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Antimicrobial activity of aqueous, acetone and methanol extracts of *Calendula officinalis* L. (Marigold) flower

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ABSTRACT

Calendula officinalis flowers were collected from the local market of Bhopal, M.P. to examine their antibacterial activity. The effect of aqueous, acetone and methanol extracts of petals of flowers was assayed in vitro on five bacteria namely Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella spp. and Pseudomonas aeruginos. All the extracts were found to show good antibacterial activity as clear growth inhibition zone were seen in all the inoculated plates. Staphylococcus aureus gave 26 mm, 15 mm and 14 mm growth inhibition zone with methanol, acetone and aqueous extract respectively. Bacillus cereus gave 10 mm, 16 mm, and 13.5 mm growth inhibition zone with methanol, acetone and aqueous extract respectively. E. coli gave 8.5 mm, 14 mm and 8 mm growth inhibition zone with methanol, acetone and aqueous extract respectively. Klebsiella spp gave 6 mm, 6.5 mm and 10 mm growth inhibition zone with methanol, acetone and aqueous extract respectively. Pseudomonas aeruginosa gave 8.5 mm, 10 mm and 8 mm growth inhibition zone with methanol, acetone and aqueous extract respectively. These results confirm the antibacterial activity of Calendula officinalis flowers and support the traditional use of the plant in the therapy of bacterial infections.

Keywords*: Antibacterial, Calendula officinalis, infection, methanol*

INTRODUCTION

Antimicrobial of plant origin has numerous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials[1]. The beneficial medicinal effects of plant materials typically results from the combination of secondary compounds present in the plants. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are capable of producing definite physiological action on the body (Kumar *et al.*, 2010). Compounds extracted from different parts of the plants can be used to cure diarrhea, dysentery, cold, cough, cholera, fever, bronchitis, etc[7].

Calendula (*Calendula Officinalis*) commonly referred to as Marigold; this annual herb is from the Family Asteraceae, the Sunflower Family[6]. It is made up of single, yellow to bright orange colored flowers that capture the strength and heat of the sun as it rises in the morning. The Calendula plant is comprised of carotenoids, flavonoids and essential oils. It has been widely used in homeopathic medicine for the treatment of many diseases[14]. This gentle and nourishing botanical possesses powerful healing and anti-inflammatory properties[3]. *C. officinalis* includes a high number of carotenoids such as flavoxanthin, lutein, rubixanthin, b-carotene, g-carotene, and lycopene[12]. It is treasured for its antifungal[9,10] antibacterial[4,13] antioxidant[11]antiviral[2] and antiseptic properties.

Calendula officinalis has now been placed in the books of cancer and anticancer cures. The present study aims to screen and evaluate antimicrobial activity of aqueous, acetone and methanol extracts of *Calendula officinalis* L. flower against bacterial pathogens.

MATERIALS AND METHODS

Collection of plant material:

Calendula flowers (*Calendula Officinalis*) were purchased from the local market in Bhopal, (M.P. India)

Preparation of plant extract:

Crude aqueous extract: 500 gm of fresh flower petals was weighed and homogenized with 500 ml of distill water. The mixture was filtered and filtrate was collected and centrifuged at 5000 rpm for 15 min at room temperature. Supernatant was collected and allowed to air dry under sterilized conditions and then the residue obtained was mixed with 10% dimethyl sulphate (DMSO) solution to obtain a final concentration of 0.5 mg/ml.

Acetone extract: 500 gm of fresh flower petals was weighed and homogenized with 500 ml of 95% acetone. The mixture was filtered and filtrate was collected and centrifuged at 5000 rpm for 15 min at room temperature. Supernatant was collected and allowed to air dry under sterilized conditions and then the residue obtained was mixed with 10%dimethyl sulphate (DMSO) solution to obtain a final concentration of 0.5 mg/ml.

Methanol extract: 500 gm of fresh flower petals was weighed and homogenized with 500 ml of 95% methanol. The mixture was filtered and filtrate was collected and centrifuged at 5000 rpm for 15 min at room temperature. Supernatant was collected and allowed to air dry under sterilized conditions and then the residue obtained was mixed with 10%dimethyl sulphate (DMSO) solution to obtain a final concentration of 0.5 mg/ml.

Microorganisms used:

Clinical isolates of pathogenic bacteria viz *Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella spp.* and *Pseudomonas aeruginosa* were stored at 4ºC.

Antimicrobial assay:

Agar disc diffusion method was used for screening of antibacterial activity of aqueous acetone and methanol extracts of *Calendula officinalis* L. flowers (Efstratios *et al.,* 2012). Bacterial cultures were spread on to nutrient agar (NA) medium. Paper discs were separately impregnated with 25µl of the 0.5 mg/mL plant extracts and placed on the inoculated agar plates. All the plates were allowed to stay at room temperature for 30 min to allow diffusion of the extract then incubated at 37ºC for 24 hrs.

RESULTS AND DISCUSSION

The aqueous, acetone and methanolic extracts of fresh flower petals were tested against different bacterial pathogens i.e. *Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella spp.* and *Pseudomonas aeruginosa.* They were found to be effective against all the bacterial pathogens. Varying degree of antimicrobial activity was shown by the growth inhibition zones. For aqueous extract bacterial pathogens showed growth inhibitory zones of diameter ranged from 8-13.5 mm. For acetone extract bacterial pathogen showed growth inhibitory zones of diameter ranged from 6.5-16 mm. For acetone extract bacterial pathogen showed growth inhibitory zones of diameter ranged from 6-26 mm. The result of the antimicrobial activity is shown in table 1.

Table 1: Zone of inhibition (mm) by extracts of fresh flower in aqueous, acetone and methanol against bacterial pathogens

S. No.	Bacterial strains used	Diameter of zone of inhibition		
		Aqueous	Acetone	Methanol
1	*Staphylococcus aureus*	14 ± 1	15± 1	26 ± 1
2	*Bacillus cereus*	13.5 ± 1	16 ± 1	10 ± 1
3	*E. coli*	8 ±1	14 ± 1	8.5 ± 1
4	*Klebsiella spp*	10 ± 1	6.5 ±1	6 ± 1
5	*Pseudomonas aeruginosa*	8 ± 1	10 ± 1	8.5 ± 1

CONCLUSION AND FUTURE SCOPE

The results of the present study signify that the aqueous, acetone and methanol extracts of the *Calendula officinalis* petals possessed good antimicrobial activity against *Staphylococcus aureus, Bacillus cereus, E. coli, Klebsiella spp.* and *Pseudomonas aeruginosa*. Further pharmacological and clinical studies are required to understand the mechanism of action and the actual efficacy of these herbal extracts in treating various bacterial diseases.

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