Antibacterial Activity of Calendula Officinalis Flowers In Vitro

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Abstract

Flower samples of Calendula officinalis were collected from the gardens of the college of Pharmacy in Baghdad city to examine their antibacterial activity .The effect of ethanol crude extract of petals and reproductive parts of flowers in different concentrations were obtained and bioassayed in vitro for its bioactivity to inhibit the growth of eight types of bacteria .The extracts of petals part were clearly superior for all bacteria especially the bacteria Pseudomonas aeruginosa (inhibition zone was 25mm in the concentration 100mg/ml) from gram negative bacteria, and Staphylococcus aureus (inhibition zone was 14mm in the concentration 50mg/ml) from gram positive bacteria, while the extracts of reproductive parts were less effective than petals part. These results confirm the antibacterial activity of Calendula officinalis flowers and support the traditional use of the plant in the therapy of bacterial infection.

Key words: antibacterial activity, petals part, reproductive parts

Introduction

The plant Calendula officinalis belongs to the family : Asteracea [1]. Commonly known as English marigold or pot marigold, field marigold , garden marigold, gold bloom, holligold, maravilla and, marybud [2].

Its name comes from the Latin word, Calends , the first day of every month, because of its long flowering period, because the flowers follow the sun , it was linked to the astrological sign of summer [3].

Its bright orange petals are visible in gardens in every month of year; such is its hardy nature and widely distributed through Europe and the Mediterranean. The orange petals and the whole flower head are used medicinally [4,5].

Marigold flowers often used in food industry for their nutritive qualities as well as coloring of several culinary products, is well known of centuries in popular medicine because of their biologic activities. Their use for the preparation of cosmetic products is well known also [6].

The major active constituents include saponins , triterpendiol esters and flavonoids including hyperoside and rutin, The orange flower contains a high content of caroteniodes including flavoxanthin and auroxanthin [7,8,9].

Calendula has antibacterial and antifungal activity [10,11] , and it has been used for the treatment of burns, abrasions, skin inflammations, ulcers, wounds and eczema[12]. It has been used internally for the treatment of gastiritis , bleeding of duodenal ulcers and colitis [13]

Researchers from Venezuela examined extracts of dried flowers from Calendula officinalis for its inhibitory effects on the human immunodeficiency virus type1(HIV-1) [14]

Calendula officinalis extracts show anti-cancer effects in vitro studies on tumor cell lines, derive from Leukemias, melanomas, fibrosacomas, breast, prostate, cervix, lung and pancreas [15].

Materials and Methods

Plant material

Flowers of Calendula officinalis were collected during April-May2009 from the garden of the college of Pharmacy in Baghdad city and was identified by Ali Abdul-hussein Al-Mosawi (Prof. in college of Sciences/University of Baghdad).

Extraction

Extraction was carried out at room temperature under normal conditions. Dried flowers of Calendula officinalis were powdered and subjected to extraction in a soxhlet apparatus at room temperature using ethanol.

The collected extracts were concentrated by evaporation under room temperature. The extracts were made in to suspensions using dimethyl sulfoxide solvent(DMSO) at concentrations (50,75,100) mg/ml from the petals part of flower and the same concentrations from the reproductive parts of flower [16].

Micro-organisms used

Gram-positive bacteria: Staphylococcus aureus, Bacillus subtilis , Staphylococcus epidermidis.

Gram-negative bacteria: Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae and Enterococcus pneumoniae.

Preparation of inoculum

Suspension of organism was prepared as per Mcfarland nephalometer standard[17].A 24hours old culture was used for the preparation of bacterial suspensions. The suspension of organism was made in a sterile isotonic solution of sodium chloride(0.9%w/v) and the turbidity was adjusted such that it contained approximately $1.5x10^8$cells/ml.

Agar well diffusion method

The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at 121˚c for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 160˚c for (1.5) hour .30ml of sterile nutrient agar was seeded by organisms (about 2ml according to Mcfarlands standard). Pores

were made on the medium using a sterile borer and 0.1ml of the extracts were added to respective pore. The Petri plates seeded with organisms containing extracts were kept in refrigerator at 4˚c for 1hour to facilitate the diffusion of the extracts into the media. After diffusion the Petri plates were incubated at 37˚c for 24 hours in an incubator and zone of inhibition was observed and measured using a scale. The results of the antibacterial activity of petals and reproductive parts of flower of Calendula officinalis are tabulated in Table(1)and Table(2).

Results and Discussion

The essential oil of the flowers inhibited the growth in vitro of Gram positive bacteria such as Bacillus subtilis and Staphylococcus aureus ,and Gram negative bacteria such as Escherichia coli and Pseudomonas aeruginosa ,these results are in agreement with some previous studies [7,18,19]. Antibacterial activity is recorded when the zone of inhibition is greater than 6mm[20].

Results as shown in (Table1) indicated the antibacterial activity of petals of Calendula officinalis to all isolates in all concentrations except the concentration (100mg/ml) can not inhibit the growth of Staphylococcus epidermidis ,Bacillus subtilis and Proteus mirabilis. The maximum inhibition zone was found in(100mg/ml) concentration and it was 25 mm in the bacterial isolate Pseudomonas aeruginosa while the minimum inhibition zone in the same concentration was 8 mm for the isolate Enterococcus pneumoniae.

In (Table2) the results shows that the antibacterial activity of reproductive parts of Calendula officinalis is less than the petals and it has no effect in all concentrations against the isolate Enterococcus pneumonia ,and also indicated that the maximum inhibition zone was observed in Pseudomonas aeruginosa in the concentration (100mg/ml) and it was 24 mm while the concentrations (50mg/ml,75mg/ml) has no effect against this bacterium , the minimum inhibition zone was 7 mm in Staphylococcus epidermidis in the concentration (50mg/ml) and there was no effect at the concentration (100mg/ml) against this bacterium .From (Table 1) and (Table 2) its clear that the effective antibacterial activity of Calendula officinalis flower concentrated in their petals more than the reproductive parts and this confirm by previous study [8].

It is not possible to make a direct correlation between the observed activity of the plant extracts in vitro and the actual effects when used in vivo for the diseases observed by the indigenous people and traditional healers [19] . Therefore , it is important that the plant should also be further investigated to evaluate the significance of these extracts , clinical role and the medical system of indigenous people . Additional deep research is necessary to isolate and characterize their active compounds for pharmacological testing.

Conclusions

Its clear that Calendula officinalis flowers as an extract may be useful as an antibacterial agent against the above mentioned bacteria . Among the organisms tested Pseudomonas aeroginosa was more susceptible to the ethanolic extract of Calendula officinalis flower . Further pharmalogical and clinical studies are required to understand the mechanism and the actual efficacy of this herb extract in treating various infections.

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Table (1) : In vitro antibacterial activity of petals part of Calendula officinalis flower

Bacteria	Concentration of the extract	Zone of inhibition(mm, diameter)
Bacillus subtilis	50mg/ml	12
	75mg/ml	13
	100mg/ml	–
Staphylococcus aureus	50mg/ml	14
	75mg/ml	12
	100mg/ml	6
Staphylococcus epidermidis	50mg/ml	10
	75mg/ml	12
	100mg/ml	–
Escherichia coli	50mg/ml	15
	75mg/ml	14
	100mg/ml	12
Klebseilla pneumoniae	50mg/ml	13
	75mg/ml	14
	100mg/ml	20
Pseudomonas aeruginosa	50mg/ml	15
	75mg/ml	17
	100mg/ml	25
Proteus mirabilis	50mg/ml	11
	75mg/ml	12
	100mg/ml	–
Enterococcus pneumoniae	50mg/ml	18
	75mg/ml	12
	100mg/ml	8

Table (2) : In vitro antibacterial activity of reproductive parts of Calendula officinalis flower

Bacteria	Concentration of the extract	Zone of inhibition(mm ,diameter)
Bacillus subtilis	50mg/ml	6
	75mg/ml	8
	100mg/ml	–
Staphylococcus aureus	50mg/ml	10
	75mg/ml	8
	100mg/ml	–
Staphylococcus epidermidis	50mg/ml	7
	75mg/ml	8
	100mg/ml	–
Escherichia coli	50mg/ml	10
	75mg/ml	12
	100mg/ml	5
Klebseilla pneumoniae	50mg/ml	13
	75mg/ml	12
	100mg/ml	11
Pseudomonas aeruginosa	50mg/ml	–
	75mg/ml	–
	100mg/ml	24
Proteus mirabilis	50mg/ml	8
	75mg/ml	10
	100mg/ml	–
Enterococcus pneumoniae	50mg/ml	–
	75mg/ml	–
	100mg/ml	–

التأثير الضد بكتيري لنبات الاقحوان Calendula officinalis خارج الجسم الحي

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الخلاصة

جمعت ازهار نبات الاقحوان Calendula officinalis من حديقة كلية الصيدلة في مدينة بغداد لفحص نشاطها الضد بكتيري . تم الاختبار الحيوي خارج الجسم الحي لمعرفة تأثير مستخلصات الكحول للبتلات والاجزاء التكاثرية للنبات بتراكيز مختلفة لتثبيط نمو 8 انواع بكتيرية.

مستخلصات البتلات كان لها تاثير أعلى وأوضح من تلك للاجزاء التكاثرية ولاسيما بكتريا Pseudomonas auroginosa منطقة التثبيط كانت (25 ملمتر عند التركيز 100 ملغم/مليلتر) من البكتريا السالبة لصبغة كرام وبكتريا Staphylococcus aureus منطقة التثبيط كانت (14 ملمتر عند التركيز 50 ملغم/مليلتر) من البكتريا الموجبة لصبغة كرام . هذه النتائج تؤيد النشاط الضد بكتيري لأزهار Calendula officinalis وتدعم الأستعمال التجاري للنبات في علاج الاصابات البكتيرية.

الكلمات المفتاحية: النشاط الضدبكتيري، البتلات، الاجزاء التكاثرية.