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ANTIBACTERIAL ACTIVITY OF FLOWER AND STEM EXTRACTS OF *CHRYSANTHEMUM CORONARIUM*

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ABSTRACT

The present study aimed at evaluating *in vitro* antibacterial activity of flower and stem extracts of *Chrysanthemum coronarium* in methanolic and acetone solvents by soaking and soxhlet method. The antibacterial activity was evaluated using the agar well diffusion method at four different concentrations (ie 2,3,4 and 5 mg/ml). DMSO was used as a solvent to dissolve the extracts and Ampicillin as a positive control. The extracts were tested against two gram positive (*S.aureus* and *B.subtilis*) and gram negative bacteria (*P. aeruginosa* and *E.coli)* . All the extracts showed a good inhibitory activity against the gram positive bacteria *(S.aureus* and *B.subtilis).* The highest antibacterial activity was shown by methanolic hot extract of *C. coronarium* stem against *B. subtilis* (36mm) and the lowest was shown by the acetone cold extract stem against *E.coli* (13mm) . The antibacterial activity against both type of bacteria indicated broad spectrum of secondary metabolites. Hence it can be used as a therapeutic drug to inhibit bacterial pathogens.

KEYWORDS: DMSO (Dimethyl sulphoxide), agar well diffusion , Ampicillin, *Chrysanthemum coronarium*

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INTRODUCTION

Plants are the most important source of chemical compounds and the extracts obtained from many plants have recently gained a great popularity and scientific interest.[1] In the present scenario there is a worldwide increase in the incidence of bacterial infections. To combat with these infections the use of plant extracts are becoming widely popular and are highly encouraged as they are cost effective and non-toxic. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.[2] The phytochemical screening of the Asteraceae plant family has revealed sesquiterpene lactones as the principal secondary metabolites responsible for their antimicrobial activities.[3] The screening of such plant extracts for antimicrobial activity has always been of great interest to scientist looking for new sources of drugs for the treatment of various diseases.[4] *Chrysanthemum coronarium* is a plant of Asteraceae family which contains various bioactive compounds that are used for the treatment of various diseases. *Chrysanthemum coronarium* has medicinal properties as the leaves are expectorant and stomachic ,while the flowers are stomachic.[5] Sesquiterpene lactones (cumambrin and dihydrocumambrin) and essential oils of the flower heads have been evaluated for their activity against insects, nematodes and plant fungal pathogens.[6] . The aim of the present research is to bring in to limelight the use of safer antibacterial agents from the natural plant products such as plant extracts to control the bacterial diseases.

MATERIALS AND METHODS

1) Sample collection

The stem and flower of *C.coronarium* species of C*hrysanthemum* were collected from the local nursery of Allahabad.

2) Preparation of extracts

a) By –Soaking method

50gm of the flower and stem of *Chrysanthemum coronarium* was subjected to shade drying. After grinding the plant material was soaked in to the solvents methanol and acetone for 3 days and was shaken intermittently. The plant material was filtered and concentrated to dryness under reduced pressure .The dried condensed extracts of stems and flower of *C.coronarium* was weighed and stored in a dry place.

b) By Soxhlet apparatus

The flower and stem of *Chrysanthemum coronarium* was shade dried, ground in to the powder form and was introduced in the soxhlet apparatus for 72 hours.. The plant extract was further concentrated to dryness under reduced pressure and weighed.

3)Test organisms for the antibacterial activity

The bacterial strains were collected from the Department of Microbiology and Fermentation Technology, SHIATS. The bacteria used were *E.coli* 0018 (gram negative) , *P. aeruginosa* 0034(gram negative), *S.aureus* 0066 (gram positive) and *B.subtilis* 0008 (gram positive).The bacterial strains were grown and maintained on nutrient agar slants at 37^{o}C, following incubation for 5 days.

4) In vitro Antibacterial activity

The antibacterial activity of extracts of *Chrysanthemum coronarium* was evaluated by using agar well diffusion method.[7] The nutrient agar plates were prepared by pouring 15ml of molten media in to sterile petriplates . Wells or cups of 5mm size were made with sterile borer in to agar plates containing the bacterial inoculums. Plant extract (25µl) of different concentration (2, 3, 4 and 5 mg /ml) was poured in to the well of inoculated plates separately. Ampicillin (10mg/ml) was used as a positive control which was introduced in to the well instead of plant extract. After incubation for 24 hours at 37ºC, the plates were observed for zone of inhibition. Antibacterial activity was recorded if the radius of zone of inhibition was greater than 4mm.[8] The antibacterial activity was considered as inactive if <4.5mm, 4.5-6mm as partially active; while 6.5-9mm as active and greater than 9mm as very active.[9]

RESULTS

Table 1
Yield of plant extracts obtained from soaking and soxhlet methods

S.No	Plant extract	Soaking method (g)	Soxhlet method (g)
1	Methanol flower extract	4.5	13.5
2	Acetone flower extract	3.2	10
3	Methanol stem extract	4	11.6
4	Acetone stem extract	2.1	7

It can be observed from the table that soxhlet extraction gave higher yield of plant extracts compared to soaking extraction and the yield of methanol extract is greater than acetone which is also reported in the literature that the cold extraction give a lower yield of plant extract compared to hot extraction in solvents having lower boiling point[10] and methanol and acetone both are polar solvents but methanol had more extractive yield than acetone.[11]

Table 2
Antibacterial activity of flower and stem extract of C. coronarium

Bacteria	Conc (mg/ml)	Flower extract				Stem extract				
		Inhibition zone(mm) Soxhlet method		Inhibition zone (mm) Soaking method		Inhibition zone (mm) Soxhlet method		Inhibition zone (mm) Soaking method		Inhibition zone of Ampicillin (mm)
		Meth	Ace	Meth	Ace	Meth	Ace	Meth	Ace	(+)control
S.aureus	**2**	18	15	16	12	21	14	20	12	28
	3	20	18	18	17	23	16	22	15	30
	4	21	19	20	18	24	17	23	16	34
	5	24	21	22	22	26	22	25	17	36
B.subtilis	**2**	29	27	25	23	28	27	27	26	30
	3	30	28	28	25	31	29	30	28	32
	4	32	31	29	27	33	31	32	30	33
	5	34	33	31	28	36	34	35	33	35
E.coli	**2**	12	11	11	10	13	11	12	10	16
	3	13	12	12	11	14	12	13	11	18
	4	15	14	14	13	15	13	14	12	19
	5	17	15	16	14	17	14	16	13	22
P.aeruginosa	**2**	21	20	-	-	12	-	-	-	27
	3	22	21	-	-	14	-	-	-	28
	4	24	23	-	-	15	-	-	-	31
	5	28	26	-	-	16	-	-	-	32

Well size* = 5mm Meth = methanol Ace = acetone +ve control = Ampicillin

DISCUSSION

It can be observed from Table 2 that the highest antibacterial activity was shown against *B.subtilis* by methanolic hot extract of *C. coronarium* flower (34mm) in comparison to the acetone extract (33mm). The lowest antibacterial activity (14mm) was shown by acetone cold extract against *E.coli* in comparison to methanolic extract (16mm). These were found in accordance with the results of the reported literature. The methanolic hot extract of *Lonicera Japonica* flower showed antibacterial activity of (20mm) against *B.subtilis* in comparison to the acetone extract (19mm).[12] The lowest antibacterial activity (7.60±0.29mm) was shown by acetone cold extract of *Calotropis procera* flower against *E.coli* in comparison to the methanolic extract (10.90±0.29 mm).[13] In stem extract it was observed that the highest antibacterial activity (36mm) was shown by methanolic hot extract of *C.coronarium* stem against *B. subtilis* in comparison to the acetone extract (34mm) . The lowest activity (13mm) was shown by the acetone cold extract stem against *E.coli* in comparison to the methanolic extract of

(16mm). This was in accordance with the results of reported literature. The highest antibacterial activity (15.6 ±0.4mm) was found in the methanolic hot extract of *Tridax procumbens* stem against *B. subtilis* compared to the acetone extract (14.0±0.6 mm). [14] The lowest antibacterial activity (10±0.8mm) was shown by the acetone cold extract in comparison to the methanolic extract (15.4±0.6mm).[15]

CONCLUSION

The methanolic and acetone extracts of flower and stem of *Chrysanthemum coronarium* were prepared by soaking and soxhlet methods . All the prepared extracts were tested against four bacteria *(S.aureus, B.subtilis, P. aeruginosa* and *E.coli*.. The activity against both the type of bacteria may be indicative of the presence of broad spectrum antibiotic compound and can be used as a therapeutic drug. On comparison of the flower and stem extracts the overall highest antibacterial activity was shown by the methanolic hot extract of *C. coronarium* stem of inhibition zone of 36mm against *B.subtilis* and the lowest antibacterial activity was shown by the acetone cold extract of *C. coronarium* stem with an inhibition zone of 13mm against *E.coli*..

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