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Full Length Research Paper

Antibacterial potential of pot marigold

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Plants continue to be an important therapeutic aid for alleviating the ailments of humankind. In the present research work different parts (root, leaf and flowers) of *Calendula officinalis* were screened for potential antibacterial activity against some important bacterial strains, namely Escherichia *coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Agrobacterium tumefaciens*. The antibacterial activity was determined in aqueous, alcohol, chloroform and petroleum ether extracts using agar disc diffusion method. Although all the plant parts showed significant anti microbial activity but the highest antibacterial activity was observed in petroleum ether extract of dried leaves against *Klebsiella pneumoniae*. Phytochemical analysis was also done.

Key words: Antibacterial activity, *Calendula officinalis*, petroleum ether extract, *Klebsiella pneumoniae.*

INTRODUCTION

Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants grow wild in different parts of our country. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vogue from ancient times (Bhattacharjee, 1998). *Calendula officinalis*, commonly known as pot marigold, is an annual herb and belongs to Asteraceae family. The flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Bees. It is noted for attracting wildlife.

It is one of the best known and versatile herbs in Western herbal medicine and is also a popular domestic remedy. The leaves, blossoms and buds are used to make a homeopathic remedy. It is used internally in order to speed the healing of wounds. Only the common deep-orange flowered variety is considered to be of medicinal value. The whole plant, but especially the flowers and the leaves, is antiphlogistic, antiseptic, antispasmodic, aperient, astringent, cholagogue, diaphoretic, emmenagogue, skin, stimulant and vulnerary. Antibacterial properties of marigold flowers and mother homeopathic tinctures of *C. officinalis* and *Calendula arvensia* have been evaluated by Dumenil et al. (1980). The sap of different organs of *Calendula* sp. has been studied for antimicrobial activity by Radioza and Lurchak (2007) . The aim of the present study was to determine the anti-bacterial activity of various extracts of *C. officinalis* which is having traditional claims for several diseases.

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MATERIALS AND METHODS

Collection of plant material

Fresh plant parts were collected from many residential gardens, local nurseries and farm houses, at different localities, in Jodhpur, during their growing seasons. Their identity was confirmed by Botanical Survey of India, Jodhpur, from the literature available on exotic plants and also from literature available in Department of Botany, J.N.V, University, Jodhpur. The voucher specimens were deposited in herbaria of Department of Botany, J.N.V.University, Jodhpur (Raj.), India.

Preparation of plant extracts from fresh plant parts

25 g of fresh plant parts, namely, leaves, roots and flowers were washed 3 to 4 times with tap water and then distilled water. Surface sterilized with 90% alcohol. Subsequently, the plant materials were grounded in 100 ml of distilled water, ethanol, chloroform and petroleum ether separately for aqueous, alcoholic extracts, chloroform extracts and petroleum ether extracts, respectively. The

macerates were kept for 24 h at room temperature to evaporate the solvents. The macerates were squeezed through double layered muslin cloth and filtered through filter paper. Aliquot was centrifuged at 10,000 rpm for 20 min, after filtration,. The supernatants were filtered through Whatman No. 1 filter paper and then sterilized by passing through 0.2 micron disposable filters. The extracts were diluted to get a concentration of 50 mg per ml and were used for the *in vitro* studies.

Preparation of plant extracts from dried plant parts

The selected plants were thoroughly washed and then dried under shade at 28±2°C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50 to 150 mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction. 25 g of dried plant powder was packed in a Whatmann No.1 filter paper and was extracted in a soxhlet apparatus using 100 ml of solvent. Solvents used for extraction were petroleum ether (60 to 80°C), chloroform (61°C), ethanol (78.5°C) and aqueous (80°C) as solvents and the extracts were dried. The dried extracts were stored in a refrigerator at 4 °C. Finally, concentration of 5 mg per disc was loaded on each disc.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loop full of cells from the stock cultures to test tubes of Nutrient Agar Medium and were incubated without agitation for 24 h at 37°C. The cultures were diluted with fresh Nutrient Agar broth to achieve optical densities corresponding to $2.0 \cdot 10^6$ colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

All the plant extracts were screened against five pathogenic bacterial strains. The tested organisms were Escherichia *coli*, (MTCC No. 729), *Salmonella typhi* (MTCC No.734), *Klebsiella pneumoniae* (MTCC No.109), *Enterobacter aerogenes* (MTCC No. 111) and *Agrobacterium tumefaciens* (MTCC No. 431), obtained from IMTECH, Chandigarh, India. The disc diffusion method (Bauer et al., 1966) was used to test the antimicrobial activity of the plant extracts. 20 ml of sterilized nutrient agar medium for *E. coli*, *S. typhi, K. pneumoniae, E. aerogenes and A. tumefaciens* were poured into each sterile petridish. The plates were allowed to solidify for 5 min and 0.1% inoculum suspension was swabbed uniformly. The entire agar surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs (5 mm in diameter) soaked in 1 ml of the plant extract (In case of fresh extract) or loaded with 5 mg/disc, of dry extract and were placed on the surface of the bacteria seeded agar plates and the compound was allowed to diffuse for 5 min and then the plates were incubated at 37 °C for 24 h. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate.

Phytochemical screening

Phytochemical screening of plant extract was carried out qualitatively for the presence of terpenoids, tannin, flavonoids, saponin, cardiac glycosides and steroids (Harborne, 1998).

RESULTS AND DISCUSSION

Both the fresh and dry plant part extracts responded to bacteria as follows:

Zelepukha and Kahans'ka (1955) studied the antibacterial activity of *C. officinalis*. Kalvatchev et al. (1997) reported anti- HIV activity of extracts from flowers of *C. officinalis*. Iauk et al. (2003) tested the antibacterial activity of plant part extracts of *C. officinalis* against anaerobic and facultative aerobic bacteria. Ramos et al. (1998) studied the geno-toxicity of an extract of *C. officinalis* towards *Aspergillus* species. Anti-inflammatory activity of flower extracts of *C. officinalis* was reported by Preethi et al. (2009). Similarly, in the present investigation root, leaves and flower extracts of *C. officinalis* were tested for their antimicrobial activity. Fresh root extracts were found to inhibit growth of *E. aerogenes* and *E. coli*. Fresh leaves proved to be toxic to *E. coli* with maximum inhibition zone in petroleum ether extract. Again fresh flower extracts were effective against *E. aerogenes* and *E. coli* (Table 1). In case of dried plant parts significant antibacterial activity was recorded (Table 2). Dried root extract was found to be most effective against *E. aerogenes*. Dried leaves exhibited antibacterial activity against *K. pneumoniae*, in all the four types of extracts. Chakarborthy (2008) studied the effect of leaf extract of *C. officinalis* against *E. coli* and *K. pneumoniae*.

Dried flower extracts exhibited high inhibition zones with maximum inhibition of *S. typhi* and *E. coli* followed by *K. pneumoniae* and *E. aerogenes*. Venikar and Jangde (1992) also reported significant effect of alcoholic and aqueous extract of *C. officinalis* against *E. coli* and *Staphylococcus aureus*. The highest antibacterial activity was observed in petroleum ether extract of dried leaves against *K. pneumoniae*. Both root and flower contain alkaloids, glycosides, flavonoids and saponins. In leaves tannin, saponins and flavonoids are present. Elias et al. (1990) isolated some saponins from *C. officinalis* and reported its antimutagenic activity.

Conclusion

Based on our results, it is concluded that plant extracts have great potential as antimicrobial compounds against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Such screening of various natural organic compounds and identification of active agents is the need of the hour because successful prediction of lead molecule and drug-like properties at the onset of drug discovery will pay off later in drug development.

Table 1. Antibacterial activity of Fresh plant parts extracts of *Calendula officinalis.*

Plant part	Plant extracts	Zone of Inhibition (mm)				
		E. coli	***Salmonella typhi***	***Klebsiella pneumoniae***	***Enterobacter aerogenes***	***Agrobacterium tumefaciens***
Root	Aqueous	4	-	-	3	-
	Alcoholic	6	4	-	11	-
	Chloroform	6	3	3	11	-
	Pet. ether	7	5	4	12	5
Leaves	Aqueous	8	6	3	4	-
	Alcoholic	6	6	7	3	5
	Chloroform	4	9	8	6	6
	Pet. ether	14	9	8	9	8
Flower	Aqueous	3	-	-	2	-
	Alcoholic	6	8	-	7	3
	Chloroform	8	6	-	8	6
	Pet. ether	10	9	6	10	6

Table 2. Antibacterial activity of dried plant parts extracts of *Calendula officinalis.*

Plant part	Plant extracts	Zone of Inhibition (mm)				
		E. coli	***Salmonella typhi***	***Klebsiella pneumoniae***	***Enterobacter aerogenes***	***Agrobacterium tumefaciens***
Root	Aqueous	5	2	-	9	-
	Alcoholic	5	4	6	4	-
	Chloroform	-	8	5	18	5
	Pet. ether	8	9	9	19	8
Leaves	Aqueous	8	9	15	7	-
	Alcoholic	11	7	6	9	4
	Chloroform	7	12	10	11	4
	Pet. ether	10	14	20	13	6
Flower	Aqueous	9	15	8	5	-
	Alcoholic	9	10	8	5	-
	Chloroform	8	10	14	10	-
	Pet. ether	19	16	15	12	5

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