

Evaluation The antibacterial activity and healing enhancer of *Calendula officinalis* extract in laboratory mice *Mus musculus*

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Abstract

The aim of the present study was to evaluate the Antibacterial and healing enhancer activity of *Calendula officinalis* using different bacterial species and laboratory mice. It appears that there a significant increase $P < 0.05$ in antibacterial activity of methanolic extract (hot and cold) compared with standard antibiotic , especially in *Klebsiella* and *E.coli*. The results was showed significant $p \geq 0.05$ decreasing in the period healing in the group treated with extract and gentamycin ointment compared with paraffin wax, A better healing pattern with complete wound closure was observed in mice treated with Methanolic extract and gentamycin within 12 days, while the wound still opened in paraffin wax group . There was a significant reduction in wound area from day three onwards in treated mice Based on our results *Calendula officinalis* extracts have great potential as antibacterial compounds against wounds bacteria and they can be used in the treatment of infectious wounds, caused by multi-drug resistant strains and could be useful for developing alternative therapy for wounds infection.

Introduction

The relationship between men and plant is as old as the history of mankind. In plant kingdom, thousands of plant yield medicines that have many uses in human life. The medicinal importance of these plants is due to presence of chemical substances in them. One of these plants is *Calendula officinalis*, commonly known as "potmarigold" also called Catha Officinalis, Golds, Mary Gowles, Oculus Christi, po marigold, Ruddes, it is an annual herb and belongs to Asteraceae family.⁽¹⁾ The plant is native to central and southern Europe, Western Asia and the US.⁽²⁾ The name Calendula stems from the latin *Kalendula* meaning first day of the month, presumably because pot marigolds are in bloom at the start of most months of the year, The common name marigold probably refers to the Virgin Mary or its old saxon name "ymbglidegold" which means "it turns with the sun".⁽³⁾ Pot marigold is a herb with different medicinal properties, the

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whole plant but especially the flowers is Analgesic, angiogenic, antiseptic Anthelmenic, antibacterial, antifungal, antispasmodichemostatic, hypotenveimmunostimulant, lymphatic, vulnerary, lymphatic and vasodilator.⁽⁴⁾ It is used externally for any skin ulcerations or inflammation, abscesses ,recent wounds and others,internally in order to speed the healing of wound.⁽⁵⁾ A laboratory study examining the effectiveness of various herbs against bacterial infections found that *Calendula officinalis* was one of the most active herbs so *Calendula* is effective not only in treating mechanical skin injuries,like scrapes but also in impetigo,acne,boils,sebaceous cysts and many other skin infections.⁽⁶⁾One of the medical worries growing to days is that compromised skin can easily be assaulted by multi drug resistant strains of bacteria especially Methicillin_resistant *Staphylococc aureus*(MRSA) and cause a difficult-to-treat supe rinfecion. ⁽⁷⁾ *Calendula* bactericida effects against *Staphylococcus aureus* are very important since a super infection can lead to lose of limb and may even threaten a persons life ,it is estimated that MRSA killed more individuals in 2005 than AIDS .⁽⁸⁾ A little prevention with *Calendula* can go a long way toward keeping multi drug resistant strains especially MRSA infections from flaring up.And since there is few report of the wound healing activities of *Calendula officinalis* through antibacterial and physiological effect.so the aim of the present study is to confirm the role of *Calendula officinalis* flower head extract in the antibacterial activity and the treatment and management of wounds

Methods

Collection and Identification

Calendula officinalis flowers were bought from a local market and identified by taxonomist Ali Aboud Shareef biology department college of Education University of Basrah The plant was dried and grounded and 40 gms of the sieved powder was weighed accurately and subjected to extraction in a soxhlet apparatus using methanol and water as a solvent extract , 2 types of extraction (hot and cold) are used successively for each solvent. Before extraction with the next solvent the powder was air dried to remove the adhering solvent. The extract obtained was filtered, concentrated in rotary flash evaporator and dried in a vacuum oven.

Collection of the tested bacteria

The bacterial isolate which is used in the present study included *Staphylococcus aureus* (MRSA), *Streptococcus spp*,*Klebsiella spp*, *E.coli*. *Pseudomonas* which brought from Al-Sader Medical City in Najaf. The isolated bacteria was identified according to⁽⁹⁾ and stored until used in the study

The Antibiotic

Each of Ampicillin, Gentamycin, Ciprofloxacin , Doxycyclin,

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Cefotaxime, Ceftriaxone, Tetracycline, Rifampin and Trimethoprim were used in susceptibility test to compare with extracts.

Antibacterial susceptibility test

The antibacterial potential of the above plant extract was seen against the test organisms using well diffusion susceptibility test ,sterile Nutrient-Agar plates were taken per plate/ bacteria tested. Five wells of about 3mm diameter were aseptically punched on each agar plate using a sterile cork- borer, with at least 30mm distance between adjacent wells and the periphery. 2-4 colonies of the test bacteria were inoculated in water and these inoculums was swabbed. Using sterile swab on the surface of above punched Nutrient-Agar plates, a fixed volumes of the plant extract was then introduced into the wells in the increasing concentration and then inoculated at 37C⁰ for 24 hours. The resulting zones of inhibitory were measured in mm.

Preparation of ointments

500mg of Dried methanolic extract was mixed with semi liquid paraffin in 1:1 ratio

The Laboratory Animal

The mice were obtained from the animal house of Basrah university College of Education and kept in standard condition. They were provided with food and water adlibitum during the period of the experiment. 18 male mice 12 week age and 20-25 Weight were used in this experiment. The dorsal skin of the mice were shaved then 7mm of the shaved skin in its full thickness was excised⁽¹⁰⁾. The mice were divided to three group of 6 mice. The first group treated with 500mg/body weight of the prepared ointment (methanolic extract mixed with semi liquid paraffin). The second group treated with gentamycin ointment. The third group treated with semi liquid paraffin and used as control group. wounds were left undressed .The mice were kept individually in separate cages. The changes in wound area were measured in mm at every 3 days interval.

Statistical analysis

The relative wound area results were compared using one- way analysis of variance (ANOVA) followed by t test using spss program.

Results

The present result showed significant $P > 0.05$ differences in antibacterial activity of different standard antibiotic against tested bacterial species. fig.(1). The figures (2,3,4,5) showed that there are significant increasing $P > 0.05$ in antibacterial activity specially in 50mg compared with other concentration against tested bacteria. The result also showed that there are significant increasing $P > 0.05$ in antibacterial activity of some antibiotic such as (doxycyclin, rifampin, ciprofloxacin, trimethoprim) and for this result it is used as

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control treatment to compare with extract .The present result showed that there is a significant $P>0.05$ increasing in antibacterial activity between the aqueous and alcoholic extract and some standard antibiotics in different used bacteria. The results also showed non significant $P>0.05$ differences between the aqueous extract(hot and cold) compared with some standard antibiotics such as ciprofloxacin in some used bacteria. And it appears that there is significant increasing $P<0.05$ in antibacterial activity of methanolic extract (hot and cold) compared with the standard antibiotic ,especially in *Klebsiella* and *E.coli* The comparison between methanolic and aqueous extracts in the antibacterial activity show significant increasing $P<0.05$ against *E.coli*, *Klebsiella* and non significant increasing $P>0.05$ in *Pseudomonas* and *streptococcus* in methanolic extract compared with water extract figure (6,7,9).The results in figure(8)showed significant $p\geq 0.05$ decreasing in the time healing in the group treated with extract and gentamycin ointment compared with paraffin wax and a better healing pattern with complete wound closure was observed in mice treated with extract and gentamycin within 12 days while the wound still opened in paraffin wax. There was a significant reduction in wound area from day three onwards in treated mice and also on later days the closure rate was much faster than when compared with control group.

Discussion

The data from the present study showed that alcohol extract were more effective than the aqueous extract in antibacterial activity, this may be due to the fact that the chemical component of *C.officinalis* flowers are may responsible of such activity. several studies explained that the chemical component of *C.officinalis* such as oleanolic acid and ursolic acid –type triterpene glycosides and its derivatives which are presented in alcohol extract especially ursolic acid was identified as one of this active components.^(11,12,13) These findings, in part, explain why Calendula is effective not only in pathogen bacteria that caused wounds infections but also in MRS astrains in these infections.⁽¹⁴⁾ Activities against the multi drug resistant strains are significant clinical importance ,inparticular, oleanolic acid can be considered to exhibit high activity against these strains compares favorably with the susceptibility of this strain to common antibiotics.^(12,15) the antibacterial investigations in this study ,hot alcohol extract of *C.officinalis* exhibited highest antibacterial activity against all the bacteria tested, this suggests that these compounds in alcohol extract(especially in hot)could reduce the growth of specific bacteria through affect the efflux pump system of them .⁽¹⁶⁾ Although many compounds have been shown to affect the activity of efflux pump.it seems oleanolic acid,a compound with catechin and epicatechin ,moderately active in modulating accumulation and efflux bacteria strains(even resistance),Oleanolic acid is

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known as an inhibitor of protein kinase ,while flavonoids have been shown to inhibit the transport of glucose ,these two mechanisms prevent the derivation of energy required for efflux pump activity, therefore accumulation takes place, in the presence of glucose, the capacity for inhibition of the efflux pump by oleanolic acid ,catechin and epicatechin .⁽¹⁷⁾Literature also reveals the use of alcohol decoctions in various skin conditions associated with bacterial infections.^(18,19)*C.officinalis* activity may be also belonged to present the triterpene acids which have both hydroxy and carboxy groups and these are very important against pathogen bacteria.⁽²⁰⁾ Another causes for the *C.officinalis* activity are presence another several classes of chemical compounds in their flowers, the main ones being essential oil,coumarines,quinines, volatile oil,amin acids,carotenoids,flavoxanthine and auroxanthin,an antioxidant and the source of the yellow–orange coloration.⁽²¹⁾ Essential oils of this herb are highly medicinal with several therapeutic activities and have very strong aromatic components, they are soluble in alcohol but to a very limited extent in water, this is another reason explain why alcohol extract has more antibacterial activity than aqueous extract.⁽²²⁾ Also carotenoids,saponins and flavonoids appear to be responsible for anti-bacterial activities.⁽²³⁾ Many of studies agreed with our results that more than35 properties have been attributed to the decoctions from the flowers, so the bactericide use of this plant are probably due to presence all of mentioned components in the decoctions.^(24,25,26,27) However most reports on the activity of *C.officinalis* extracts have focused mainly on the microorganisms and pathogen bacteria, while information's of the activity against wounds based resistant pathogen bacteria is scanty .

Wound healing properties

The results also showed that wound healing and repair, was accelerated by applying methanolic extract and gentamycin compared with control group Study on animal models showed enhanced rate of wound contraction and drastic reduction in healing time than control, which might be due to enhanced epitheliasation.⁽²⁸⁾ A number of secondary metabolites/active compounds isolated from plants have been demonstrated in animal models (in vivo) as active principles responsible for facilitating healing of wounds Some of the most important ones include oleanolic acid from *Anredra diffusa*.⁽²⁹⁾.the result in our study also agree with⁽³⁰⁾ who reported that *Calendula* has been found to stimulate the growth of new tissues and blood vessels when applied externally to wounds. Professional homeopaths often recommend ointments containing homeopathic doses of calendula to heal first-degree burns and sunburns. In fact, some homeopaths considered this remedy is the treatment for children. Homeopathic calendula ointments may also be used in the healing stages of second and third degree burns to stimulate re growth of skin and to diminish scar

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formation.

Conclusion:.

Based on our results *Calendula officinalis* extracts have great potential as antibacterial compounds against wounds bacteria and it can be used in the treatment of infectious wounds caused by Multi-drug resistant strains and could be useful for developing alternative therapy for wounds infection and thus opened a gate way for the possibility to finding cures for many strains of bacteria which are now resistant to many usable and common antibiotics in our countries.*C.officinalis* is a promising cure for human wounds.

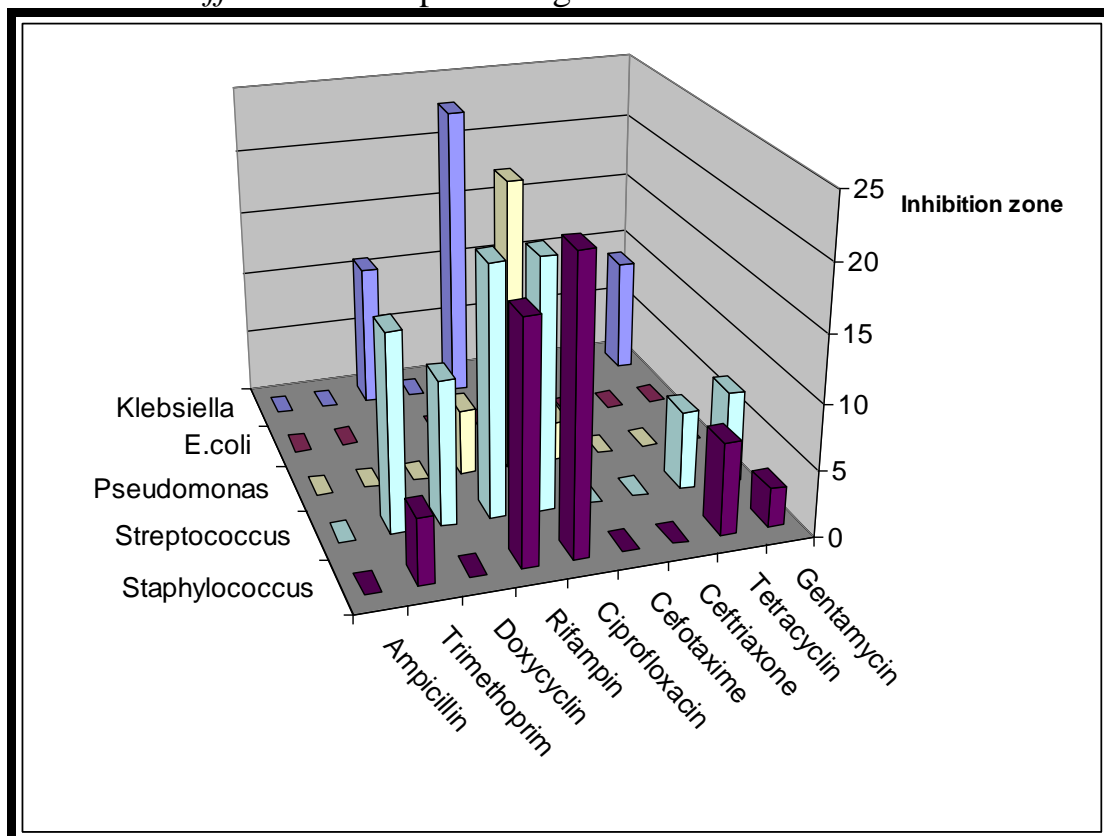
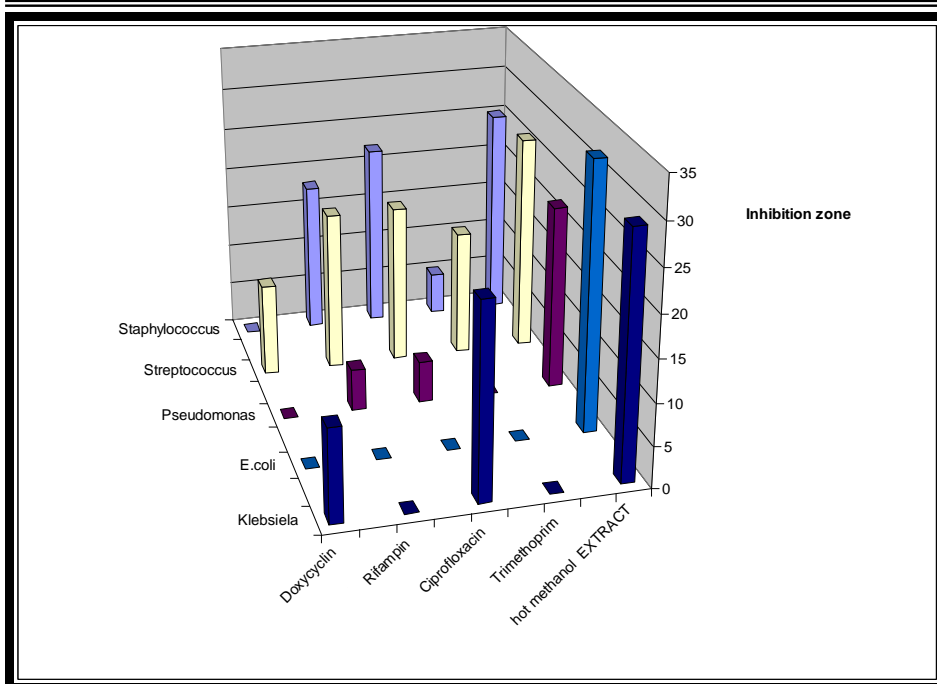


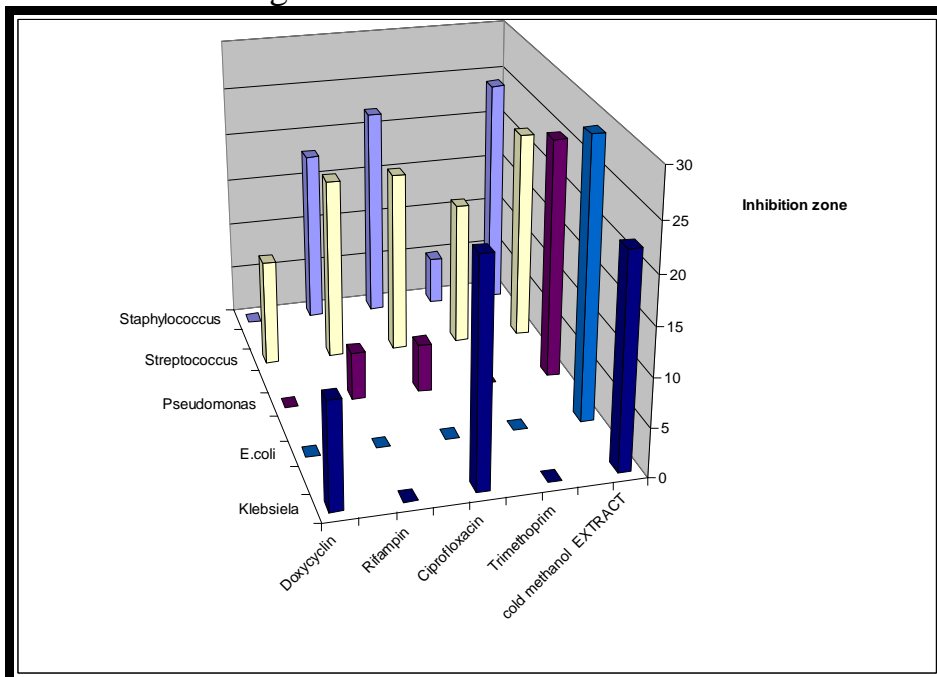
Fig.(1) Antibacterial activity of standard antibiotic against tested bacteria

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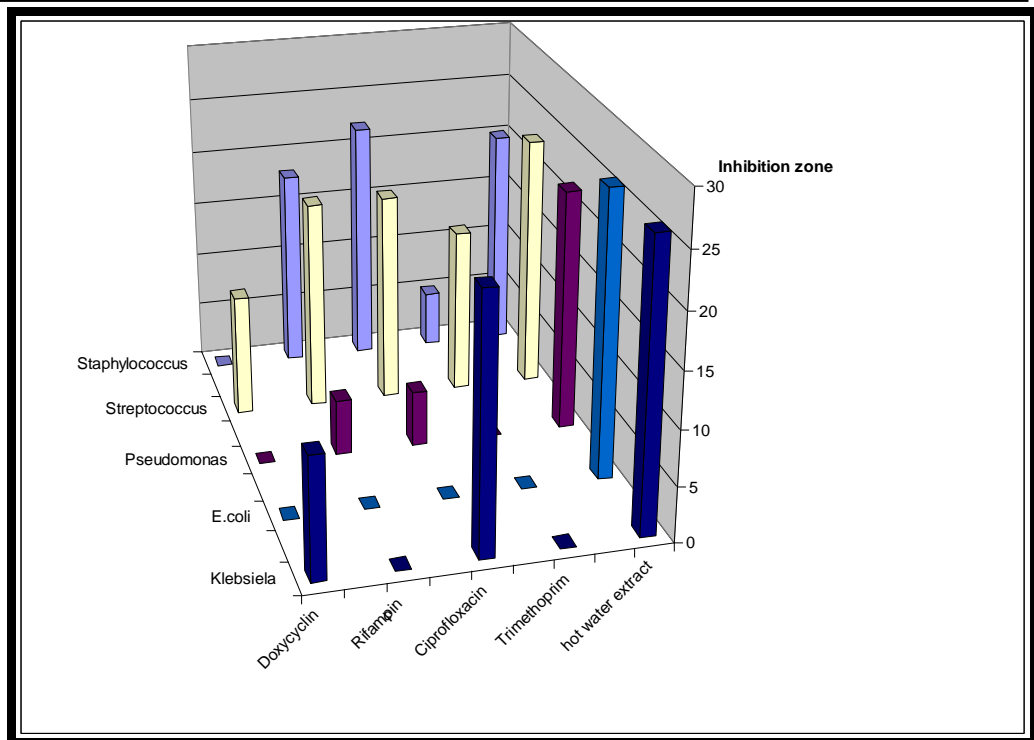
Fig(2) Antibacterial activity of hot methanolic extract and standard antibiotic against tested bacteria



Fig(3) Antibacterial activity of cold methanolic extract and standard antibiotic against tested bacteria

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Fig(4) Antibacterial activity of hot water extract and standard antibiotic against tested bacteria

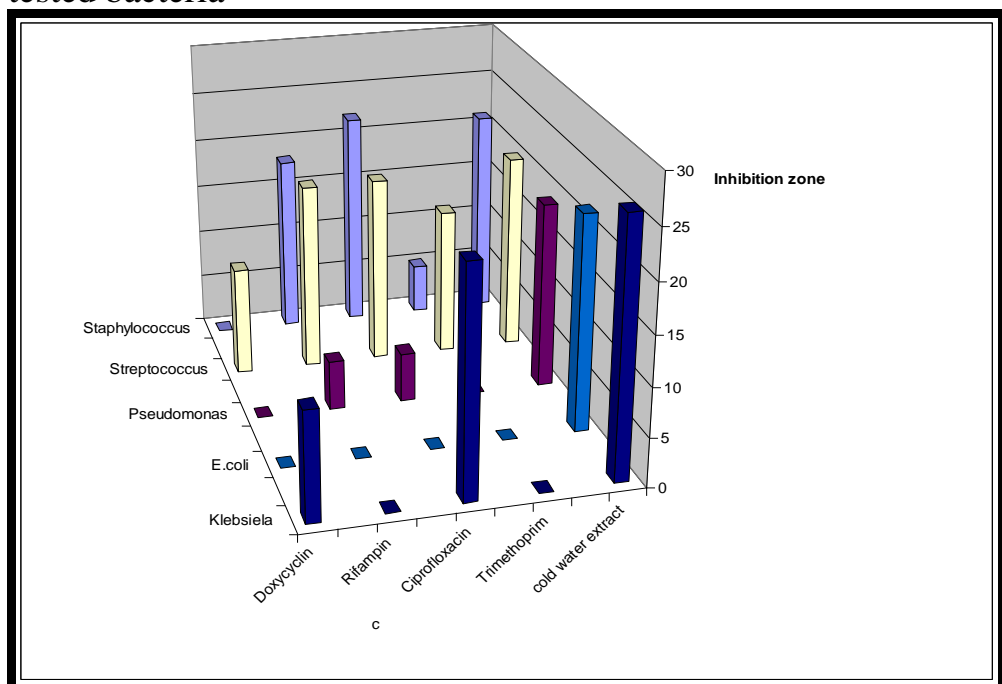


Fig.(5) Antibacterial activity of cold water extract and standard antibiotic against tested bacteria

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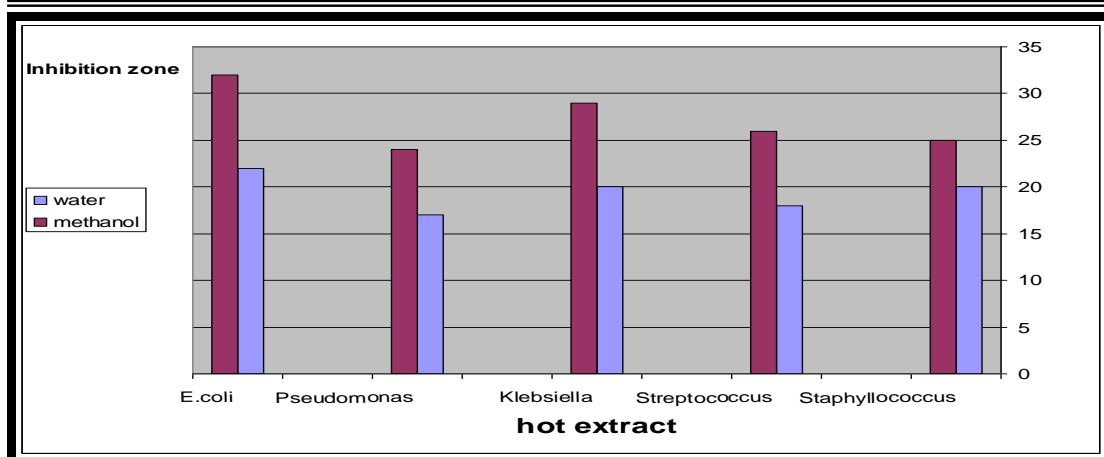


Fig.(6)Comparison the antibacterial activity of methanolic and water hot extract

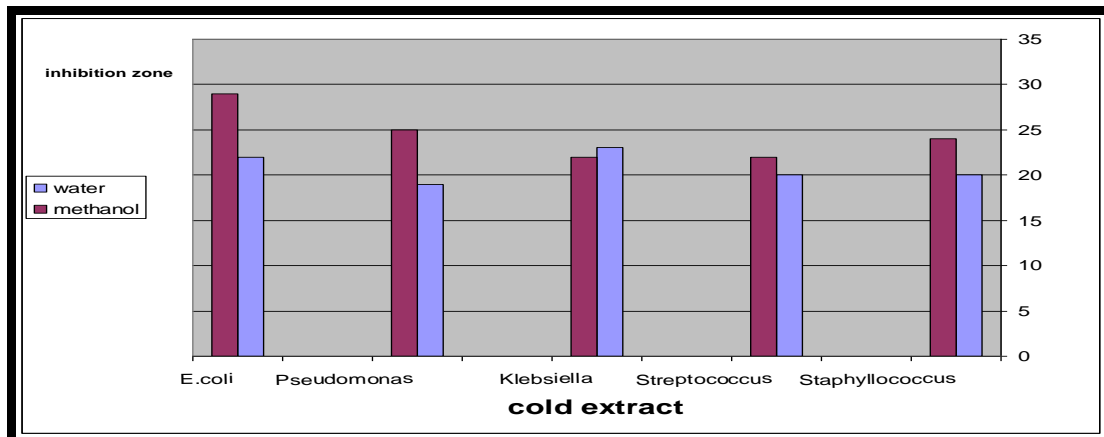
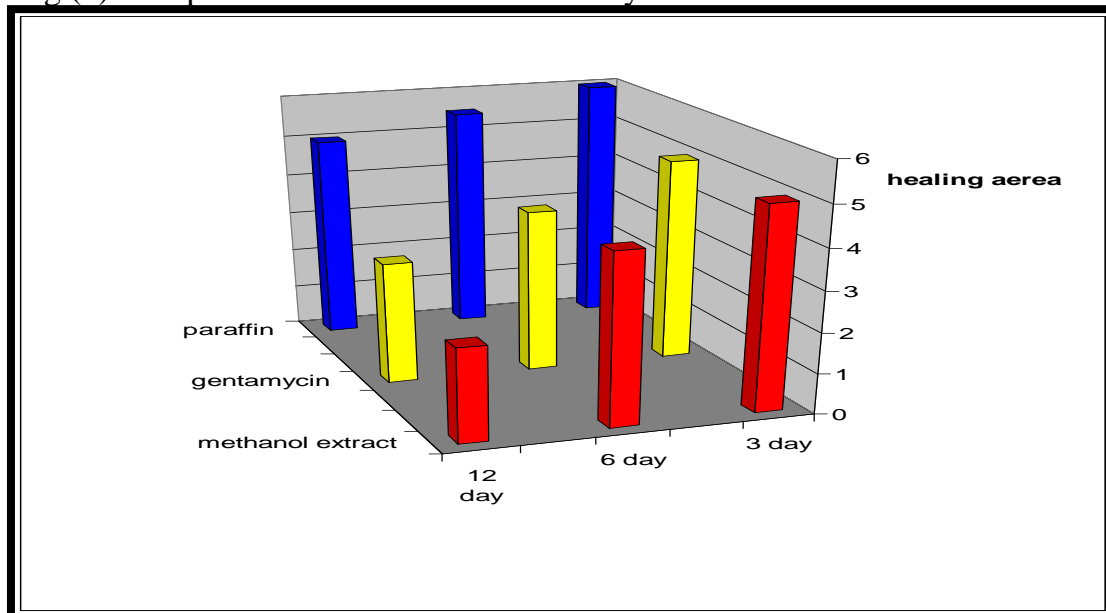


Fig.(7) Comparison the antibacterial activity of methanolic and water hot extract



Fig(8) Comparison of the healing activity between methanol extract gentamycin and semiliquid paraffin

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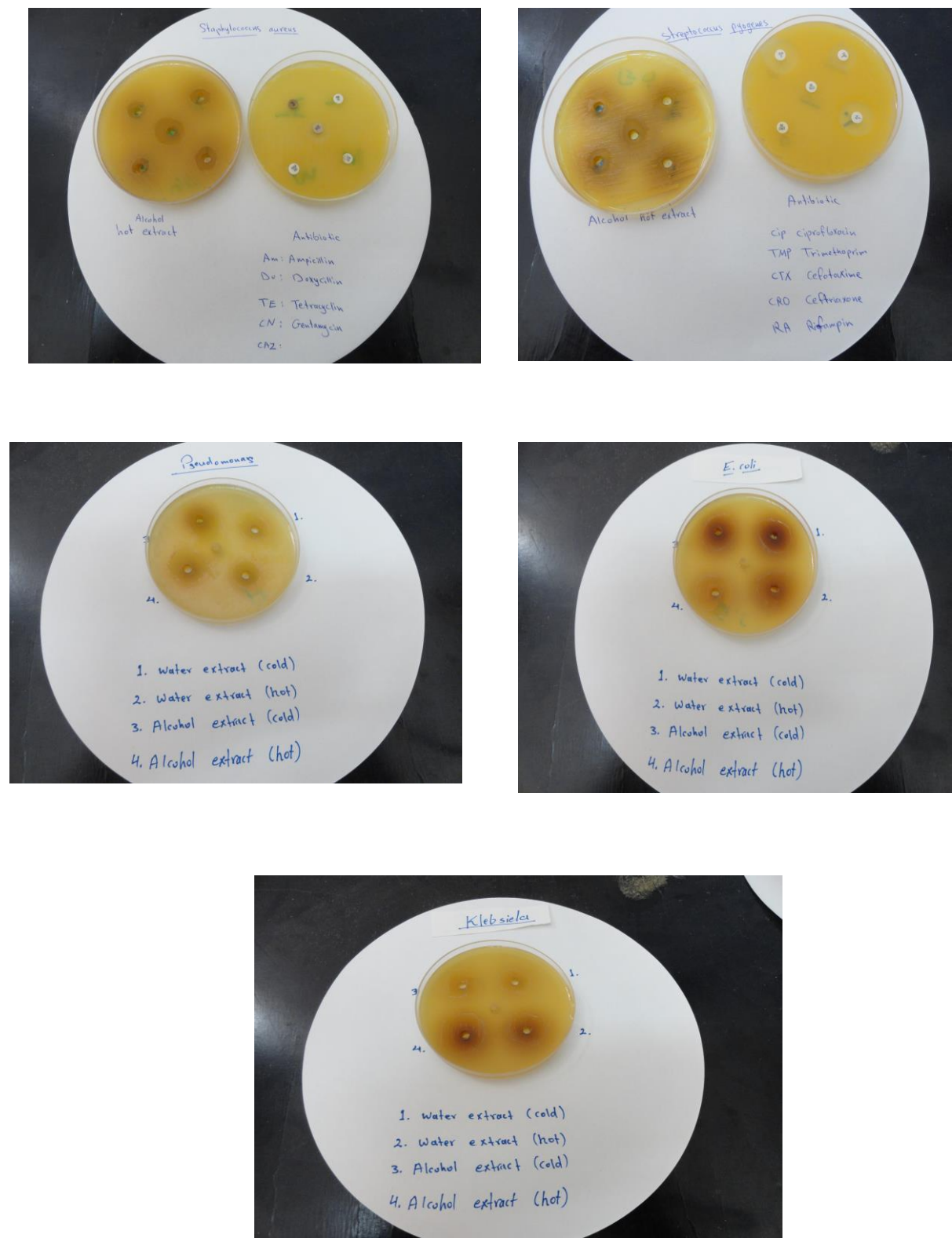


Fig.(9) Comparison the antibacterial activity of methanol and water extract with standard antibiotic

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تقييم الفعالية ضد بكتيرية ومعزز الشفاء لمستخلص *calendula officianalis* في الفئران

المختبرية *Mus musculus*

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الملخص:

الهدف من الدراسة الحالية هو لتقييم الفعالية ضد بكتيرية والفعالية المعززة لشفاء الجروح لمستخلص *calendula officianalis* وباستخدام انواع بكتيرية مختلفة والفئران المختبرية. اظهرت النتائج وجود زيادة معنوية $p \geq 0.5$ في الفعالية ضد بكتيرية لمستخلص الميثانول (الحار والبارد) وخاصة في بكتريا *E coli*, *klebsilla* كما اظهرت النتائج انخفاض معنوي في الفترة الزمنية لشفاء الجروح ومرهم الجنتاميسين خلال 12 يوم من المعاملة بينما بقي الجرح مفتوحا في المجموعة المعاملة بشمع البرافين كما وهناك اختزال معنوي في مساحة الجرح بدأ من اليوم الثالث في المجاميع المعاملة بالمستخلص الكحولي ومرهم الجنتاميسين وتأسيسا على هذه النتائج فان لمستخلص *calendula officianalis* فعالية عالية ضد بكتريا الجروح ويمكن ان يستخدم لمعالجة التهابات الجروح الناتجة من مقاومة العزلات للدوية ويمكن ان يكون نافعا لتطويع بدائل علاجية لالتهاب الجروح.