



**A COMPARATIVE STUDY ON ANTIMICROBIAL EFFECT OF AQUEOUS, ACETONE
AND ETHANOL EXTRACTS OF CALENDULA OFFICINALIS L. (MARIGOLD)
FLOWER**

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ABSTRACT

Infectious diseases have always been one of the important concerns of human and have continuously attracted the attention of a large number of various medical and laboratory professionals. On the other hand, treatment with antibiotics has other problems such as drug resistance and side effects, so the use of new herbal medicines with fewer side effects can be a great help in treating these types of infections. The present investigation was carried out to evaluate the antibacterial activities of *Calendula officinalis* Linn. The effect of aqueous, acetone and ethanol crude extract of petals of flowers in different concentrations were obtained and bioassay in vitro for its bioactivity to inhibit the growth of eight types of bacteria's gram positive bacteria; *Bacillus Subtilis*, *S. aureus*, *Enterococcus* and gram negative bacteria; *P. Aeruginosa*, *K. Pneumoniae*, *E. Coli*. The extracts of petals part were clearly superior for all bacteria especially the bacteria *Bacillus Subtilis* (inhibition zone was 20 mm in the concentration 100 mg/ml) from gram negative bacteria, and *E. Coli* (inhibition zone was 16 mm in the concentration 75 mg/ml) from gram positive bacteria, while the extracts of ethanol was more effective as compared to aqueous, acetone extract. These results confirm the antibacterial activity of *Calendula officinalis* flowers and support the traditional use of the plant in the therapy of bacterial infection. These findings corroborate the traditional usage of *Calendula officinalis* flowers in the treatment of bacterial illnesses by confirming their antibacterial activity.

KEYWORDS: Antibacterial, *Calendula officinalis*, infection, ethanol, Flower Extract.

INTRODUCTION

Plant-based antimicrobials have a wide range of medicinal applications. They are efficient in the treatment of infectious disorders while avoiding many of the negative side effects associated with synthetic antimicrobials (Bhattacharjee, et al., 2005). Plant materials favourable therapeutic effects are usually due to a mix of secondary chemicals found in the plants. These substances are generally secondary metabolites found in plants, such as alkaloids, steroids, tannins, and phenol compounds, which can have a physiological effect on the body (Kumar, et al., 2010). Compounds derived from various plant parts can be used to treat diarrhoea, dysentery, colds, coughs, cholera, fever, bronchitis, and other ailments (Hasan, et al., 2007). The plant *Calendula officinalis* belongs to the family: Asteracea (Muley, et al., 2009). Commonly known as English marigold or pot marigold, field marigold, garden marigold, gold bloom, holligold, maravilla and, marybud (Henrich, et al., 2004). It is composed of solitary yellow to brilliant orange blossoms that capture the sun's intensity and heat when it rises in the morning.

Carotenoids, flavonoids, and essential oils make up the *Calendula* plant. It has long been used in homoeopathic medicine to treat a variety of ailments (Zitterl-Eglseer, et al., 1997). This mild and nutritious plant has potent anti-inflammatory and therapeutic qualities (Della, et al., 1994). Flavoxanthin, lutein, rubixanthin, b-carotene, g-carotene, and lycopene are among the carotenoids found in *C. officinalis*.^[12] Antifungal (Kasiram, et al., 2000; Nadkarni, et al., 1982), antibacterial (Dumenil, et al., 1980), antioxidant (Preethi, et al., 2006), antiviral (Barbour, et al., 2004), and antiseptic qualities are prized. *Calendula officinalis* is increasingly included in cancer and anti-cancer treatment literature. *Calendula officinalis* extracts show anti-cancer effects in vitro studies on tumor cell lines, derive from Leukemias, melanomas, fibrosarcomas, breast, prostate, cervix, lung and pancreas (Jimenez-M edina, et al, 2006). The goal of this work is to screen and assess the antibacterial activity of *Calendula officinalis* L. flower extracts against bacterial pathogens using aqueous, acetone, and ethanol extracts.

MATERIALS AND METHODS

Plant material collection

Calendula flowers (*Calendula officinalis*) were taken from the local garden of Raipur, Chhatisgarh, India and authenticated by the Institute.

Preparation of extract

The *Calendula officinalis* flower were dried for few days and converted into powdered form. Then 20g of powdered *Calendula Officinalis* flower was treated with different solvents i.e. 50% aqueous, 50% acetone and 50% ethanol separately with the help of soxhlet extraction unit. The excess solvent was removed and the liquid extract was dried in hot air oven at 50°C. The dried extract was kept in air tied box and used for further testing.

Microorganisms

The tested microorganisms included the gram positive bacteria; *Bacillus Subtilis*, *S. aureus*, *Enterococcus* and gram negative bacteria; *P. Aeruginosa*, *K. Pneumoniae*, *E. Coli*. These bacterial strains were produced from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37°C and maintained in nutrient agar slant at 4°C.

Antibacterial Assay

Antibacterial activity of *Calendula officinalis* in all solvents extract was determined by agar disk diffusion method (Rizvi and Pandey, 2022) at four concentrations i.e. 100, 75, 50 and 25mg/ml. Then Muller Hinton agar was prepared and the plates were prepared with appropriate microorganism (Gram positive bacteria; *Bacillus Subtilis*, *S. aureus*, *Enterococcus* and gram negative bacteria; *P. Aeruginosa*, *K. Pneumoniae*, *E. Coli*. After that discs of 6mm diameter were prepared with Whatman filter paper No. 24 and sterilized. The discs were impregnated with the extract and DMSO. Antibiotics for gram positive bacteria (NX- Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX-Norfloxacin, OF-Ofloxacin, E-Erythromycin, CFM- Cefixime). Then the plates were

incubated at 37 degree C for 24hrs and zone of inhibition were measured.

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 (Janssen, 1986; Muhammad and Muhammad 2005). Antibacterial activity is recorded when the zone of inhibition is greater than 6mm (Goun, et al., 2003). Results as shown in (Table 1) indicated the antibacterial activity of *Calendula officinalis* ethnolic flower extract. The ethanolic extract of *Calendula officinalis* flower extract has shown the positive results antimicrobial activity against gram positive bacteria; *Bacillus Subtilis*, *S. aureus*, *Enterococcus* and gram negative bacteria; *P. Aeruginosa*, *K. Pneumoniae*, *E. Coli*. The initiation of microbial growth was considered as zero hour and further accordingly readings were taken. The ethnolic extract of *Calendula officinalis* flower inhibits the microbial growth in different concentration (25%, 50%, 75% and 100%). But it was observed that the ethanolic extract of *Calendula officinalis* flower have shown the best results against all the bacteria in 100% concentration.

Results as shown in (Table 2 and 3) indicated the antibacterial activity of *Calendula officinalis* aqueous and acetone flower extract. Thus we can consider *Calendula officinalis* flower as a good source of natural antimicrobial compounds. 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 (Muhammad and Muhammad 2005). 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.

Table 1: The study of anti-bacterial activities of *Calendula officinalis* flower ethnolic extract using disk diffusion method (Mean± SE).

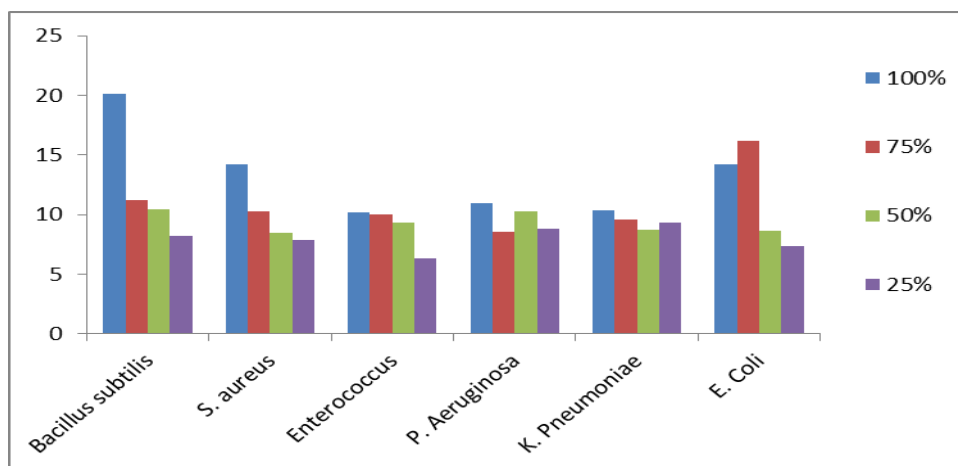
SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram Negative (+)	<i>Bacillus subtilis</i>	20.10± 0.87	11.20 ± 0.45	10.45 ± 0.78	8.25 ± 0.88
		<i>S. aureus</i>	14.25 ± 1.45	10.25 ± 0.78	8.45 ± 0.77	7.89 ± 0.98
		<i>Enterococcus</i>	10.23 ± 0.74	10.01 ± 0.12	9.36 ± 0.25	6.35 ± 0.78
2.	Gram positive (-)	<i>P. Aeruginosa</i>	11.00 ± 0.25	8.52 ± 0.44	10.25 ± 0.47	8.78 ± 0.12
		<i>K. Pneumoniae</i>	10.37 ± 0.77	9.56 ± 0.8	8.77 ± 0.55	9.35 ± 0.44
		<i>E. Coli</i>	14.25 ± 0.78	16.20 ± 0.78	8.66 ± 1.04	7.33 ± 0.97

Table 2: showing the study of antibacterial activity of *Calendula officinalis* flower aqueous extract using Disk Diffusion Method (Mean±SE).

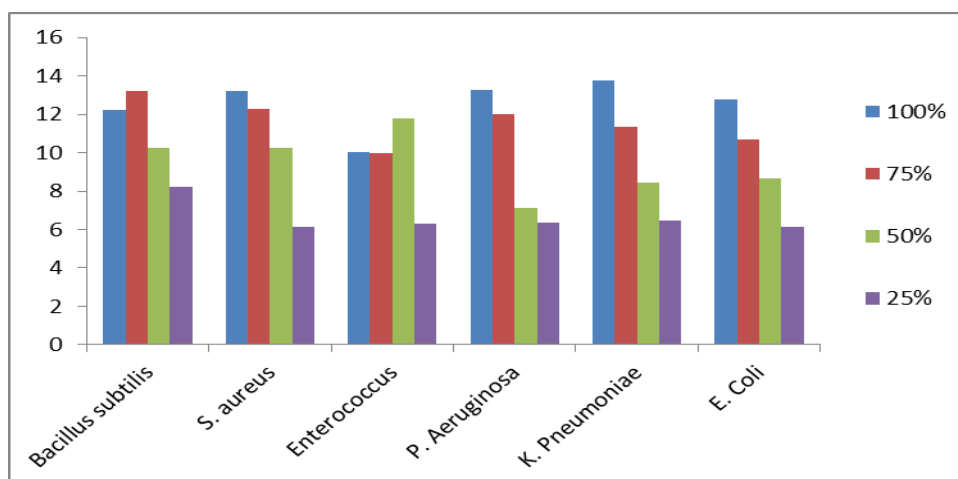
SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram Negative (+)	<i>Bacillus subtilis</i>	10.25±0.87	14.23±1.82	11.33±2.32	08.00±0.20
		<i>S. aureus</i>	12.66±0.65	11.00±0.10	14.66±2.40	06.00±1.15
		<i>Enterococcus</i>	11.00±1.15	09.00±1.73	14.66±2.60	05.66±2.40
2.	Gram positive (-)	<i>P. Aeruginosa</i>	14.10±0.87	13.23±0.46	07.0±0.00	06.23±1.32
		<i>K. Pneumoniae</i>	12.16±0.87	08.00±0.98	7.66±0.65	06.73±0.32
		<i>E. Coli</i>	13.66±1.32	09.43±0.847	7.66±1.19	07.73±1.90

Table 3: showing the study of antibacterial activity of *Calendula officinalis* flower acetone extract using Disk Diffusion Method (Mean±SE).

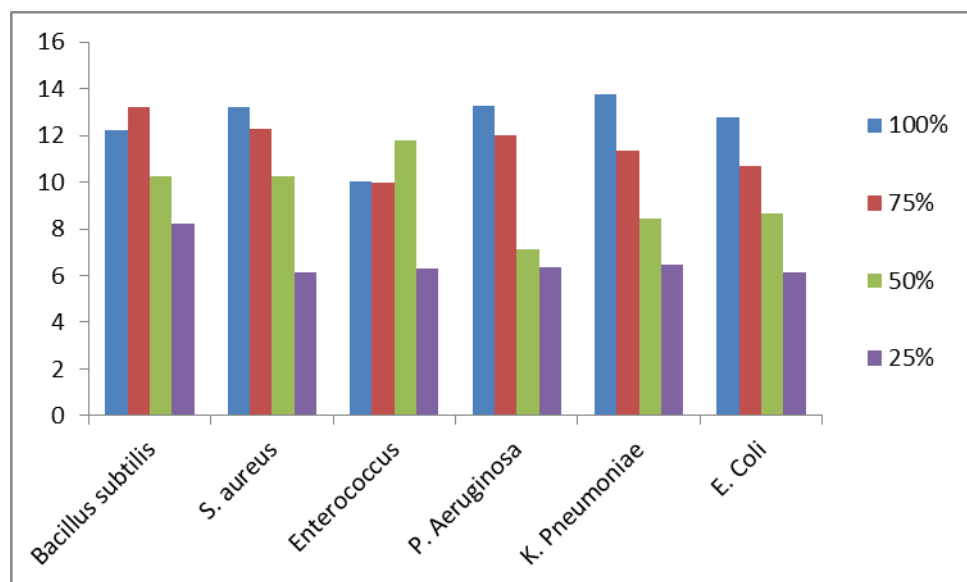
SI	Bacterial Stain	Bacteria use	Zone of inhibition (In MM)			
			100%	75%	50%	25%
1.	Gram Negative (+)	<i>Bacillus subtilis</i>	12.20±0.87	13.20±1.82	10.25±1.32	08.20±0.78
		<i>S. aureus</i>	13.20±0.85	12.30±0.78	10.23±0.47	06.12±1.05
		<i>Enterococcus</i>	10.02±1.20	10.00±0.23	11.78±1.23	6.30±0.45
2.	Gram positive (-)	<i>P. Aeruginosa</i>	13.25±0.82	12.03±0.76	07.12±0.78	06.33±1.78
		<i>K. Pneumoniae</i>	13.76±0.44	11.33±0.77	8.44±0.48	06.45±0.44
		<i>E. Coli</i>	12.77±0.25	10.7±0.84	8.66±0.23	6.12±0.33



Graph 1: Antibacterial activities of *Calendula officinalis* flower ethanolic extract using disk diffusion method.



Graph 2: showing the study of antibacterial activity of *Calendula officinalis* flower aqueous extract using Disk Diffusion Method.



Graph 3: showing the study of antibacterial activity of *Calendula officinalis* flower acetone extract using Disk Diffusion Method.

CONCLUSION

As we know spices are not only used in cuisines but also used as traditional medicines since ancient time due to the presence of phytonutrients which have anti-inflammatory, anti-bacterial and antioxidant properties. These naturally occurring antibacterial compounds have fewer side effects than the synthetic ones, so it is important to discover new natural antibacterial drugs. *Calendula officinalis* flower extract has shown strong antibacterial effect against both gram positive bacteria; *Bacillus Subtilis*, *S. aureus*, *Enterococcus* and gram negative bacteria; *P. Aeruginosa*, *K. Pneumoniae*, *E. Coli.*, thus we can consider it as a good source of natural antimicrobial compounds which can be used for the treatment of illness caused by the food pathogens. However further investigation are still necessary to determine the toxicity, their side effects and other pharmacokinetic properties. It is clear that *Calendula officinalis* flowers as an extract may be useful as an antibacterial agent against the above mentioned bacteria. Further pharmacological 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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