

## Assessment of the Biological Activities of Calendula officials Extracts

## تقييم الفعالية البيولوجية لمستخلص زهرة الاقحوان

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## المستخلص:

**الهدف:** يهدف البحث الى تقييم نوعين من الاستخلاص المائي والكحولي لنبات زهرة الاقحوان باستخدام الزهور و الاوراق ودراسة الفعالية المضادة للبكتريا لهذه المستخلصات باختبار خمسة انواع من البكتريا.

**المنهجية:** تم اختيار نبات زهرة الاقحوان لاجراء هذه الدراسة حيث جمعت الزهور، الاوراق والسيقان من الاسواق المحلية في بغداد وجففت في الظل لمدة ٧ ايام ثم طحنت الى مسحوق ناعم وتم تحضير مستخلص مائي حار لفترة ساعتين عند درجة حرارة (١٠٠°C) للزهور، سيقان واوراق زهرة الاقحوان، كما تم اجراء استخلاص كحولي لفترة ٤٨ ساعة عند درجة حرارة (٨٠°C) للزهور، سيقان واوراق النبات وتم دراسة فعالية هذه المستخلصات كمادة مضادة للبكتريا على خمسة انواع من البكتريا المعزولة.

**النتائج:** من خلال هذه الدراسة تم التوصل الى نتائج تفيد ان المستخلص المائي الحار لمدة ساعتين لنبات زهرة الاقحوان ليس له أي تأثير ضد مايكروبي بينما المستخلص الكحولي لفترة ٤٨ ساعة لنبات زهرة الاقحوان اعطى تأثير مضاد لكل انواع البكتريا المستخدمة، كما اظهرت النتائج ان هذا المستخلص له تأثير واضح كمضاد للبكتريا ولوحظ اعلى قيمة لمنطقة التثبيط عند نوعي بكتريا *Staphylococcus aureus* و *Escherichia coli*

**التوصيات:** اقترحت هذه الدراسة ضرورة اعتماد مستخلص نبات زهرة الاقحوان كعلاج بديل مضاد لانواع من البكتريا التي تظهر مقاومة للعلاج التقليدي. تعد هذه الدراسة خطوة اولية لدراسات لاحقة لذا من الضروري استخدام طرق استخلاص اخرى ممكن ان تستخلص المادة الفعالة بنسبة اكثر فضلا عن الاستعانة بمذيبات عضوية مختلفة وتطبيق المستخلصات ضد انواع بكتريا اخرى ذات مقاومة عالية للعلاج التقليدي.

## Abstract:

**Objective:** To assess two kinds of extraction (aqueous and methanolic) for Calendula officials using flowers, leaves and stems and studying their antibacterial activity against five different bacteria.

**Methodology:** Calendula officials were selected to carry out this study. Flowers, leaves and stems were collected from local markets in Baghdad then dried in shade for 7 days and grinded to fine powder. Aqueous hot extracts for 2hr. at (100°C) and alcoholic extracts for 48 hrs at (80°C) were performed using flowers, leaves and stems then studied antibacterial effect against five different bacterial genuses by using well diffusion technique.

**Results:** This study showed that hot aqueous extracts for 2hr. to all parts of Calendula officials indicated no antibacterial activity. While, methanolic extracts of flowers, leaves and stems for 48hrs had antibacterial activity and the highest values for inhibition zone shown in staphylococcus aureus and Escherichia coli.

**Recommendations:** The present study has been suggested to use Calendula officials flowers extract as alternative medical therapy for microorganisms which may resist conventional treatment. This study is a first step for another future studies. It is necessary to use various extraction methods to give active materials with high percentage, although different organic solvents to be used with Calendula officials plant to obtain extracts used for testing different kinds of microorganisms which have highly resistance to conventional treatment.

**Keywords:** Medicinal plants, Biological activities.

**Introduction :**

**C**alendula *officinalis* is a natural herb used for treating many diseases like Antiphlogistic; Antiseptic; Antispasmodic; Aperient; Astringent; Cholagogue; Diaphoretic; Emmenagogue; Homeopathy; Skin; Stimulant; Vulnerary; Warts.

*Calendula officinalis* is one of the best known and versatile herbs in Western herbal medicine and a popular domestic remedy like bites stings, sprains, wounds, sore eyes, and varicose veins etc<sup>(1-2)</sup>. It is also a cleansing and detoxifying herb and is taken internally in treating fevers and chronic infections<sup>(1-2)</sup>, also It is a remedy for skin problems and is applied externally<sup>(1)</sup>. The whole plant, especially the flowers and the leaves, is antiphlogistic, antiseptic, antispasmodic, aperient, astringent, cholagogue, diaphoretic, emmenagogue, skin, stimulant and vulnerary<sup>(1-3-4-5-6-7-8)</sup>. The leaves can be used fresh or dried; they are best harvested in the morning of a fine sunny day just after the dew has dried from them<sup>(1)</sup>. The flowers are also used fresh or dried, for drying they are harvested when fully open and need to be dried quickly in the shade<sup>(1)</sup>. A tea of the petals tones up the circulation and, taken regularly, can ease varicose veins<sup>(8)</sup>. An application of the crushed stems to corns and warts will soon render them easily removable<sup>(3)</sup>. The leaves, blossoms and buds are used to make a homeopathic remedy<sup>(9)</sup>. It is used internally in order to speed the healing of wounds<sup>(9)</sup>.

A *calendula officinalis* that contains alkaline one of these alkaloids is ctedoron and volatile oils monolactones hemiterpenes and flavonedes and vitamin B.

**Methodology:****Plant Materials**

Flowers, leaves and stems of *Calendula officinalis* were collected from local markets in Baghdad. Leaves and stems were washed with distilled water, dried in shade for at least 7 days, and then all parts of the plant were grinded to fine powder and stored in airtight container at room temperature in the dark until used.

**Extraction of plant materials**

*Calendula officinalis* were collected from natural habitats during flowering. Air dried plant sample

rinsed with water and dried. After evaporation of the solvent, the residues were powdered (250 g) and extracted with 500ml, 70% ethanol or methanol in a soxhlet apparatus and the extracts were evaporated to dryness by a rotary evaporator.

**Agar Diffusion Assays**

Different strains of bacteria were used which are: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella sp.*, *Proteus vulgaris* and *Staphylococcus aureus*. All strains were collected from samples such as blood, CSF, urine. The identity of all these strains was confirmed. A bacterial suspension was prepared and added to the sterilized medium before solidification. The media with bacteria was poured into sterilized Petri dishes under aseptic condition. Different weights of the extractants (ethanolic and methanolic 0.02 M) in N,N-dimethylformamide (DMF) solvent were placed on the surface of the culture and incubated at 37 °C for 24 hours. After incubation the average of inhibition zones was recorded<sup>[10-11]</sup>.

**Minimum inhibitory concentration (MIC) evaluation:**

The MIC was evaluated on plant extract that showed antimicrobial activity. This test was performed at four concentrations of the extract employing the same agar well diffusion method.

**Procedure of Thin layer chromatography (TLC)**

The TLC plate prepared with recoated silica gel aluminum plate 60F 254 and the stationary face having a thickness of about 0.5 mm. 5µL each of test solution was applied on silica gel plate (20x 10 cm). The TLC plate was saturated chromatographic tank containing chloroform: benzene: methanol (60:25:15) solvent systems.

**Mayers test:** we add 3 drops of Mayers reagent<sup>[12]</sup> to 1% solution of *Calendula officinalis* extract a white precipitate was appears.

**Flavonoids test:** we add 10mL of 50% alcohol and 10mL of (50%) potassium hydroxide to 5mL. of (1%) *Calendula officinalis* extract a yellow precipitate was appears<sup>(13)</sup>.

**Terpens test:** we add 2 drops of sulfuric acid and 2 drops of acetic acid to a mixture of 1mL. (1%) *Calendula officinalis* alcoholic extract and 2mL of chloroform a brown color was appears<sup>(14)</sup>.

**Result:****Table 1.** The antibacterial activity of *Calendula officinals* in agar diffusion assay (for organic solvent extract)

	5mg/ml	2.5mg/ml	1.25mg/ml	0.6mg/ml	0.3mg/ml
1- <i>E.coli</i>	3.5cm	3.5cm	3.5cm	3.5cm	3.5cm
2- <i>S. aureus</i>	2.5cm	1.5cm	1.5cm	1.5cm	1.5cm
3- <i>Klebsiella sp.</i>	3.0cm	2.5cm	1.5cm	0.5cm	0.5cm
4- <i>Pseudomonas aeruginosa</i>	2.0cm	2.0cm	1.5cm	0.5cm	0.5cm
5- <i>Proteus vulgaris</i>	2.5cm	2.0cm	1.5cm	1.0cm	1.0cm

This table above shows the antibacterial activity of *Calendula officinals* alcoholic extract by means of agar diffusion assay against different types of bacteria (gram positive and gram negative).

**Table 2.** The most important active compounds that found in *Calendula officinals* alcoholic extract

The compound	Test	Color	Result
Alkaloids	Mayers reagent	White ppt.	ve+
Terpens	Chloroform+acetic acid+H <sub>2</sub> SO <sub>4</sub>	Brown	ve+
Flavonoids	KOH+alcohol	Yellow ppt.	ve+

+ve: positive

This table above shows the most important active compounds that found in *calendula officinals* alcoholic extract which have antibacterial activity against different types of bacteria.

**Discussion:**

The present study indicates antimicrobial activity of *Calendula officinals* alcoholic extract by means of the agar diffusion assay showed that plant extract tested exhibited an antimicrobial effect against Gram positive bacteria, *S. aureus* and Gram negative, *Klebsiella*, *P. vulgaris*, *P.aeruginosa* and *E.coli*.

The antibacterial activity of *Calendula officinals* extract were evaluated by measuring the inhibition zone observed around the tested materials. In agar diffusion assay, the methanolic extract of the plant showed considerable activity against all tested bacteria. [10-11]

This indicates that alcoholic extract has broad spectrum of antimicrobial activity and a wide therapeutic window. The isolated tested in this study responsible for many diseases, nosocomial infections and bacteremia due to multidrug resistant staphylococcal infections, and diarrheal diseases caused by *E. coli* [19-20]. The sensitivity of these isolates to *Calendula officinals* extract also implies that the intrinsic biosubstances in this extract naïve to the various drug resistance factors of the isolates.

The *Calendula officinals*' plant contain many chemical compounds for example alkaloids, volatile oils monolactones hemiterpenes,

flavonoids and vitamin B. The presence of terpens which considered as antibacterial [15-16]; the inhibitory activity of the methanolic extract refer to the content of *Calendula officinals* plant of terpens [17] against gram negative bacteria with a concentration (0.3%) which have the antibacterial activity. This extract also contains flavonoids and alkaloids which have the antibacterial activity [18].

**Recommendations:**

The present study has been suggested to use *Calendula officinals*' extracts as alternative medical therapy for microorganisms which may resist conventional treatment. The future studies will be considered to evaluate some organic solvents extracts of *Calendula officinals* flowers. Also it could be use different methods of extraction for testing various kinds of microorganisms which have highly resistance against the conventional therapy .This will offer a great help in facing the emergence spread of bacteria

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