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Original Research Paper

ANTIMICROBIAL ACTIVITY OF EXTRACTS OF THE MEDICINAL PLANT *COLEUS FORSKOHLII*

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ABSTRACT

Kumaon Himalaya is rich in biodiversity and home of several medicinal plants. Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular. Medicinal plants are the wealthy source of antibacterial agents and curatives. The present study aimed at evaluating the antimicrobial activity of the extracts of medicinal plants *Coleus forskohlii* against bacteria *Staphylococcus aureus, Pseudomonas fluorescens, Sericea, Kelebsiella pneumonia, Bacillus pumilus* and fungi *Aspergillus flavus, Aspergillus parasiticus, Trichoderma rubrum, Microsporum gypseum.* However, this study revealed maximum growth inhibition and effectiveness was remarkably observed in the extracts of *Coleus forskohlii.* These results indicate that roots have a potential broad spectrum antibacterial activity.

Keywords: *Coleus forskohlii*, Extracts, Antibacterial activity, Antifungal activity, Minimal Inhibitory Concentration, Paper disc diffusion method.

INTRODUCTION

Coleus forskohlii (wild) Briq [Syn. *C. barbatus* (Andr.) Benth.] is a plant of Indian origin (Valdes *et al*. 1987) and belongs to the family Lamiaceae (previously Labiatae). It is the most important species of genus *Coleus* popularly known as *Mainamool* or *Manganiberu* or *Makandi beru* in Karnataka and garmar in Maharashtra. It is distributed in sub-tropical Himalayas from Gharwal to Nepal up to an altitude of 2500 m above mean sea level including Pakistan and Sri Lanka. Traditionally, the roots have been used for preparation as condiments in pickles and preparation of pickles (Anon., 1950) and also for medicinal purposes by the Ayurvedic schools of medicines (Ammon and Muller, 1985). Root juice is given to children suffering from constipation (Singh *et al.,* 1980). Kothas, the native tribes of Trichigadi in Nilgiri, South India consider the decoction of tuberous roots as tonic (Abraham, 1981). Roots are eaten for curing cough in Kumaon Himalayas and one to three teaspoonful of root decoction is recommended for treatment of asthma in Maharashtra. Paste prepared from the roots is

mixed with mustard oil and used in the treatment of skin infection by the natives of Kumaon Himalayas.

In India the crop is cultivated in the parts of Gujarat, Maharashtra, Rajasthan, Karnataka and Tamil Nadu and is being grown in an area of more than 2500 hectares for its tuberous roots. Many herbs comprise remarkable properties and functions on multiple biochemical pathways capable to control several organ systems simultaneously. No doubt, many medicinal herbs still holds valuable active compounds of medicinal value which have yet to be discovered. The need of the hour is to screen enormous medicinal plants for its potential biological activity. On the basis of traditional use, Herbs are selected and combined for their ability to inhibit microbial growth in various part of the body and support organ systems responsible for detoxification and immune function. Herbal medicine is also renowned as Phytomedicine - the use of whole plants or part of plants such as seeds, berries, roots, leaves, barks and flowers to prevent or treat illness. A survey of World Health Organization (WHO) indicates that about 70-80% of the world population in the developing countries depends on herbal sources as their primary healthcare system (Fransworth, 1985), (Geneva. 1998). Phytoconstituents such as flavonoids, alkaloids, tannins and triterpenoids are rich source of many medicinal plants challenges the modern medicine and stimulating opportunity for the expansion of modern chemotherapies against wide range of microorganisms (Lutterodt, 1999), (Marjorie, 1999). Due to the increasing failure of chemotherapeutics and rapid development of multiresistant bacterial strains of clinically important medical pathogens acquired the interest of scientist to develop newer broad spectrum antimicrobial agents (Ritch Kro, 1996), (Weisser, 1966). The less availability and unaffordable cost of new generation antibiotics initiated to look for alternative phytomedicine to discover plant derived constituents with claimed antimicrobial activity. The extractable bioactive compounds in medicinal plants are a significant alternative approach to synthetic antibiotics, which could be used as valuables in human disease management. Many herbs with significant antimicrobial activity have been reported in different traditional literatures (Balandrin, 1985), (Jones, 1996). The therapeutic properties of this volatile oil in skin care are anti-inflammatory, antiphlogistic, antiseptic, astringent, cicatrisant, cytophylactic, diuretic and tonic. The fresh leaves have medicinal value and are used as a decoction with other drugs to treat nausea, diarrhea, cold and headache (Arpana, 2008). The objective of this research was to authenticate the antibacterial activity of the extracts obtained from the roots of *Coleus forskohlii.*

MATERIALS AND METHODS

Experimental Section

All the chemicals and reagents used were from C.D.H and Ranchem. Glass wares used were from borosil. The media and broth used for microbial culture were from Hi-Media Pvt. Limited, Bombay, India.

Collection of Plant

The plant *Coleus forskohlii* was collected from G. B. Pant University Ranichauri, Chamba Uttarakhand (India). The root of the plant *Coleus forskohlii* was removed and washed in tape water finally dried.

Preparation of Extracts

Five hundred grams of the air-dried and coarsely powdered plant material was exhaustively extracted for 12 hours with petroleum ether (40-60°C) in soxhlet apparatus. The petroleum ether extract was filtered and evaporated under reduced pressure using Rota-vapor (Heidolph, Heizbad, Laborota 4001, Germany, 2000). The extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with diethyl ether (34ºc) for 10 hours. Again extracted plant material was then air-dried, repacked in the soxhlet apparatus

and exhaustively extracted with Chloroform (99.5%, 60-61ºC) for 12 hours. Further extracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with Ethanol (95%, 78ºC) for 10 hours. And the atleastextracted plant material was then air-dried, repacked in the soxhlet apparatus and exhaustively extracted with methanol (98.8%) for 12 hours. The methanol extract was filtered and evaporated under reduced pressure using Rota-vapor. The extracts were dissolved in dimethylsulphoxide to make the final concentrations which kept in refrigerator till used.

Culture Media and Strains

The media used for antifungal test was Potato dextrose agar/broth of Hi media Pvt. Bombay, India. And also media used for antibacterial test was nutrient agar media of Hi media Pvt. Bombay, India. Pathological strains, i.e. bacteria *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Sericea, Kelebsiella pneumonia, Bacillus pumilus* and fungi *Aspergillus flavus, Aspergillus parasiticus, Trichoderma rubrum, Microsporum gypseum* were tested for antimicrobial activity of the extracts. These pure cultures of strains were collected from Microbial Tissue Culture Collection (MTCC) Chandigarh India.

Disc Diffusion Method

The antimicrobial activity of the extracts was determined by disc diffusion method (NCCLS, 1997) in petriplates containing NA and PDA medium (20 mL media/plate), respectively. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism. Ampicillin was used as a positive reference for bacteria while Gentamicin for fungi. Discs without samples were used as a negative control. Plates were kept at 4 ºC for 1h. The plates were incubated at 37 ºC for 24 h for bacteria and at 30 °C for 48 h for fungal strains. Antimicrobial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

Determination of Antibacterial Activity

The extracts were individually tested against a panel of microorganisms selected. Bacterial strains were cultured overnight at 37 ºC in nutrient agar (NA). The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism. The diameters of zone of inhibition observed were measured.

Determination of Antifungal Activity

The disc diffusion method (NCCLS, 1997) was modified. Potato dextrose agar (PDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Potato dextrose broth. The paper discs (6 mm in diameter) were separately impregnated with 15 µL of extracts placed on the agar which had previously been inoculated with the selected test microorganism. Fungal plates were incubated at 37 ^{o}C for 72 h. The diameters of zone of inhibition observed were measured.

Determination of MIC

The antifungal plant extracts were then after evaluated to determine MIC value. The broth dilution method was adopted by using N-saline for diluting the plant extracts and was incubated for 48 h. The minimum dilution of the plant extracts that kills the fungal growth and microbial growth. The minimum dilution of plant extracts that inhibits the growth of the organism was taken as MIC.

RESULTS AND DISCUSSION

Antibacterial Activity

The antibacterial activity was determined by measuring the diameter of zone of inhibition recorded. The different extracts of the plant *Coleus forskohlii* were found to have maximum antibacterial activity. The results obtained in the evaluation of the antibacterial activity of the

different extracts against some bacteria *Staphylococcus aureus, Pseudomonas fluorescens, Sericea, Kelebsiella pneumonia* and *Bacillus pumilus* are listed in Table 1. The root extracts (Pet. ether, Diethyl ether, chloroform, methanol and ethanol) were screened for antibacterial (Figure1).

Table 1: Anti bacterial activity of different fraction of *Coleus forskohlii* root with standard

S. No.	Test Organism/s	Inhibition zone in diameter (mm)					Methanol
		Ampicillin	Pet. ether	Diethyl ether	Chloroform	Ethanol	
1.	*Staphylococcus aureus*	18	10	7	7	22	12
2.	*Pseudomonas fluorescens*	20	6	7	_	24	8
3.	*Sericea*	18	10	10	7	11	14
4.	*Kelebsiella pneumonia*	15	_	_	_	20	15
5.	*Bacillus pumilus*	16	_	_	_	12	11

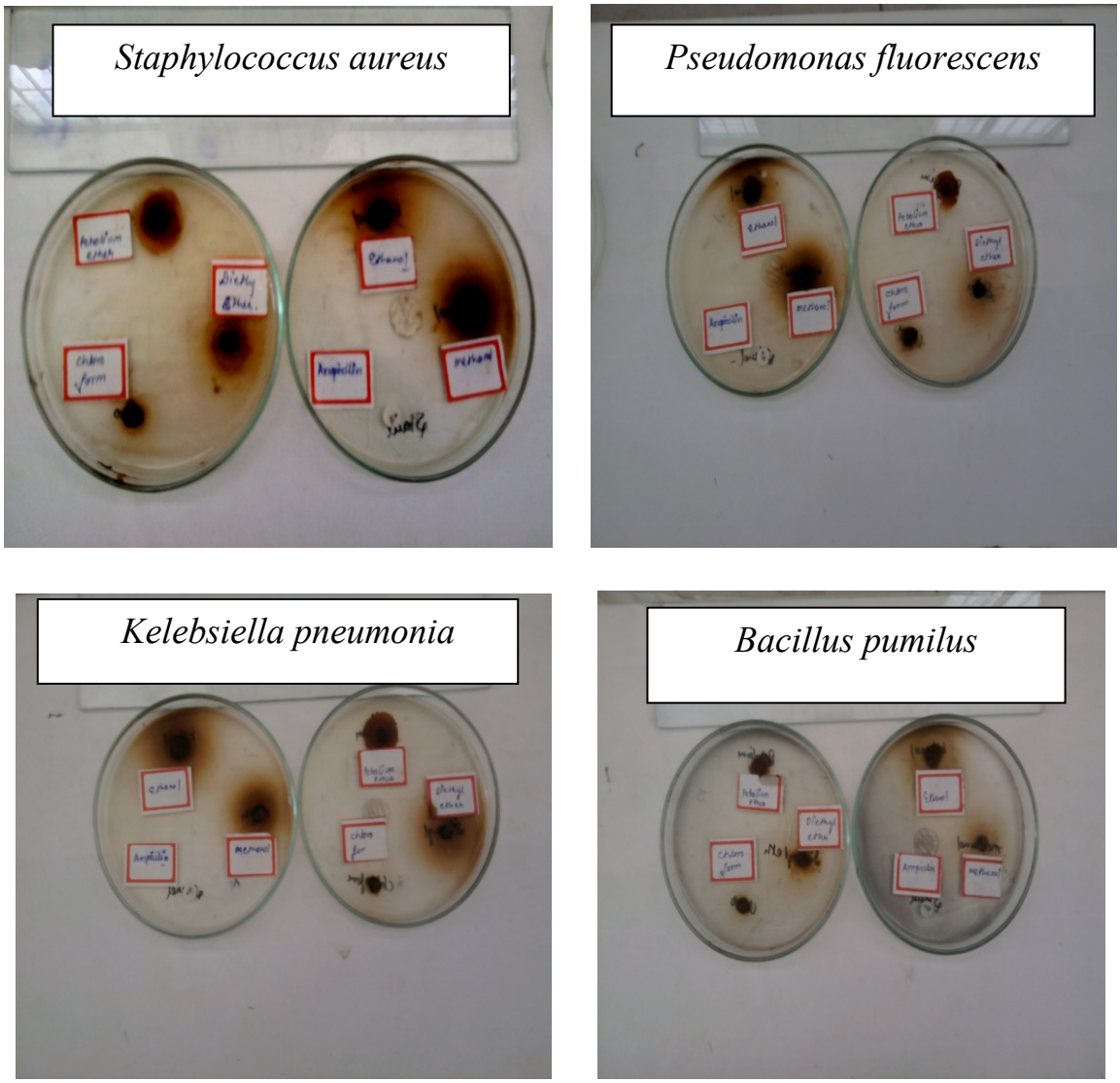

Figure1: Pictures showing Anti bacterial activity of different fraction of *Coleus forskohlii* root with standard

Table 2: Minimum inhibitor concentration (MIC) of Ethanolic extract of *Coleus forskohlii* root against various bacterial strains

S. No.	Bacteria Strain	Ethanolic extract *Coleus forskohlii* Roots					
		100ml	50ml	25ml	12.5ml	6.25ml	3.125ml
1.	*Staphylococcus aureus*	15	10	6	-	-	-
2.	*Pseudomonas fluorescens*	24	20	17	15	10	7
3.	*Sericea*	6	-	-	-	-	-
4.	*Kelebsiella pneumonia*	10	15	10	7	-	-
5.	*Bacillus pumilus*	11	7	-	-	-	-

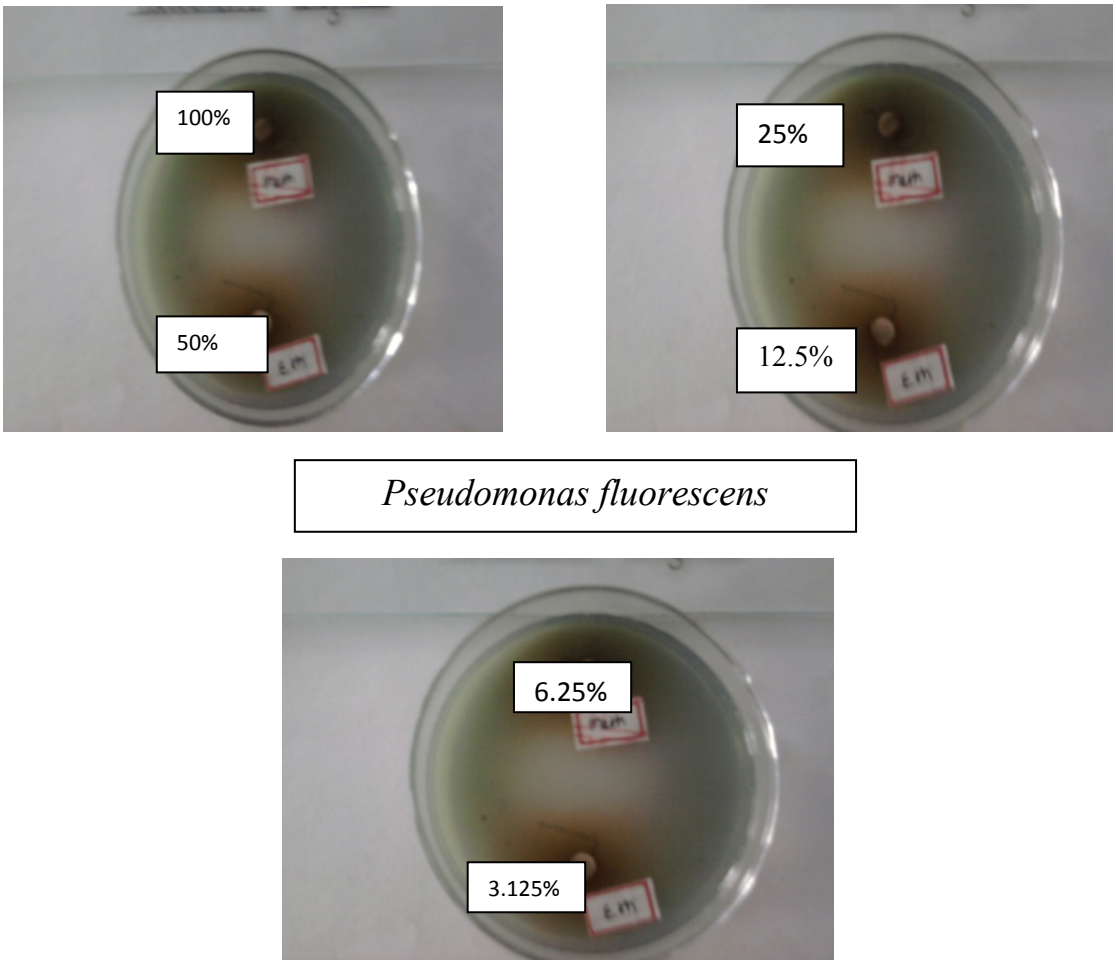

Figure2: Pictures showing Minimum inhibitor concentration (MIC) of Ethanolic extract *of Coleus forskohlii* root against various bacterial strains

The extracts shown zone of inhibition against bacteria *Staphylococcus aureus, Pseudomonas fluorescens, Sericea, Kelebsiella pneumonia* and *Bacillus pumilus*. But Ethanolic extract shown maximum zone of inhibition against bacteria *Staphylococcus aureus, Pseudomonas fluorescens, Sericea, Kelebsiella pneumonia* and *Bacillus pumilus*. The inhibition zones are 22mm, 24mm, 11mm, 20mm and 12mm respectively (Figure2).

Antifungal Activity

The different extracts of the plant *Coleus forskohlii* were found to have maximum

antifungal activity. The results obtained in the evaluation of the antifungal activity of the different extracts against some fungi *Asperigilus flavs, Asperigilus parasiticus, Trichoderma rubrum,* and *Microsporum gypseum* are shown in Table 3.

Table 3: Antifungal Activity of different fraction of *Coleus forskohlii* with standard drug

S. No.	Organism	Inhibition Zone (mm)					
		Gentamicin	Petr. ether	Diethyl ether	Chloroform	Ethanol	Methanol
1.	*Aspergillus flavus*	20 mm	11mm	15	20	25	11
2.	*Aspergillus parasiticus*	18 mm	11mm	-	-	-	-
3.	*Trichoderma rubrum*	20 mm	11mm	22	25	10	-
4.	*Microsporum gypseum*	22 mm	11mm	10	25	20	10

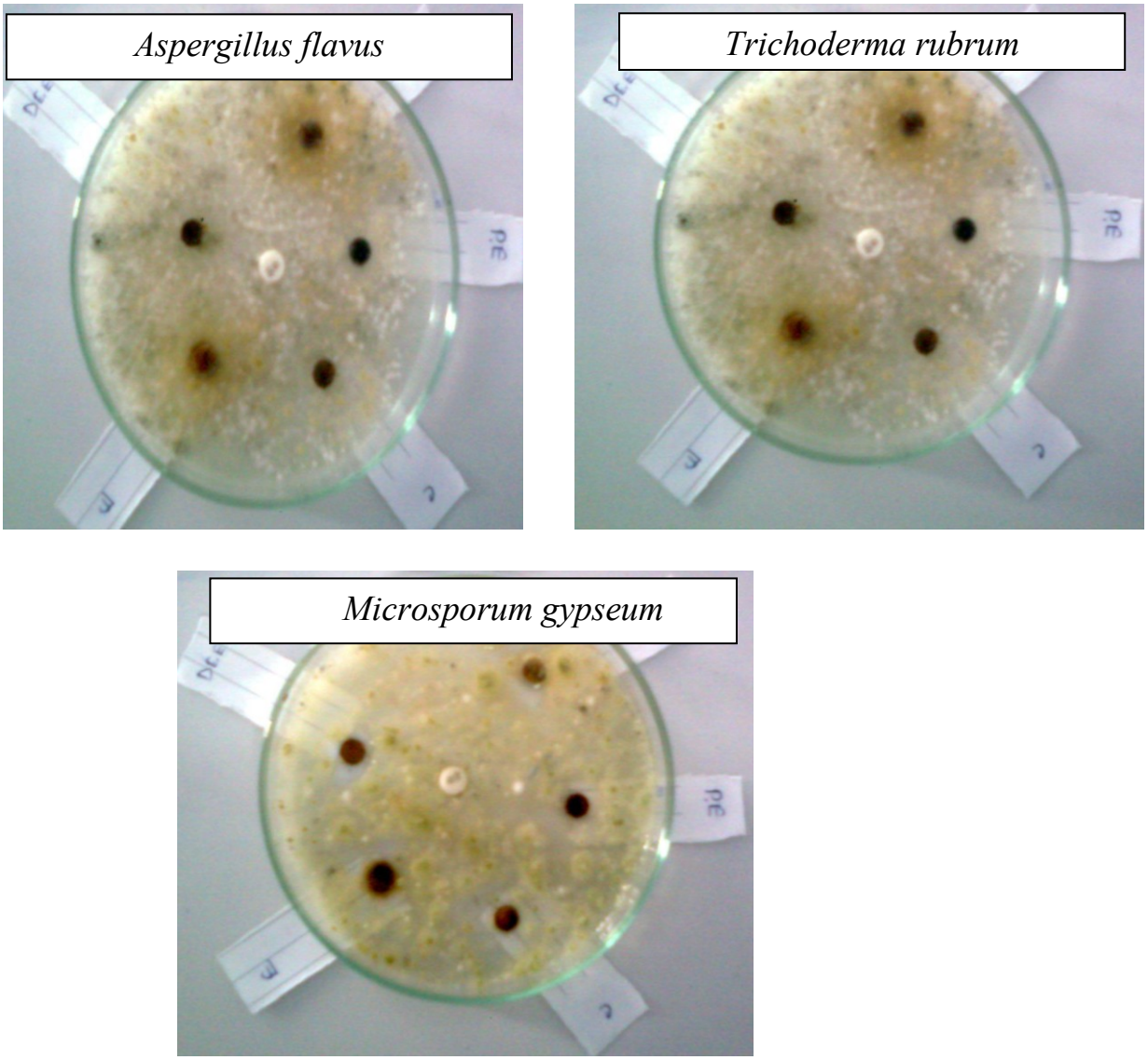

Figure3: Pictures showing antifungal activity of different fraction of *Coleus forskohlii* with standard drug

Table 4: MIC of chloroform extract of root of *Coleus forskohlii*

Organism	100ml	50ml	25ml	12.5ml	6.25ml	3.125ml
Aspergillus flavus	20	10	12	6	-	-
Aspergillus parasiticus	-	-	-	-	-	-
Trichoderma rubrum	20	15	10	7	-	-
Microsporum gypseum	25	20	17	15	10	7

The extract shown zone of inhibition against fungi *Aspergillus flavus, Aspergillus parasiticus, Trichoderma rubrum,* and *Microsporum gypseum* whereas chloroform extract shown maximum zone of inhibition against fungi *Aspergillus flavus, Aspergillus parasiticus, Trichoderma rubrum,* and *Microsporum gypseum.* The inhibition zones were 20mm, 0mm, 25mm and 25mm respectively (Figure3).

CONCLUSION

In the present study crude extracts of the plant material obtained in polar and less polar organic solvent were tested against six standard bacteria (*Staphylococcus aureus*, *Pseudomonas fluorescens*, *Sericea, Kelebsiella pneumonia, Bacillus pumilus)* and four fungi *Aspergillus flavus, Aspergillus parasiticus, Trichoderma rubrum, Microsporum gypseum* causing skin diseases. All the crude extracts have significant antibacterial activity on most of the bacteria whereas ethanol extract had maximum inhibition activity as compared to chloroform, methanol, ethyl acetate and petroleum ether. The antifungal activity on most of the fungi, but chloroform extract had maximum inhibition activity as compared to ethanol, methanol, ethyl acetate and petroleum eater. From the present study, it is evident that *Coleus forskohlii* could play an important role in the management of hypotensive and cardiotonic. The above result opens the possibility of finding new clinically effective antioxidant drug and could be useful in understanding the relationship between traditional cures and current medicines.

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