFU) used in this study were 10⁶ CFU/ml for bacteria, 10⁵ CFU/ml for *C. albicans* and 10⁴ spore/ml for *A. flavus* (12).

Disc-diffusion assay

The dried plant extract were dissolved in Dimethylsulphoxide (DMSO) to a concentration of 30mg/ml and sterilized by filtration (0.54µm). Antimicrobial tests were carried out by disc diffusion method by using 100µL of tested isolates suspensions by using pour plate technique (14). The discs (5mm in diameter) were impregnated with 10µL of the 300 µg /mL of extracts placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ampicillin (10µg/disc), Cephazolin (30µg/disc), Ofloxacin (10µg/disc), Ciprofloxacin (5µg/ disc) and Nystatin were used as positive reference standards to determine the sensitivity of each isolated microbial species tested and the inoculated plates were incubated at 37°C for 24h for bacteria, 48h for *C. albicans*, 72h for *A. flavus* and *A. niger*. Antimicrobial activity was evaluated by measuring the zone of inhibition against the tested organisms and each assay in this experiment was repeated twice.

Minimum Inhibitory Concentration (MIC)

a- For bacteria

MIC values for alcoholic and aqueous extracts against sensitive isolates of bacteria were studied by using disc diffusion assay. The inoculum of the bacterial strains were prepared from 12h broth of *A. falcata*, dissolved in 50% DMSO was first diluted to the highest concentration (1000µg/ml) to be tested, and then serial 2-fold dilutions were made in order to obtain a concentration range from 62.5 µg/ml to 1000 µg/ml in 10 ml sterile test tubes containing nutrient broth.

MIC values of *A.falcata* against bacterial strains were determined based on serial dilution method by using liquid media (12). All tubes were incubated at 37°C for 24h. microbial growth in each test tube was determined visually for turbidity. The MIC values were defined as the lowest concentration required for inhibiting the growth of microorganisms.

b. For fungi

MIC values for *A. flavus* were determined by serial dilution method as described by (12). The extracts were added aseptically to sterile molted SDA media containing tween 80 (0.5%v/v) to produce the concentration range of 62.5-1000µg/ml. The plates were spot inoculated with 10 ul (10^4 spore/ml) of *A. flavus*, incubated at 37^o C for 72 hrs. The plates were evaluated for the presence or absence of growth. MIC Values were determinate as the lowest concentration of the *A. falcata* extracts where absence of growth was recorded, test replicated twice.

Data analysis

Determination of all the growth inhibition and MICs value were repeated twice. Comparisons of influence of alcoholic, aqueous extracts, antibiotics, and type of microorganisms were performed by ANOVA test and LSD. A P value of ≤ 0.05 considered significant.

Result

***In-vitro* antimicrobial activity of *A .falcata* extracts**

The antimicrobial tests of the alcoholic and aqueous *A.falcata* L. extracts, assayed against a wide range of human microorganisms and their potency was qualitatively and quantitatively assessed by evaluating the presence of inhibition zones and MIC values. The results of the antimicrobial activity of the aqueous and alcoholic extracts of *A. falcata* were given in table (1). These data revealed that the aqueous extract did not exert any antimicrobial activity against tested microorganism, except against *E. coli* which showed weak activity (8 mm inhibition zone). However, the alcoholic extract showed significant antimicrobial activity against 4 bacterial and 2 fungal species tested (figure 1,2). The alcoholic extract inhibited the growth of both G^{+Ve} and G^{-Ve} bacterial species included *E. coli* , *K. pneumonia*, *Pro. Vulgaris*, *E. faecalis*, *S. aureus*, *B. cereus* , *C. albicans* and *A. flavus* at concentration (300 µg / disc) (24,25,12,12,22,10, 14,8) mm inhibition zones respectively as appeared in table (1) .While, the alcoholic extract showed no activity against *P. aeruginosa*, *S.typhi*, *S. epidermidis*, *S. pyogenes*, *B. subtilis* and *A. niger* at concentration 300 µg /disc .

The MICs values of alcoholic extract against different types of susceptible microorganisms

The results of MIC values of alcoholic extract of *A. falcate* was varied and the

values of alcoholic plant extract for bacteria were ranged between 110-250 µg/ml , while it was 250µg/ml and 1000 µg/ml for both *C. albicans* and *A.flavus* respectively as showed in Figure(3).

Discussion

The data of antimicrobial potential of the *A. falcata* alcoholic and aqueous extracts were presented in table (1). The alcoholic extract inhibits 75% of tested microorganisms, while the aqueous extract didn't show any antimicrobial activity (Fig. 1,2). However, *E. coli* was susceptible to alcoholic and aqueous extracts (24 and 8 mm inhibition zones) respectively, these results disagree with (15) which they found that *E. coli* was more resistant to different plant extracts. On the other hand, the results of present study showed that *P. aeruginosa* , *S.typhi*, *S. epidermidis*, *S. pyogenes*, *B. subtilis* and *A. niger* were resistant to alcoholic ,aqueous extracts and antibiotics and this result was similar to (15, 11).

The microorganism susceptibility to aqueous and alcoholic extracts correlated with susceptibility to drugs showed the higher susceptibility to extracts than those of resistant species, this fact was evident for all tested microorganisms, this result was similar to (16, 17) investigated the antifungal and antibacterial activities of flower head extract which contain amides and essential oil.

Indeed , *Achillea* has been used in a popular medicine for its antihemorrhagic , healing and analgesic properties in the various regions throughout the word. Various species of the genus are traditionally used in Turkey for wound healing, against diarrhea and flatulence, as a diuretic, as emmenagog agents, and for abdominal pain (18,19). Also it was used by northern European and North American Native people as contraceptive, a abortifacient and emmenagogue . *Achillae* plants are considered for high hypoglycemic activity. Among the medicinal properties of *Achillea*, their cytotoxic and antiulcer effects are important . The activity of these plants against different bacteria, fungi and parasites might be due to the present of a broad range of secondary active metabolites such as flavonoids, phenolic acids, essential oil, coumarins terpenoids and sterols as well as presence of anti-inflammatory such as sesquiterpenes and alkalamides is another reason for importance of the plant as the potential source of medicinal compounds and drugs in future (20 , 21).

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Table (1): Inhibition zones (mm) of alcoholic and aqueous extracts of *A. falcata* against different tested bacterial and fungal strains.

Tested microorganisms	Inhibition Zones (mm)						
	<i>A. Falcata</i> extracts		Reference Standard Antibiotics				
Bacteria	Alcoholic	Aqueous	Amp	Cep	Ofx	Cip	NYS
<i>Escherichia coli</i>	*24	8	18	27	R	R	-
<i>Klebsiella pneumoniae</i>	*25	R	R	18	12	18	-
<i>Proteus vulgaris</i>	*12	R	14	12	12	R	-
<i>Pseudomonas aeruginosa</i>	R	R	R	R	R	27	-
<i>Salmonella typhi</i>	R	R	R	22	R	28	-
<i>Enterococcus faecalis</i>	*12	R	R	20	R	22	-
<i>Staphylococcus aureus</i>	*22	R	18	20	R	21	-
<i>Staphylococcus epidermidis</i>	R	R	20	20	R	12	-
<i>Streptococcus pyogenes</i>	R	R	18	18	13	20	-
<i>Bacillus cereus</i>	*10	R	12	20	16	18	-
<i>Bacillus subtilis</i>	R	R	18	20	28	12	-
Fungal							
<i>Candida albicans</i>	*14	R	-	-	-	-	24
<i>Aspergillus flavus</i>	8	R	-	-	-	-	18
<i>Aspergillus niger</i>	R	R	-	-	-	-	18

*There are significant differences (at $P \leq 0.05$)

R: Resistant strain

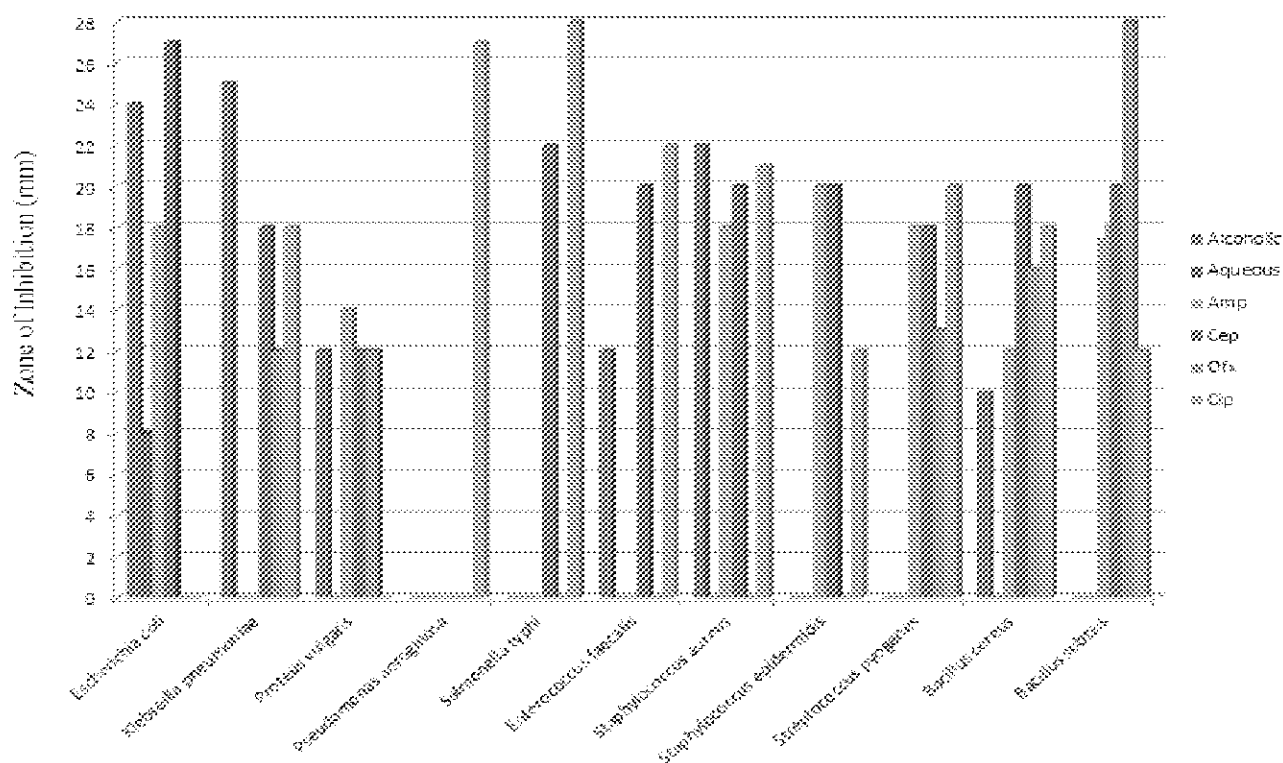


Fig. (1): Alcoholic and Aqueous extracts comparison with antibiotics

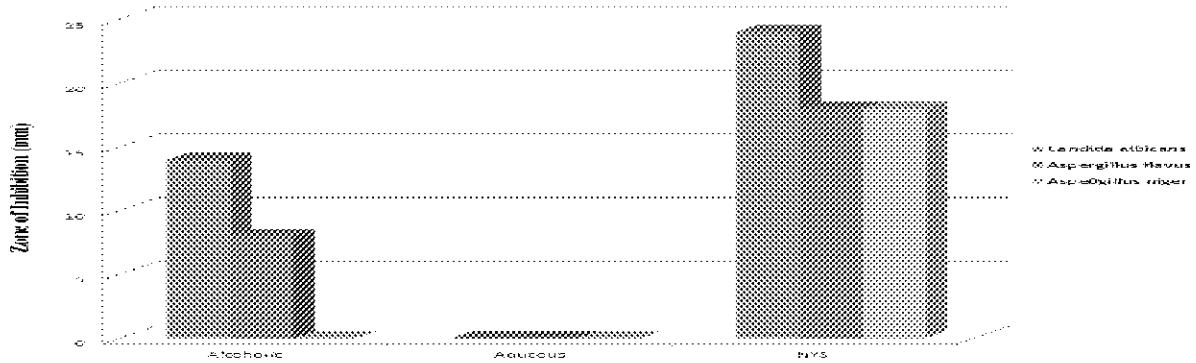


Fig (2): Alcoholic and Aqueous extracts comparison with Nystatin

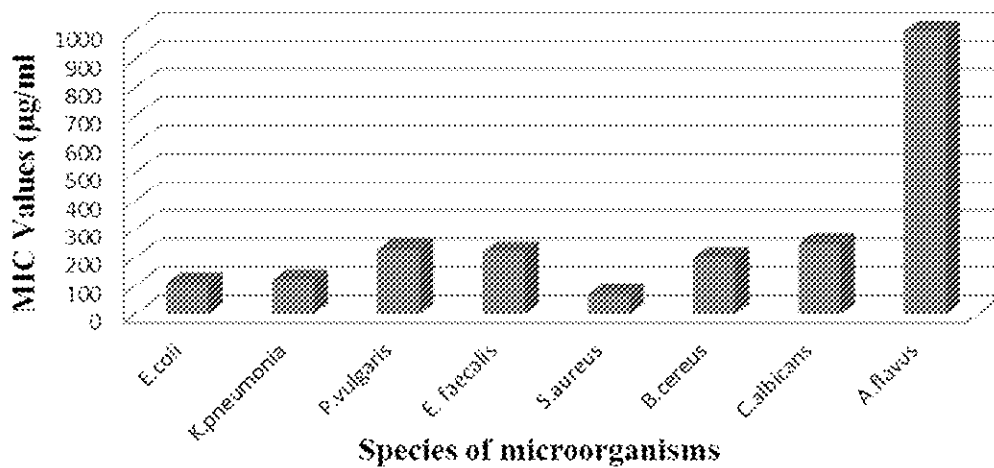


Fig. (3): The MIC values of alcoholic extract of *A. falcata* against different bacterial and fungal species.

فعالية التضاد الميكروبي لمستخلص *Achillea falcata* ضد بعض العوامل المسببة للمرض

الخلاصة

تم دراسة التأثير المضاد للخلاصة المائية والكحولية لنبات القيسوم ضد العوامل المسببة للمرض والتي هي *Salmonella typhi* ، *Proteus vulagris* ، *Klebsella pneumonia* ، *Eschesichia coli* هي *Staphylococcus epidermidis* ، *Staphylococcus aureus* ، *Enterococcus faecalis* و *Bacillus subtilis* ، *Bacillus cereus* ، *Streptococcus pyogenes* وانواع فطرية مثل *Candida albicans* ، *Asspergillus flavu* ، *Aspergillus niger* . اظهرت الدراسة ان الخلاصة المائية لا تملك فعالية مضادة للبكتريا الموجبة و السالبة لصبغة الغرام والفطريات باستثناء فعالية ضعيفة ضد *E. coli* و كان قياس قطر منطقة التثبيط (8) mm ، في حين اظهرت الخلاصة الكحولية مستويات مختلفة من الفعالية و كان معدل اقطار منطقة التثبيط لكل *S. aureus* ، *E. faecalis* ، *Pro. Vulgaris* ، *K. pneumonia* ، *E. coli* و *C. albicans* ، *B. cereus* ، *A. flavus* و (8 ، 14 ، 10 ، 22 ، 12 ، 12 ، 25 ، 24) ملم وعلى التوالي وباستثناء *B. sutilis* و *S. pyogenes* ، *S. epidermidis* ، *S. typhi* ، *P. aeruginosa* ، النتائج امتلاك الخلاصة الكحولية فعالية مضادة للاحياء المجهرية اكبر من الخلاصة المائية وبفارق معنوي تحت احتمالية (P<0.05). كما ان قيم التركيز التثبيط الادنى MIC للخلاصة الكحولية لنبات القيسوم ضد الانواع البكتيرية كانت تتراوح بين 110-250 ميكروكرام/مل ، بينما كانت 250 ميكروكرام/مل و 1000 ميكروكرام/مل لكل من *A. flavus* و *C. albicans* على التوالي.