AN EVALUATION OF ANTIMICROBIAL ACTIVITIES OF ROOT EXTRACT OF *CALENDULA OFFICINALIS* (LINN.)

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Summary

In view of increasing resistance to existing antimicrobial agents, herbal drugs are being looked as very importance source for discovery of new agents for treating various ailments related to bacterial infections. *Calendula officinalis* are well known plants in Asia including India which posses wide range of pharmacological activities. The present investigation was carried out to evaluate the antibacterial activities of Calendula officinalis Linn. Root extract of Calendula officinalis was successively extracted with petroleum ether using Soxhlet to form extract. Extracts were screened for its antibacterial activity using agar well diffusion method. The microorganisms used for antibacterial were *V3-MPCST*, *Staphylococcus Cohni, Salmonella typhae, Aeromonas hydrophila, Serratia fecarria, Pseudomonas vericularis, Streptococcus fecallis, E.coli, Pseudomonas and Salmonella*. Tetracycline 50µg/ml was used as standards. The extracts showed antimicrobial activity were subjected to minimum inhibitory concentration assay. Petroleum ether extract exhibited in-vitro antibacterial activity. This study may be a lead for further ethnopharmacognostic investigation to identify new compounds with therapeutic promise.

Key words: *Calendula officinalis (Linn.)*, Antibacterial activity.

Introduction:

Traditional medicines hold a great promice as source of easily available effective therapy for skin diseases to the people, particularly in tropical developing counties, including India. It is in this context that the people use several plant derived preparations to cure skin diseases (1). It is used because of the broad area of biological activities like anti-inflammatory, anti-mutagenic, diuretic, antispasmodic activities. It is also used for in gastrointestinal, gynecological, eye diseases, skin injuries and in some cases of burns. The plant is rich in many pharmaceutical active ingredients like flavonoids, carotenoids, glycosides and sterols (2).

The limited life span of antimicrobials due to resistance because of indiscriminate use necessitates the continuous search for alternatives. Awareness for misuse of antibiotics and also the potential risk of using synthetic form of phytochemicals have been reported (3). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. The use of traditional plant extracts as well as other alternative forms of medical treatments have been getting momentum since the 1990s (4) In present study antimicriboal activity of petroleum ether extracts of root of *Calendula officeinalis* Linn was studied. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it.

Material and Methods:

Plant Material: The roots of *C.officinalis* were collected from the local areas of Bilaspur, Chhattisgarh, India. It was authenticated from Institute of Pharmacy, Raipur Institute of Technology, Raipur, Chhattisgarh.

Preparation of Extracts: Collected roots were cleaned and shade-dried. The dried roots were pulverized by a mechanical grinder and passed through a 20-mesh sieve.

A powdered root (400 g) was successively extracted with petroleum ether using a Soxhlet apparatus. The extraction was carried out for 24 hrs at room temperature with mild shaking. The extracts were filtered and concentrated at 35° C, and the weight of each residue was recorded and percentage yield was calculated (5)

Preliminary Phytochemical Screening: All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites (6)

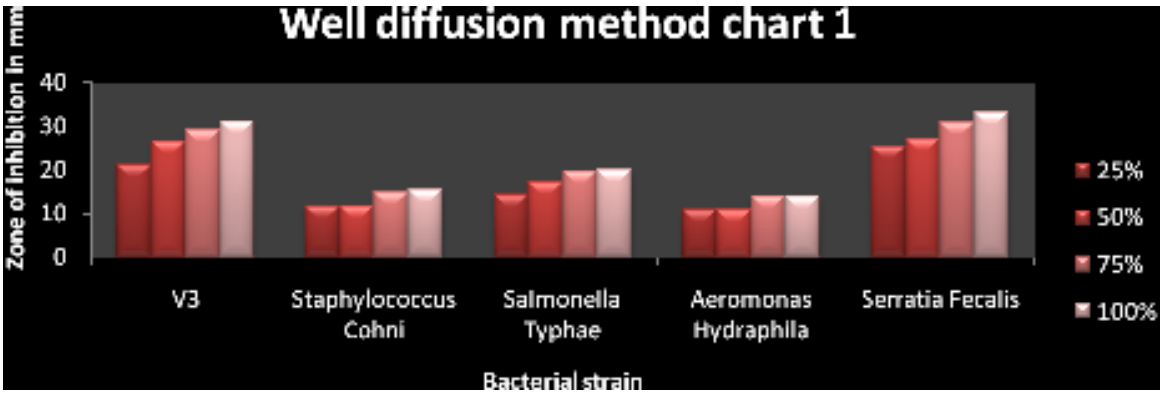
Preparation of Inoculums: Suspension of organism was prepared as per McFarland nephelometer standard (7). A 24 hour old culture was used for the preparation of bacterial suspension. Suspension of organism was made in a sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted such that it contained approximately 1.5X 10 8 cells/ml. It was obtained by adjusting the optical density of the bacterial suspension to that of a solution of 0.05ml of 1.175% of barium chloride and 9.95 ml of 1% sulphuric acid.

Procedure: The medium was prepared by dissolving all the ingredients in distilled water and subjected to sterilization in an autoclave at1210C for 15 minutes. The Petri plates were washed thoroughly and sterilized in hot air oven at 1600C for 1 ½ hours. 30 ml of sterile molten agar medium was seeded by organisms (about 2 ml according to Mc Farland's standard), in semi hot conditions (400C) was poured aseptically in sterile Petri plate and allowed to solidify at room temperature. Bores were made on the medium using sterile borer and 1 ml of the extracts were added to respective bore and 1ml of the standard Tetracycline at a concentration of 50 µg / ml was taken as standard. The Petri plates seeded with organisms, containing extracts and the standard were kept in refrigerator at 40C for 1 hour to facilitate the diffusion of the extracts and the standard in to the media. After diffusion the Petri plates were incubated at 37 ± 10C for 24 hours in an incubator and zone of inhibition was observed and measured in mm. The results of the antibacterial activity of *C.officinalis* are tabulated in (Table 1). Minimum inhibitory concentrations of extracts against the tested organisms of *C.officinalis* extracts exhibited MIC (Table 2) against the tested organisms.

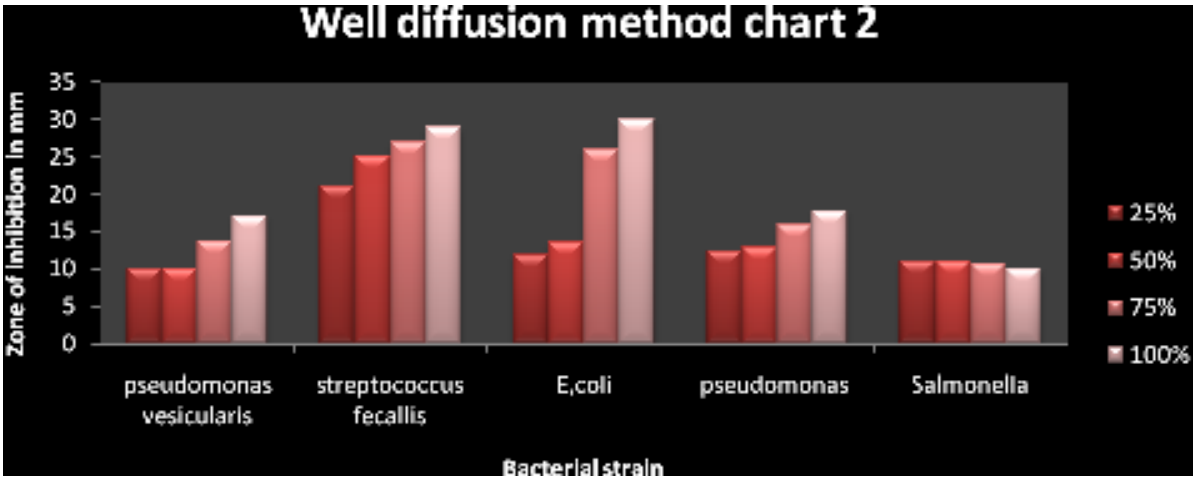
Table-1: Observations of antimicrobial assay of root of *C.officinalis* by well diffusion method.

S/NO.	BACTERIAL STRAIN	DIAMETER OF ZONE OF INHIBITION (in mm)			
		25%	50%	75%	100%
		SEM	SEM	SEM	SEM
1.	V3-MPCST	21.125±0.075	26.625±0.075	29.000±0.000	31.000±0.000
2.	*Staphylococcus cohni* (121-MPCST)	11.750±0.087	11.875±0.087	14.875±0.075	15.375±0.075
3.	*Salmonella typhae* (109-MPCST)	14.250±0.087	17.250±0.087	19.625±0.144	19.875±0.075
4.	*Aeromonas hydrophila* (104-MPCST)	11.000±0.000	11.000±0.000	14.000±0.000	14.000±0.000
5.	*Serratia fecarria* (076-MPCST)	25.375±0.075	27.000±0.000	30.750±0.087	33.125±0.075
6.	*Pseudomonas vericularis* (088-MPCST)	10.000±0.000	10.000±0.000	13.750±0.087	17.125±0.075
7.	*Streptococcus fecallis* (072-MPCST)	21.000±0.000	25.125±0.075	26.875±0.075	29.000±0.000
8.	*E.coli*	11.875±0.075	13.500±0.123	25.875±0.075	30.000±0.000
9.	*Pseudomonas*	12.375±0.075	12.875±0.075	15.875±0.075	17.750±0.087
10.	*Salmonella*	11.000±0.000	11.000±0.000	10.750±0.075	10.000±0.000

Each data presents mean±SE (n = 4).



Graph-1: Well diffusion method chart 1



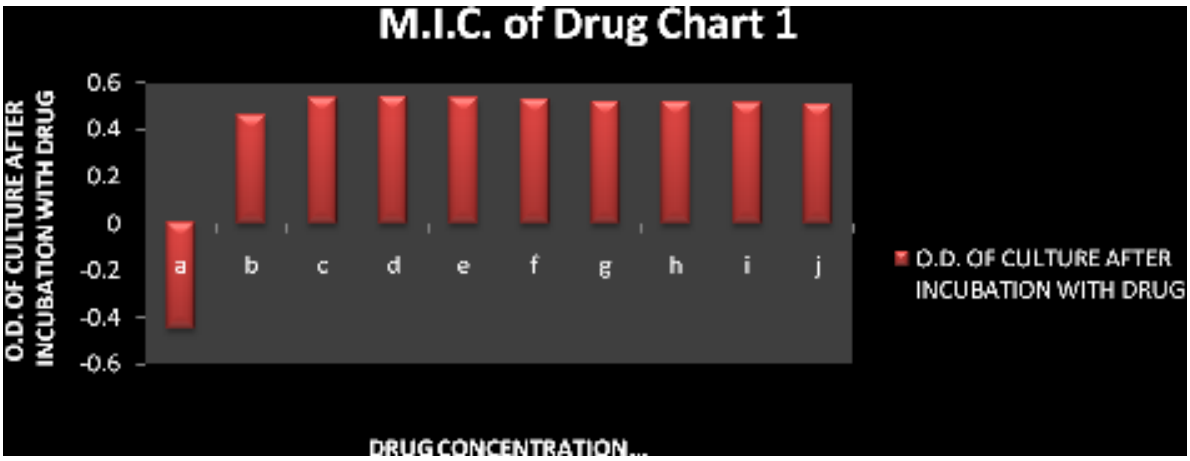
Graph-2: Well diffusion method chart 2

Table 2: Minimum inhibitory Concentration of root of *C.officinalis*

S/No	DRUG CONCENTRATION IN CULTURE (ml)	No. OF CELLS ($\times 10^7$)	
		SEM	SEM
1.	10^{-1}	-0.445±0.001	-0.608±0.002
2.	10^{-2}	0.460±0.002	0.633±0.004
3.	10^{-3}	0.535±0.003	0.757±0.004
4.	10^{-4}	0.542±0.001	0.770±0.002
5.	10^{-5}	0.532±0.001	0.754±0.002
6.	10^{-6}	0.527±0.001	0.745±0.002
7.	10^{-7}	0.517±0.001	0.729±0.002
8.	10^{-8}	0.517±0.002	0.729±0.004
9.	10^{-9}	0.512±0.001	0.721±0.002
10.	10^{-10}	0.507±0.001	0.712±0.002

Note: 1ml of plant extract mixed with 9 ml (10^{-1}) of culture media and further diluted upto 10^{-10}.

Each data presents mean±SE (n = 4).



Graph-3: M.I.C. of drug chart 1

Graph-3: Determination of Minimum Inhibitory Concentration of *C.officinalis*

Result and Discussion:

The antimicrobial assay showed that petroleum ether extracts of *C.officinalis* root exhibited *in-vitro* antibacterial activity against Gram-positive and Gram-negative bacteria, showany activity with the antimicrobial organisms (Table 1). Minimum inhibitory concentration of the active extracts is shown in (Table 2). The results reveal that extracts of *C.officinalis* root were significantly effective against both Gram-positive and Gram-negative organism. Preliminary phytochemical screening of the extracts showed the presence of Alkaloids, terpenoids, flavonoids, sterols, carbohydrates and tannins. Thus furtherwork can be carried on the isolation procedure for finding out the exactmoiety responsible for the biological activity. On the basis of the data obtained in the present investigation, conclusion may be drown that the crude extracts obtained from root of the *C.officinalis* may be used as drug to treat the disease caused by those organisms, which are sensitive to the above mentioned samples. But before use in human being isolation of pure compound, toxicological study and clinical trial in animal model should be carried out thereafter. However, further and specific studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

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