Original Article

Antibacterial and antioxidant activity of flower extracts of aster and calendula sp. against skin pathogens

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Abstract

Wound infections are problematic because it delays healing encourages scarring and may result in bacteraemia, sepsis or multiple organ dysfunction. Bacteria are most common pathogens colonizing wounds. Aster (*Callistephus chinenesis*) and *Calendula officinalis* (Asteraceae) is an aromatic annual herb, which is used in traditional system of medicine to treat various diseases like anti-inflammatory, antispasmodic and antitumor activity. In the present study antibacterial and antioxidant activity of cold floral extracts of Aster and Calendula species are determined. The skin pathogens were isolated from clinical sample of various types of skin aliments. The antibiotic sensitivity pattern was determined. The selected isolates were resistant to various antibiotics like Tetracycline, Erythromycin, Penicillin, Amoxicillin, Naladixic acid and Ciprofloxacin. The antibacterial activity was assessed using modified agar cup method followed by Agar dilution method. Although all the extracts showed significant antibacterial activity but highest antibacterial activity was observed with cold water and alcohol extract of Aster followed by Calendula. The antioxidant activity was determined using DPPH method. All the extracts showed significant antioxidant activity. Thus, these extract would be an ideal alternative that could be used in therapeutic preparation against skin infections.

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

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INTRODUCTION

With the emergence and spread of multidrug resistant pathogens, skin and soft tissue infections are posing a great challenge. To start empirical therapy it is important to know prevailing susceptibility pattern and alternate medicine[1]. Excessive use of drug results in early development of bacterial resistance[2]. Plant extracts have been used for a wide variety of purposes for thousands of years[3] Beneficial effects of medicinal plants include healing of wounds, burn, injuries, antifungal, antibacterial, antiviral and anticancerous activities[4]. *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 flavonoids, carotenoids, glycosides and phenols[5]. Calendula is used in Ayurveda for treatment of fever and cancer[6]. Calendula has antibacterial and antifungal activities and it has been used for the treatment of burns, abrasions, skin inflammation, ulcers, wounds and eczema[7]. Aster is perennial ornamental herbused as expectorant, stimulant with antifungal and antibacterial activity[8]. Aster is among the 112 Chinese medicines associated with anticancerous activity[9]. Asteraceae flowers are 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[10]. Antioxidants are extensively studied for

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their capacity to protect organisms and cell from damage that is induced by oxidative stress[11]. 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[12]. Hence, the objective of present study is to determine the antibacterial and antioxidant activity of *Callistephus chinenesis* and *Calendula offcinallis*. These plants extracts were tested for their efficacy to inhibit the causativeagents of various skin infections using Agar dilution method. Preliminary phytochemical analysis was done to correlate with antioxidant activity.

MATERIAL AND METHOD

Collection of plant material

Fresh plants were collected from More Nursery (Vangani). The taxanomic identification of these plants was done by Dr. Pravin, Blatter Hebarium, St. Xaviers College, Mumbai. The voucher specimens were preserved.

Method of extraction

The flowers from plant of *Calendula officinalis*and *Callistephus chinensis*were collected, cleaned and dried in oven at 50^{o}c. The dried flowers were pulverized by mechanical grinder and passed through mesh sieve. Powdered material were mixed with respective solvent 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^{o}c[5,13]

Microorganisms used

Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae Escherichia coli, Salmonella typhi and *Pseudomonas aeruginosa* were collected from patients suffering from various types of skin infections. Various hospitals, clinics and pathological laboratories were approached for the same. The isolates were identified by studying morphological, cultural and biochemical characteristics[14].

Antibioticsensitivity test

The antibiotic sensitivity of the organism was tested using the "Agar diffusion method"[15]. The MAR (Multiple Antibiotic Resistance index of each isolate was calculated and only the most resistant bacteria were selected for the study.

Antibacterial screening of extracts

The antimicrobial potential of the above plant extracts was tested against the test organisms using agar well diffusion test. 20 ml of sterile molten Muellar and Hinton agar was bulk seeded with test culture (0.2 ml of cultures according to Mcfarlands standard). Wells of 6mm are made on the medium using a sterile borer and 65 µl of the extracts were added to respective pores. The petri plates seeded with organisms containing extracts were incubated at 37^{o}c for 24 hours after prediffusion. The zone of inhibition was measured[16]. The extracts that showed antibacterial activity were subjected to minimum inhibitory concentration (MIC) assay using agar dilution method[17]. MIC was interpreted as the lowest concentration of the extracts showing inhibition of growth of cultures.

Antioxidant activity

The invitro antioxidant activity of test extracts was estimated using standard 1, 1- diphenyl-2-picryl-hydrazyl (DPPH) method. The reaction mixture contains 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^{o}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[18,19].

Preliminary phytochemical analysis

Qualitative phytochemical analysis of crudepowder of flowers of both plants was used for determining presence of Tannins, Alkaloids, Flavonoids, Glycosides and Steroids[20]. The presence of above phytochemicals was further confirmed by TLC (Thin Layer Chromatogrophy). Silica gel 60 F254 TLC aluminium, Merck was used to perform analysis[21].

OBSERVATION AND RESULT

Out of 42 resistant isolates collected from more than 50 samples of skin infections like impetigo, carbuncle, burn wounds, sepsis, cellulitis, impetigo etc, the most multiple antibiotic resistant isolates having MAR index greater than 0.8 were selected for the study as shown in Table 2. The organisms mainly associated with skin infections were found to be *Staphylococcus aureus, Psedomonas aeruginosa*, and *E.coli*. Klebsiella and Salmonella species may be the secondary invaders.The qualitative phytochemical analysis of the plant extracts was carried out. The results are as shown in Table 1. The presence of respective phytoconstituents was confirmed using Thin Layer Chromatography. The MIC of all the extracts were carried out within the range of 1 to 10 mg/ml. Appropriate medium control and solvent controls were maintained, which showed complete growth of organisms. The antibacterial assay showed that water, ethanol and petroleum ether extracts of *Calendula officinalis* and *Callistephus chinensis*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. The lowest MIC values were observed for ethanol extract of *Calendula officinalis* i.e. 5

mg/ml against *S. aureus, S. epidermidis,* and *E.coli*. Whereas 7-10 mg/ml for *K.pneumoniae*, *S. typhi,*and *P. aeruginosa.* Aqueous and Petroleum ether extracts of Calendula flower has MIC within the range of 10 - 20mg/ml. The antioxidant activities observed with complete colour reduction at minimum concentration of extracts by DPPH method are given in Table 3.

Table 1: Phytoconstituents of Aster and Calendula

	Family	Parts used	Phytochemical Analysis					
			Saponins	Alkaloids	Flavonoids	Glycosides	Tannins	Steroids
Aster	Aster-aceae	Flower	+	+	+	+	+	+
Calendula	Aster-aceae	Flower	-	+	+	+	+	-

Table 2: 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
Klebsiella sp	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 G.

Table 3: Minimum Inhibitory Concentration Flower extracts

Bacteria	MIC of extracts mg/ml					
	Callistephus chinensis			*Calendula officinalis*		
	Aquoeus extract	Ethanol extract	Petroleum ether extract	Aquoeus extract	Ethanol extract	Petroleum ether extract
S.aureus	4	5	12	14	4	15
S.epidermidis	4	4	12	14	4	15
Klebsiella sp.	6	6	18	16	8	20
P. aeruginosa	6	6	18	17	7	20
E. coli	5	5	15	15	6	16
S.typhi	10	10	18	15	10	20

Table 4: Antioxidant activity of flower extracts

Plants	Extracts	Minimum effective concentration µg/ml
Calendula officinalis	Aquoeus extract	50
	Ethanol extract	50
	Petroleum ether extract	50
Callistephus chinensis	Aquoeus extract	30
	Ethanol extract	30
	Petroleum ether extract	30

DISCUSSION

The results of antibacterial activity are in agreement with M.H Hamad *et al*[22,23]. The *Callistephus chinensis*aqueous and ethanol flower extract showed significantly efficacy against both Gram positive and Gram negative bacteria at MIC concentrations of 4 mg/ml for *S. aureus, S. Epidermidis* and *E. Coli.*and 6 mg/ml for *K.pneumonia, P. aeruginosa* and *10mg/ml* for *S.typhi.*[24]. All alcohol, petroleum ether and water extracts of flowers of *Calendula officinalis* and *Callistephus chinensis* 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 molecule[25].

CONCLUSION

The result clearly indicates that all extracts of *Calendula officinalis*and *Callistephus chinensis*posses broad spectrum antibacterial activity against microorganisms

responsible for common skin infections. Ethanolic and aqueous flower extract have better activity as compared to petroleum extracts. Presence of Alkaloids, Flavonoids and Glycosides 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 *Calendula officinalis* and *Callistephus chinensis* extracts can be utilised for development of topical herbal formulations for treatment of skin pathogens.

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