ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF CRUDE EXTRACTS OF *CALENDULA OFFICINALLIS*

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Abstract: Burn wound infections are problematic because it delays healing and encourages scarring and may also result in bacteraemia, sepsis or multiple organ dysfunction syndrome, Bacteria and fungi are most common pathogens colonizing burn wounds. Calendula (Asteraceae) is an aromatic annual herb, which is used in traditional system of medicine to treat various diseases like anti-inflammatory, antispasmodic, antitumor. In the present study antibacterial and antioxidant activity of leaves and floral extracts of Calendula species were determined. The petroleum ether, alcohol and water extracts of leaves and flowers were used. The skin pathogens were isolated from clinical sample obtained from hospitals. The antibiotic sensitivity pattern was determined. The isolates were obtained and identified using morphological, cultural and biochemical characteristics. The antibacterial activity was assessed using modified agar cup method. Although all the extracts showed significant antibacterial activity, highest antibacterial activity was observed in alcohol extract of flowers of calendula. The antioxidant activity was determined using DPPH method. All the extracts have good antioxidant activity. Thus these extract would be an ideal alternative that could be used in therapeutic preparation against skin infections.

Keywords: Calendula, Antibacterial, Antioxidant, Agar cup method, DPPH method.

Introduction: Inspite of chemotherapeutic revolution, plant origin drugs are preferred over synthetic ones because of emergence and spread of multidrug resistant pathogens. [1]. Beneficial effects of medicinal plants include healing of wounds, burn, injuries, antifungal, antibacterial, antiviral and anticancerous activities [2]. *Calendula officinalis Linn.* (Asteraceae), commonly known as pot Marigold is an important medicinal plant used in our traditional system of medicine to treat various diseases. The plant is rich in many pharmaceutical active ingredients like flavanoids, carotenoids, glycosides and phenols [3].Calendula is used in ayurveda for treatment of fever and cancer [4]. Calendula has antibacterial and antifungal activities and it has been used for the treatment of burns, abarasions, skin inflammation, ulcers, wounds and eczema [5]. It has been used internally for the treatment of gastritis, bleeding of duodenal ulcers and colitis [6]. Marigold flower is often used in food industry for their nutritive qualities as well as colouring of several culinary products. Their use for the preparation of cosmetic product is also well known [7]. Calendula tea is used as eyewashes, gargles or compresses to treat conjunctivitis, pharyngitis, gingivitis, diaper rash and other inflammatory conditions of the skin and mucous membranes [8]. Calendula cream is also a favourite homeopathic remedy to treat abarasions and minor burns [9].

Antioxidants are extensively studied for their capacity to protect organisms and cell from damage that is induced by oxidative stress [10]. There are number of synthetic antioxidants like

butylated hydroxyl anisole, butylated hydroxyl toluene, propyl gallate and gallic acid esters, which are available but are suspected to cause negative health effects and are also unstable at elevated temperatures[11]. Hence, the objective of present study is to determine the antibacterial and antioxidant activity of *Calendula offcinallis*.

Material and Method:

Method of extraction:

The flowers and leaves from plant of *Calendula officinalis* were collected, cleaned and dried in oven at 50°c. The dried leaves and flowers were pulverized by mechanical grinder and passed through mesh sieve. Powdered material were mixed with respective solvents like petroleum ether, ethanol, and water and kept on shaker for 24 hours at room temperature. The extracts were filtered and evaporated and concentrated at 45°c [3, 12].

Microorganisms used:

Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus spp. Escherichia coli, Salmonella typhi and *Pseudomonas aeruginosa* were isolated from patients of wound infections from hospitals and pathological laboratories. The isolates were identified by studying morphological, cultural and biochemical characteristics [13]. Their antibacterial susceptibility pattern was done by Kirby Bauer method and most resistant pathogens were selected [14]. The MAR index of each isolate is calculated (Multiple Antibiotic resistant) and only most resistant bacterias are selected.

Antimicrobial screening of extracts:

The antimicrobial potential of the above plant extracts was seen against the test organisms using agar well diffusion susceptibility test. 20 ml of sterile molten Muellar and Hinton ager was bulk seeded with test culture (2 ml of cultures according to Mcfarlands standard). Wells of 6mm were made in the medium using a sterile borer and 65 µl of the extracts were added to respective wells. The petri plates seeded with organisms containing extracts were incubated at 37°c for 24 hours after prediffusion. The zone of inhibition was measured [15]. The extracts that showed antibacterial activity were subjected to minimum inhibitory concentration (MIC) assay using agar dilution method [16]. MIC was interpreted as the lowest concentration of the extracts showing inhibition of growth of cultures.

Antioxidant activity:

The in vitro antioxidant activity of test extracts was estimated using standard 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) method. The reaction mixture contained 1ml of 0.1 mM solution of DPPH added to 3 ml of extract in methanol at different concentrations. The mixture was then vigorously shaken and incubated at 37°c for 30 minutes, later the absorbance was measured at 517 nm. A blank was prepared without adding extract. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity [17].

Result and Discussion:

The clinical isolates used for the study are multiple drug resistant with MAR index greater than 80% (resistant to more than 80% of antibiotics used for the study). The sensivity pattern of each isolate was determined using 10 standard antibiotics results are shown in Table 1.

Table 1: Antibacterial susceptibility pattern of Clinical isolates using Disc diffusion method

Bacteria	Antibiotics µg/ml									
	ER-15	ST - 10	AX - 10	CR-30	VA-30	NX-10	NA-30	KA-30	CT-30	PG-10 units
S.aureus	R	R	R	R	I	R	R	R	R	R
S.epidermidis	R	R	R	R	I	R	R	R	R	R
Streptococcus	R	R	R	R	R	R	R	R	R	R
P. aeruginosa	R	R	R	R	R	R	I	R	R	R
E. coli	R	R	R	R	I	R	R	R	R	R
S.typhi	R	R	R	R	I	R	R	R	R	R

Key: 'R' = Resistant, 'I' = intermediately sensitive

ER = Erythromycin, ST = Sterptomycin,

AX = Amoxycillin, CR = Chloramphenicol,

VA = Vancomycin, NX – Norfloxacin,

NA = Naladixic acid, KA = Kanamycin

CT = Cephotaxime, PG = Penicillin

The antibacterial assay showed that water, ethanol and petroleum ether extracts of *Calendula officinalis* leaves and flowers exhibited in vitro antibacterial activity against both Gram positive and Gram negative bacteria. Minimum inhibitory concentration of the active extracts against the clinical isolates is shown in Table2.

Table2: Minimum Inhibitory Concentrations of *Calendula officinalis* Flower & Leaves extracts

Bacteria	MIC of extracts mg/ml					
	Flower			Leaves		
	Aquoeus extract	Ethanol extract	Petroleum ether extract	Aquoeus extract	Alcohol extract	Petroleum ether extract
S.aureus	50	30	50	200	200	200
S.epidermidis	50	30	50	200	200	200
Streptococcus	100	50	100	200	200	200
P. aeruginosa	200	100	200	300	300	300
E. coli	50	50	100	300	300	300
S.typhi	200	100	200	300	300	300

The lowest MIC values were observed for ethanol extract of *Calendula officinalis* i.e 30 mg/ml against *S. aureus* and *S. epidermidis,* 50 mg/ml against *streptococcus* and *E.coli*. and, 100 mg/ml for *S. typhi, p. aeruginosa.* Aqueous and Petroleum ether extracts of Calendula flower also had similar MIC as ethanol

extract except for *S.typhi* and *P. aeruginosa* i.e 200 mg/ml. These results of antibacterial activity are in agreement with results of Hamad et al

[18]. The calendula leaves extract are also significantly effective against both Gram positive and Gram negative bacteria but at higher concentrations. MIC was 200 mg/ml for *S. aureus, S. epidermidis, streptococcus*, *E. coli* and 300 mg/ml for *S. typhi* and *P. aeruginosa* [19].

Alcohol, petroleum ether and water extracts of leaves and flowers of *Calendula officinalis* exhibited potent antioxidant activity when DPPH radical was used as a substrate to evaluate the free radical scavenging activity. The antioxidant reacted with DPPH, a purple colour stable free radical which accepts an electron or hydrogen radical to become a stable diamagnetic molecule [20].

The best antioxidant activity observed is for flower extract of Calendula with complete colour reduction at minimum concentration of 50 mcg/ml as compared to leaves extracts effective at concentration of 100 mcg/ml . Detail results of all extracts are given in Table 3.

Table 3: Antioxidant activity of *Calendula officinalis* Flower & Leaf extracts

Plant parts	Extracts	Minimum effective concentration µg/ml
Flower	Aquoeus extract	50
	Ethanol extract	50
	Petroleum ether extract	50
Leaves	Aquoeus extract	100
	Ethanol extract	100
	Petroleum ether extract	100

Conclusion: The result clearly indicates that all extracts of *Calendula officinalis* leaves and flowers posses broad spectrum antibacterial activity. Ethanolic extract of flower has best activity among all the extracts. Floral extracts are more effective than leaves extracts [18, 21]. Presence of phenolic compounds in these test extracts make them strong free radical scavenger, which indicates that these plants can be a good source of natural antioxidants. Therefore further investigations are required to explore the parameters essential for formulation so that antibacterial and antioxidant potential of these calendula extracts can be utilised for development of topical herbal formulations for treatment of skin pathogens.

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