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# Chemical Content and Antibacterial Activity of Some Extracts of *Anastatica hierochuntica* leaves

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## Abstract

The present study was undertaken to study the chemical content and antibacterial activity of *Anastatica hierochuntica* leaves by preparation extracts (n-hexan, ethanolic 70% and aqueous) and tested against Gram-positive bacteria (*Streptococcus pyogens* and *Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris*). The results of chemical analysis showed that hexan extract contains phenols , terpenes and sterols only, while extracts (ethanolic 70% and aqueous) contain carbohydrates , phenols , flavonoids , tannins , saponins and alkaloids also observed contained this plant on minerals (Cu, Zn and Fe ) in concentrations (8.65±0.30, 47.51±1.31, 169.48±7.11) ppm respectively. The antibacterial activity of the preparation extracts was evaluated using agar well-diffusion method, the results indicated that plant extracts were more active against Gram-negative bacteria than Gram-positive bacteria, alcoholic extract has antibacterial activity stronger than hexan and aqueous extracts. From this results we concluded that *Anastatica hierochuntica* leaves can used in treatment of various disorders by have a number of active components and antibacterial activity. .

**Key words:** *Anastatica hierochuntica* leaves, antibacterial activity, Chemical Analysis**,** Cu, Zn, Fe

# المحتوى الكيميائي و الفعالية الضد بكتيرية لبعض مستخلصات أوراق نبات كف مريم *(Anastatica hierochuntica)*

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## الخلاصة

تم دراسة المحتوى الكيميائي و الفعالية الضد بكتيرية لاوراق نبات كف مريم من خلال تحضير مستخلصات (الهكسان ، الايثانولي 70% و المائي ) و أختبرت ضد البكتيريا الموجبة لصبغة كرام ( *Streptococcus Pyogens* و *Staphylococcus aureus*) و البكتيريا السالبة لصبغة كرام ( *Pseudomonas aeruginosa* , *Escherichia coli* و *Proteus vulgaris* ) وقد أظهرت نتائج التحليل الكيميائي

احتواء مستخلص الهكسان على (الفينولات ، التربينات و الستيرولات ) فقط بينما مستخلصات ( الايثانولي 70% و المائي ) احتوت على (الكاربوهيدرات ، الفينولات ، الفلافونيدات ،التانينات ، الصابونين و القلويدات) كما لوحظ احتواء هذا النبات على عناصر (الزنك ، النحاس و الحديد) بتراكيز( 47.51±1.31 ,8.65±0.30, 169.48±7.11) ppm على التوالي و قيمت الفعالية الضد بكتيرية للمستخلصات المحضرة بأستخدام طريقة الحفر و أظهرت نتائج الدراسة ان مستخلصات النبات اكثر فعالية ضد البكتيريا السالبة لصبغة كرام مقارنة بالبكتيريا الموجبة لصبغة كرام .المستخلص الكحولي له فعالية ضد بكتيرية أقوى مقارنة بمستخلصات ( الهكسان و المائي ). من هذه النتائج يمكن الاستنتاج بأن أوراق نبات كف مريم يمكن ان تستخدم في علاج اضطرابات مختلفة من خلال احتوائها على عدد من المكونات الفعالة وامتلاكها فعالية ضد بكتيرية .

## Introduction

*Anastatica hierochuntica*, is a member of the family Brassicaceae [1].Is commonly called "Kaff maryam" or "Rose of Jericho", which is a small grey winter annual herb, found in the arid regions of Saudi Arabia, Egypt, Jordan, Iraq, Kuwait and North Africa [2]. The plant is prescribed in folk medicine for uterine and hemorrhage[3] .It is also used as a tea to treat asthuma and respiratory disease, dysentery, salmonella typhi , typhod fever, diabetes, heart disease and headaches [4].The medicinal value of this plant lie in phytochemical components which produce definite physiological actions on human body [5],so this study was undertaken to find chemical composition of *Anastatica hierochuntica* leaves and evaluation activity of some plant extracts as antibacterial .

## Materials and Methods

### Plant material

*Anastatica hierochuntica* was brought from local market of Basra city. The plant was separated into leaves and stems, after that the leaves were washed with tap water and dried at room temperature (25 ˚ C) .The dried leaves material was ground to fine powder.

### Bacterial strains

The isolates of bacteria used in this study were obtained from Microbiology Department, College of veterinary medicine, Basra University as shown in table (1).

Table 1: Bacterial strains

| Gram Reaction | Indicator Strain |
|---|---|
| Gram Positive bacteria | *Staphylococcus aureus*<br>*Streptococcus pyogens* |
| Gram Negative bacteria | *Escherichia coli*<br>*pseudomonas aeruginosa*<br>*Proteus vulgaris* |

### Preparation of Extracts

**n-hexan Extract:** 50 gm of *Anastatica hierochuntica* leaves powder was added to a thimble and then placed in a soxhlet extractor.Heat was applied to a round bottom flask which contain n-hexan solvent was placed at the base of the soxhlet extractor.The extract was concentrated using a rotary evaporator (Puchi Rotavapor-Re) then dried at room temperature [6] .

**Alcoholic Extract:** The alcoholic extract was prepared by extracting (50 gm) of *Anastatica hierochuntica* leaves with 70% ethanol using reflux method. The suspension was filtered through whatman filter paper No. 31, the filtrate was concentrated using rotary evaporator [7].

**Aqueous Extract:** Aqueous extract was obtained by extracting (50 gm) of *Anastatica hierochuntica* leaves with deionized distilled water using reflux method. The suspension was filtered through whatman filter paper No. 31, the filtrate was concentrated by rotary evaporator [7].

## Chemical Analysis

**Phytochemical analysis:** Quantitative phytochemical tests were carried out on the preparation extracts using standard procedures to identify the presence or absence of carbohydrates , phenols , flavonoids , tannins , saponins , alkaloids , terpenoids and sterols according to the methods described by [8] and [9] .

**Minerals Analysis:** *Anastatica hierochuntica* leaves in the powdred form was weighed and digested in (5:1) mixture of nitric acid and perchloric acid [10] . After digestion few drops of concentrated HCl was added. The solution was heated and then filtered. The filtrate was diluted suitably with distilled deionized water.The dilute filtrate solution was used for analysis of minerals of interest by atomic absorption spectrophotometer. The measurement of minerals was performed in triplicate at the minimum [11].

**Antibacterial Activity:** five types of pathogenic bacteria were previously isolated and identified by other work. Muller-Hinton agar medium was used for bacteria growth plates were incubated at 37 °C for 24-48 hours. The antibacterial activity of n-hexan, alcoholic and aqueous extracts of *Anastatica hierochuntica* leaves used Agar-Well diffusion method by adding 100 µl containing 20µg / ml from each extract to each well, the extracts were allowed to diffuse into the medium for 1 hour at room temperature, then incubated at 37 ° C for 24 hours, the zone of growth inhibition were measured and recorded in mm [12]. The control was set up in a same manner except that the extract replaced with n-hexan and 70% ethanol.

## Statiatical analysis

The results were expressed as mean ± SD was performed by ANOVA procedures [13] .

## Results and Discussion

The results of phytochemical analysis of *Anastatica hierochuntica* leaves are presented in Table 2 which show that contained alcoholic and aqueous extracts on same components carbohydrates, phenols, flavonoids, tannins, saponins and alkaloids while, n-hexan extract contained phenols , terpenoids and sterols as refer [14] .

Table 2: Phytochemical analysis of *Anastatica hierochuntica* leaves.

| Phytochemicals | n-hexan extract | Alcoholic extract | Aqueous extract |
|---|---|---|---|
| carbohydrates | - | + | + |
| phenols | + | + | + |
| Flavonoids | - | + | + |
| Tannins | - | + | + |
| Saponins | - | + | + |
| Alkaloids | - | + | + |
| Terpenoids | + | - | - |
| Sterols | + | - | - |

**Absence = - , Presence = +**

The results of minerals analysis revealed that contained *Anastatica hierochuntica* leaves on copper (Cu ), zinc (Zn) and iron (Fe) as shown in Table 3 this result is in agreement with [15] .

Table 3 : Mineral concentration of Anastatica hierochuntica leaves .

| Minerals (ppm) | | |
|---|---|---|
| **Cu** | **Zn** | **Fe** |
| 8.65±0.30 | 47.51±1.31 | 169.48±7.11 |

**Data are mean ± standard deviation, ppm = parts per million (1 mg / Kg = 1 ppm)**

In this study we obtained data for minerals concentration were difference than those reported by [15], this may be attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plant is cultivated. The presence of these essential minerals implies *Anastatica hierochuntica* leaves could be utilized as a nutritionally valuble and healthy ingredient. These nutrients may no be strictly medicinal but could be valuable in preventing

disease that are related to malnutrition. Copper is growth and development but can be toxic at excessive levels [16] .Copper deficiency results in anemia and congenital inability to excrete copper resulting in wilson' s disease [17] .

Zinc is an essential trace element for plant growth and also plays an important role in various cell processes including normal growth, brain development, behavioural response, bone formation and wound healing [18].

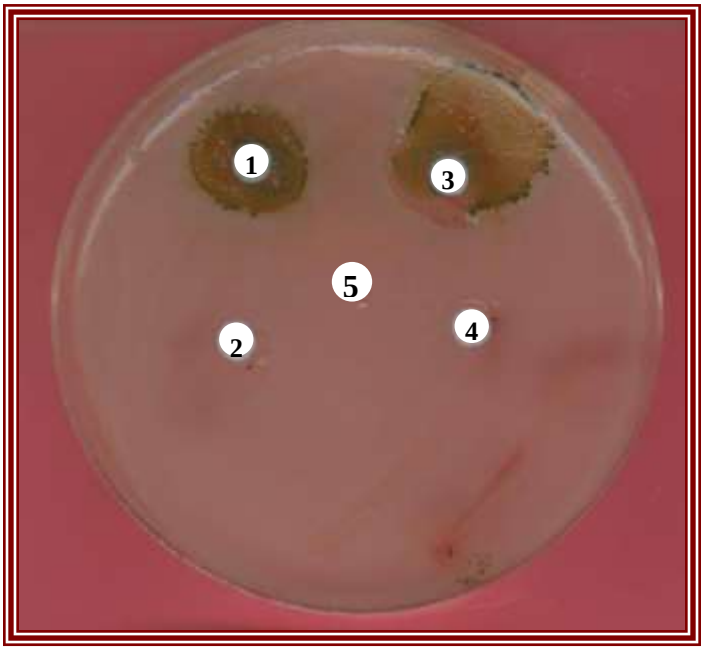
Iron is an essential element for human beings and animals, the role of iron in the body is clearly associated with hemoglobin and the transfer of oxygen from lungs to the tissue cells and is very important factor in diabetes [19].

The antibacterial activity of the crude extracts were assessed quantitatively by determining the zones of inhibition as given in table 4 and figures 1 and 2.

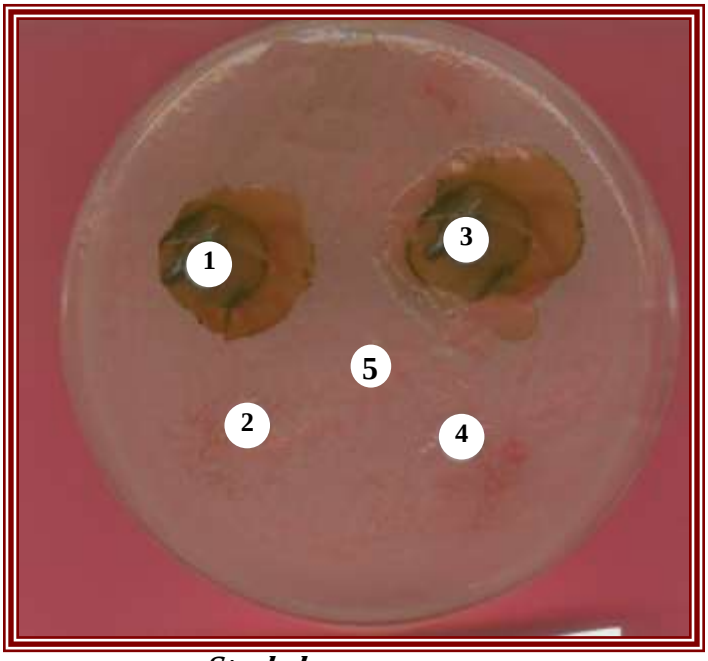
an essential enzymatic element for normal plant

Table 4: Antibacterial activity of n-hexan, ethanolic and aqueous extracts of Anastatica hierochuntica leaves using the well diffusion method.

| **Test Organisms** | **Inhibition Zone (mm)** | | |
|---|---|---|---|
| **Gram-positive bacteria** | **n-hexan Extract** | **ethanolic Extract** | **Aqueous Extract** |
| *Streptococcus pyogens* | 17 | 20 | 0 |
| *Staphylococcus aureus* | 23 | 28 | 0 |
| **Gram-negative bacteria** | | | |
| *Escherichia coli* | 35 | 35 | 9 |
| *Pseudomonas aeruginosa* | 29 | 29 | 17 |
| *Proteus vulgaris* | 25 | 27 | 15 |

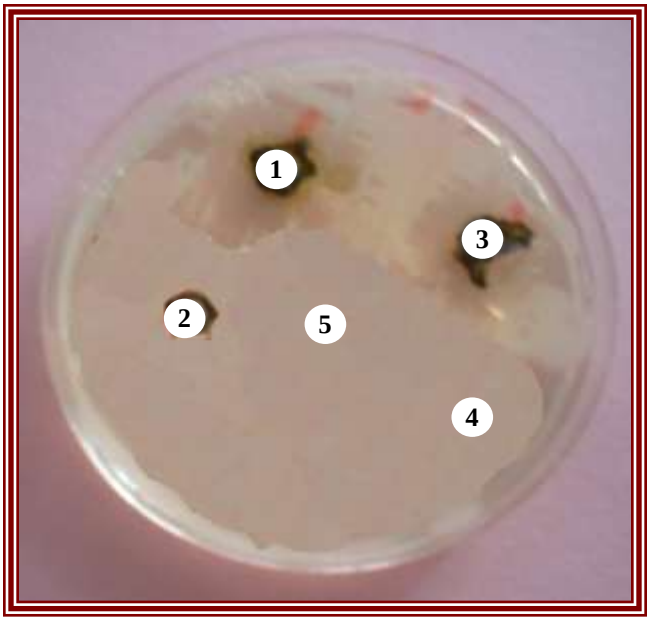



***Streptococcus pyogens***

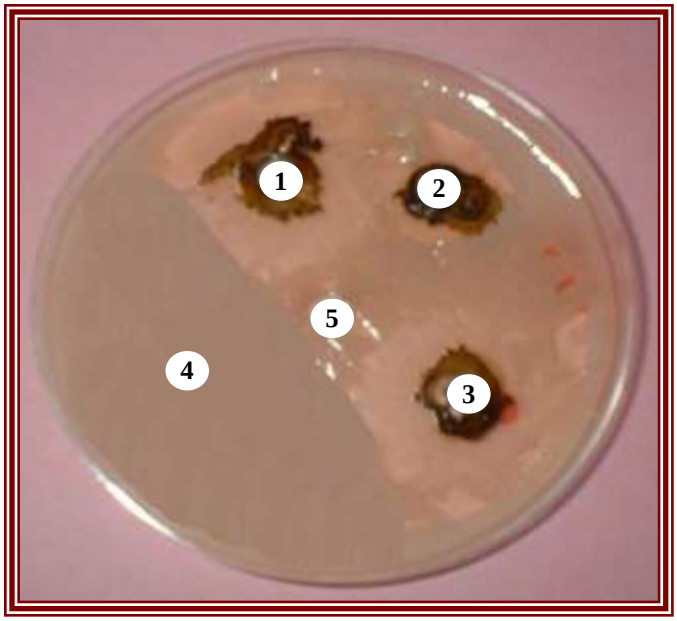



***Staphylococcus aureus***

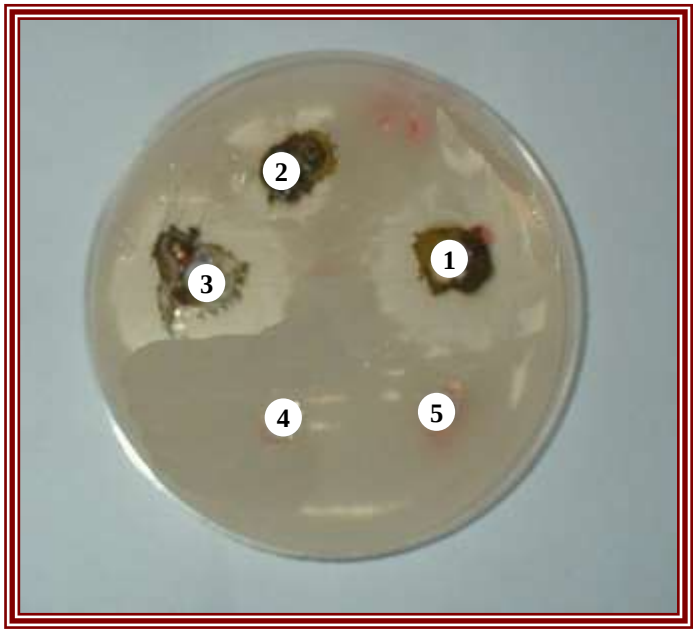
Figure 1: Antibacterial activity of n-hexan, ethanolic and aqueous extracts of *Anastatica hierochuntica* leaves against Gram-positive bacteria.
1- n-hexan extract 2- aqueous extract 3- ethanolic extract 4- n- hexan 5- ethanolic 70%




***Escherichia coli***




***Pseudomonas aeruginosa***




***Proteus vulgaris***

Figure 2: Antibacterial activity of n-hexan , ethanolic and aqueous extracts of *Anastatica hierochuntica* leaves against Gram-negative bacteria .
1- n-hexan extract 2- aqueous extract 3- ethanolic extract 4- n- hexan 5- ethanolic 70%

The n-hexan and alcoholic crude extracts of *Anastatica hierochuntica* leaves showed variable inhibition effects against both the Gram-positive bacteria (*Streptococcus pyogens* and *Staphylococcus aureus* ) and Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus vulgaris* ) as compared with control. While aqueous extract showed antibacterial activity against Gram-negative bacteria only. In general, ethanolic extract has antibacterial activity as compared with control this result may be due to these types of bacteria not sensitive for this concentration of alcohol. Ethanolic extract on the other hand showed stronger inhibition effect than n-hexan and aqueous extracts. This suggests that some of the active compounds in the ethanolic extract exist in higher percent than those in n-hexan and aqueous extracts. Previous studies have noted alcohols to be reliable and consistent solvents for the extraction of antimicrobial substances from medicinal plants [20], the n-hexan extract may contain non-polar compounds that inhibits the growth of bacteria, they might have novel bioactive phytochemical compounds present in low concentration.

The potency of *Anastatica hierochuntica* leaves as antibacterial is attributed to the action of phytochemical compounds is contains, which are secreted by plant naturally, in response to environmental pressure or as a defense mechanism to animal attacks or plant diseases, these compounds normally extracted in polar extracts included alkaloids, flavonoids and some phenls, or non-polar compounds may include terpenes and sterols. Antibacterial activity may involve complex mechanisms, like the inhibition of the synthesis of cell walls and cell membranes and proteins [21]. This results help to explain the therapeutic effect of the plant.

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