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Full Length Research Paper

Study on the medicinal plant *Calandula officinalis*

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The antibacterial and anti-fungal activities of the methanol and its sub fractions of chloroform, ethyl acetate, and water extracts of the roots, stem and leaves of *Calendula officinalis* were carried out against bacterial strains, *Staphylococus aureus, Klebsiella pneumoniae, Salmonella typhymurium* and *Escherichia coli,* and fungal species including *Aspergilla fumigates, Fusarium solani, Aspergillus niger*, and *Aspergilla flavus*. Phytochemical analysis was also performed using the literature methods. The studied medicinal plant extracts against the tested bacterial strain, *C. officinalis* showed very promising results against both the Gram positive and Gram negative bacterial and fungal species. The significant antibacterial activity of active extracts was compared with the standard antimicrobics, gentamicin (10 µg/disc). The methanolic extracts showed inhibitory effects of 16 mm each of the stem and leaves samples against *S. typhymurium* and *S. aureus.* The water extract of leaves has 17 mm against *S. typhymurium* and very high activity among all the extracts 18 mm inhibition of the roots against *E. coli*. The antifungal activity of the extracts showed significantly variable results against the tested fungal strains. Phytochemical studied indicated that the roots, stem and leaves contain secondary metabolites such as alkaloids, flavonoids, saponins, anthraquinone, terpenoids, tannins ,reducing sugar, cardiac glycosides etc. These phytochemicals are present predominantly in the roots, stem and leaves of *C. officinalis*. A fluorescence characteristic of the roots stem and leaves powders and their extracts were also investigated.

Key words: Antibacterial, *Calendula* officinalis, phytochemicals.

INTRODUCTION

The world is lush with naturally grown medicinal plants. Medicinal plants are now more focused than ever because they have the capability of producing many benefits to society indeed to mankind, especially in the line of medicine and pharmacological (Hussain et al., 2011). Plant based drugs have been in use against different ailments since time immemorial (Hussain et al., 2011). Any parts of plant: Stem, root, leaves etc which have in one or more of its organs containing constituents that can be utilized for therapeutic purposes, are called medicinal plants (Hussain et al., 2011).

Medicinal plants are used as herbs or traditional medicines for various types of diseases since ancient times. Phytochemicals that are isolated from the medicinal plants have multiple functions as a drug, as a protecting agent, as a pest etc. The results provided by them are very promising and have showed no side effects or damage to other part of the body. The potential of medicinal plants as a source for search of new drugs still remained unexplored. The synthetic drugs are not

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only expensive and inadequate for the treatment of diseases but also often with adulteration and side effects. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). The increasing failure of chemo-therapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (Colombo and Bosisio, 1996). In recent years, secondary plant metabolites (phyto-chemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents (Iwu et al., 1999). Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Martins et al., 2001). The selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds (Parekh et al., 2007) Such screening of various plant extracts has been previously studied (Afolayan, 2003, Kasamota et al., 1995). Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated (Erdogrul, 2002). *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 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* been evaluated previously (Dumenil et al., 1980). The sap of different organs of *Calendula* sp. has been studied for antimicrobial activity by Radioza and Lurchak (2007). The present study will however explore new frontiers and will open new doors for the herbal industries, local practioners and for other users and a scientific data basis for the traditional claims of this ethnic medicinal plant.

MATERIALS AND METHODS

Reagents and chemicals

All the chemicals used were of analytical grade and were used as such without further purification. Nutrient agar media, sterile yeast were purchased from Defco.

Post harvest treatment of plant materials

All the plants were washed in tap water and then rinsed with the de-ionized water properly; the rinsed plants were then dried under shade. The dried whole plants were pulverized by sterile electric blender to get powered plant materials. The powdered form of these plants were then stored in air-tight glass containers and protected from sun light till required for analysis.

Anti-microbial activity

Preparation of crude extract

100 g of each of the roots, stems and leaves powdered plant material were taken and extracted with methanol. The solution was then after filtration evaporated under reduced pressure. The mathanolic residue was then extracted successively with chloroform, ethyl acetate and water. All the four extracts after drying at reduced pressure were kept in refrigerator for further processes (Parek et al., 2006).

Preparation of standard bacterial suspension

Antibacterial activity

The antimicrobial activity of the prepared extracts was determined by using well agar diffusion method. One Gram positive *Escherichia coli*, and three Gram negative bacterial stains *Klesiela pneuumoniae, Salmonella typhi* and *Staphylococcus aureus* were used as the test organism. The standard bacterial stock suspension 10^8 to 10^9 CFU/ ml was mixed with 60 ml of sterile nutrient agar thoroughly. 20 ml inoculated nutrient agar was poured into sterile Petri dishes. The agar left to set and four well (10 mm in diameter) were made in each of these plates using sterile cork borer No. 8 and then agar discs were removed. The entire well were filled with 0.1 ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were incubated at 37℃ for 24 h.

Two replicates were also performed for each extract against each of the test organism. Simultaneously, addition of the respective solvent instead of extract was carried out as controls. After incubation, the zone of inhibition was measured (in mm) (Balandrin et al., 1985; Xie and Huang, 1998).

Preparation of standard fungal suspension

The fungal cultures, *Aspergillus fumigates, Aspergillus niger*, *Aspergillus flavus* and *Fusarium solani* were maintained on saboraud dextrose agar, incubated at 25℃ for four days. The fungal growth was harvested and washed with sterile normal saline and the suspension was stored in refrigerator till used (Balandrin et al., 1985; Xie and Huang, 1998).

Anti fungal activity

The antifungal activities of the prepared extracts were determined by using well agar diffusion method. The fungal cultures including *A. fumigates,*, *A. niger*, *A. flavus* and *F. solani* were used as the test organism The 0.6 ml standard fungal stock suspension 10^8 to 10^9 CFU/ ml was mixed with 60 ml of sterile yeast and mould extract agar thoroughly. 20 ml inoculated yeast and mould extract agar

Table 1. Antibacterial activities of different extract of *C. officinalis* against one Gram positive and three Gram negative bacterial strains.

Plant part	Zone of Inhibition (mm)				
	Methanol fraction	Chloroform fraction	Ethyl acetate fraction	Aqueous fraction	Micro-organism
Roots	10	9	13	16	*S. aureus*
	13	-	10	-	*K. pneumonia*
	-	14	-	-	*S. typhymurium*
	-		12	18	*E. coli*
Stems	-	11	14	14	*S. aureus*
	9	7	13	-	*K. pneumonia*
	16	11	-	17	*S. typhymurium*
	10	-	-	-	*E. coli*
Leaves	16	12	11	14	*S. aureus*
	12	13	9	-	*K. pneumonia*
	14	15	8	17	*S. typhymurium*
	-	-	16	-	*E. coli*

was poured into sterile Petri dishes. The agar was left to set and four wells (10 mm in diameter) was made in each of these plates using sterile cork borer No 8, and then agar discs were removed. The entire well were filled with 0.1 ml of each extracts using microtiter-pipette and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 25℃ for 4 days simultaneously; addition of the respective solvent instead of extract was carried out as controls. After incubation, the result was measured as positive or negative (Balandrin et al., 1985; Xie and Huang, 1998).

Phytochemicals analysis

Preliminary phytochemicals analysis of the extracts in various solvents using the standard procedures has been performed (Jit and Nag, 1988; Bose et al., 1963).

Physicochemical analysis

The powder and extract of the powders in various solvents were examined under ordinary light and ultraviolet (UV)-light (365 nm) and the fluorescence characters were determined .The percentage of loss of weight on drying, total ash, water soluble ash, acid insoluble ash, base insoluble ash and methanol insoluble ash were determined.

RESULTS AND DISCUSSION

The antibacterial activity of *C. officinalis* roots, stem and leaves extracts with different solvents was performed using the Agar well diffusion method by measuring the growth of inhibitory zones. The results showed that all the four solvent extracts possess antibacterial activity against the tested pathogens. As can be seen from Table 1, the root, stem and leaves sample extracted with methanol have different activities against the examined pathogens. For example in case of the root samples of methanol fraction, 10 mm activity was found against *S. aureus* followed by 09 mm against *S. aureus* and 14 mm against *S. typhymurium* aqueous fraction has high activity 16 mm against *S. aureus* and 18 mm inhibition against *E. coli* among all the samples. The stem samples of methanol and water extracts exhibited promising inhibitory results of 16 mm and 17 mm against *S. typhymurium*. An equal zone of inhibition of 14 mm of the ethyl acetate and water extracts was demonstrated against *S. aureus*. The leaves extracts of all the solvents were active with significant zone of inhibition 16 mm of methanolic and 14 mm of water extracts against *S. aureus* and 15 and 17 mm of chloroform and water extracts and the mentioned pathogenic stains. Interestingly only the ethyl acetate fraction was found active against *E. coli* with zone of inhibition of 16 mm.

The mathanolic extract of roots of *C. officinalis* was found active against the three fungal stains except *A. niger*. The chloroform extract was recorded as active against *A. fumigates* and *A. niger* while the ethyl acetate exhibited positive activity against *F. solani*. The water extracts have different results than the chloroform and ethyl acetate fraction and was found positive against *F. solani* and *A. flavor* (Table 2). In case of the stem extracts of the methanol and water similar activity was recorded and exhibited positive results against *F. solani, A. niger* and *A. flavor*. The chloroform extract was found positive against *A. fumigates,* however the ethyl acetate extract was positive against the leaves extract of methanol has positive activity only against *A. fumigates.* The chloroform fraction was inactive only against *A. fumigates* and have positive results against the rest of fungal strains. Similarly, the ethyl acetate and water extract have positive activity against *A. fumigates* and *A.*

Table 2. Antifungal activity of roots, stem and leaves of *C. officinalis*.

Test organisms extract	Part used extract	Methanolic extract	Cholroform extract	Ethyl acetate	Water
A. fumigates	Roots	+	+	-	-
F. solani		+	-	+	+
A. niger		-	+	-	-
A. falvur		+	-	-	+
A. fumigates	Stems	-	+	-	-
F. solani		+	-	-	+
A. niger		+	-	+	+
A. falvus		+	-	+	+
A. fumigates	Leaves	+	-	+	+
F. solani		-	+	-	-
A. niger		-	+	+	+
A. falvus		-	+	-	-

Table 3. Physico-chemicals characters of *C. officinalalis.*

Particular	Plant (%)
Loss of weight on drying	2.90
Total ash	1.24
Acid insoluble ash	35.60
Base insoluble ash	88.55
Water insoluble ash	44.05
Acid soluble ash	62.07
Base soluble ash	73

niger (Table 2). Thus from Table 2, it is apparent that the leaves extracts are more active than the stem and roots extracts. Thus the order is leaves > stem = roots. Many antibacterial and antifungal agents are available for the treatment of bacterial and fungal infections, and these are available in several pharmaceutical forms for either topical or systemic use. Some of the agents that are available commercially but with limited functions and side effects are always associated.

However, because of the need for extended treatments, the high cost, toxicity and limited actions of the classical drugs, and also out of the reach of common man, new and desirable products with low cost, promising results are desirable and are the need of the day to treat these bacterial and fungal infections.

Physiochemical determination

Quantitative determination

The percentage of loss of weight on drying, total ash, water soluble ash, acid insoluble ash, base soluble ash, and residue obtained on ignition were obtained by employing standard method of analysis. The percentage of extractive values in petroleum ether, benzene, chloroform, ethyl acetate and water were also determined Table 3. Fluorescence character of powder and their extract in different solvents were examined under ordinary light and UV-light (365 nm). The powder was also treated with various chemical reagents and the change in colour of the powder and the extract in different solvents were recorded. The results are presented in Table 4.

Phytochemicals screening

5 g of powder of was extracted with benzene, petroleum ether, chloroform, ethyl acetate, and water. The different extracts were tested for the percentage of alkaloids, flavonoids, saponins, anthraquinone, terpenoids, tannins, reducing sugar, and cardiac glycosides. The phyto-chemical tests were performed and the results obtained were presented in Table 5

Conclusion

The results of the antibacterial and antifungal activity

Table 4. Fluorescence characters of powder and their extracts in different solvents.

S/N	Particulars of treatment	Ordinary light	UV light
1	Powder as such	Greenish	Green
2	Powder+1NNaOH	GrayBrown	Light green
3	Powder+1NH_2SO_4	Green	Yellowish
4	Powder+H_2O	Light brown	Green
5	Powder+Methanol	Green	White
	Extracts		
a	Petroleum ether	Light yellowish	Grey
b	Benzene	Green	White
c	Chloroform	Light green	Blackish green
d	Ethyl acetate	Light green	Green
e	Water	Brown	Green

Table 5. Shows the phytochemical test result (+) Positive, (-) negative.

S/N	Test	Results
1	Alkaloids	+
2	Flavonoids	+
3	Saponins	+
4	Antraquinone	+
5	Terpenoids	+
7	Reducing sugar	+
8	Cardiac glycosides	+

contributed by *C. officinale* is very promising and encouraging. Further studies on the fresh and dry plant comparison will further enhance its applications and pharmacological studies which will play very important role in commercializing this highly valuable medicinal plant for its effective results and antimicrobial functions.

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