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# Investigations on phytochemical analysis and antifungal activity of *Chromolena odorata* (L) king and robinson on human dermatophytic fungi

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## ABSTRACT

The present investigation highlights phytochemical screening and antifungal activity of *Chromolena odorata* kings and robinson. The shade dried plant materials (root and leaf) were subjected to soxhlet extraction using petroleum ether, ethanol and chloroform as solvents. The solvent extracts were subjected to preliminary phytochemical screening and *in vitro* antifungal activity against human dermatophytic fungi by Well diffusion method. Leaf extracts exhibited the presence of more phytoconstituents than root extracts. Marked antifungal activity was observed in case of leaf extracts. The antifungal activity could be due to the phytoconstituents present in the solvent extracts. Further studies in animal models could possibly reveal the potential of plant to inhibit fungal pathogens *in vivo*.

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## KEYWORDS

*Chromolena odorata*;
Dermatophytic fungi;
Solvent extracts;
Phytochemical analysis;
Agar well diffusion.

## INTRODUCTION

Mankind has been continuously using the plants in one or the other way in the treatment of various ailments. The developing countries still using traditional medicines for health care. The modern pharmacopeia contains medicines obtained from plant and many other synthetic analogues built on prototype compounds isolated from plants. *Chromolena odorata* (L) King and Robinson, belongs to the family Asteraceae and commonly known as Siam weed, is a fast growing perennial and invasive weed native to south and central America and later it has been introduced to tropical region of Asia, Africa and other parts of world. It is an aggressive competitor that occupies different types of lands where it forms dense stands that prevents the establishments of other flora. It is a menace in plantations and other ecosystems. In recent decades, it has become a serious weed in the humid tropics of south East Asia, Africa and Pacific Islands. Despite the negative sides to the plant, it still has patronage from practitioners of traditional. It has been reported to have anti-plasmodic, anti-protozoal, anti-trypanosomal, antibacterial and anti-hypersensitive activities. It has also been reported to possess anti-inflammatory, astringent, diuretic and hepatoprotective activities. The present investigation highlights the potential of phytochemical constituents of *Chromolena odorata* in terms of antifungal properties against the most aggressive human pathogenic dermatophytic fungi which causes several types of skin diseases in human beings.

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## MATERIALS AND METHODS

### Collection of plant materials

Plant material was collected in and around Shankaraghatta, Kuvempu University Shivamogga district. Plant material was authenticated to identity in the Dept. of Applied Botany, Kuvempu University and a voucher specimen was kept in the department. The collected material was washed with distilled water thoroughly, leaves and roots were separated and kept for shade drying. Shed dried plant materials were powdered mechanically.

### Solvent extraction

About 250g of powdered material was subjected to soxhlet extraction and exhaustively extracted with various solvents namely petroleum ether, chloroform, and ethanol for about 48 hours. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator[1].

### Phytochemical screening

Qualitative phytochemical analysis of the crude solvent extracts of root and leaf of *Chromolena odorata* was determined as follows: Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate+2 ml $FeCl_3$, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate+1% HCl+steam, 1 ml filtrate+6 drops of Mayor's reagents/Wagner's reagent/ Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate+5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate +1 ml glacial acetic acid + $FeCl_3$ + conc. $H_2SO_4$); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. $H_2SO_4$. Blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids[2].

### Antifungal activity

### Test fungi

The human dermatophytes namely *Trichophyton equineum, Chrysosporium zonatum, Microsporium gypsum* and *Tricophyton kannin* were used as target fungi. The fungi were maintained on Sabouraud's dextrose agar slants and preserved in refrigerator. The suspension of spores of the test fungi was prepared in a test tube containing 0.85% sterile normal saline containing 0.01% Tween 80 detergent[3].

### Antifungal susceptibility test

The antifungal susceptibility test was performed by Agar well diffusion (Cup plate assay) method[4] with minor modifications. The spore suspensions of test fungi were inoculated on to Sabouraud's dextrose agar medium by swab inoculation. Using sterile cork borer, 6 mm diameter wells were bored in the agar and the different solvent extracts (100mg/ml) of both root and leaves reconstituted in 5% DMF were transferred into the wells. The plates were allowed to stand for two hours and then incubated at room temperature for up to 72 hours and zone of inhibition was measured to the nearest millimeter. The test was done in triplicates to arrive concordant results.

## RESULTS AND DISCUSSION

TABLE 1 reveals phytochemical constituents

**TABLE 1: Phytoconstituents in different solvent extracts of root and leaf of *Chromolena odorata***

| Phytoconstituent | Leaf extract | | | Root extract | | |
|---|---|---|---|---|---|---|
| | Pet. ether | Chloroform | Ethanol | Pet. ether | Chloroform | Ethanol |
| Saponin | + | + | - | + | + | - |
| Terpinoids | - | - | + | - | + | + |
| Steroids | + | + | + | - | - | - |
| Alkaloids | - | - | - | - | - | - |
| Tannins | + | + | + | - | - | - |
| Flavonoids | - | - | + | - | + | + |
| Glycosides | + | + | - | - | - | - |

'+' Detectable; '-' Not detectable

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TABLE 2: Antifungal activity of different solvent extracts of root and leaf of *Chromolena odorata*

| Test fungi | Zone of inhibition in mm | | | | | |
|---|---|---|---|---|---|---|
| | Leaf extract | | | Root extract | | |
| | Ethanol | Chloroform | Pet.ether | Ethanol | Chloroform | Pet.ether |
| *Trichophyton equineum* | 18 | 16 | 13 | 13 | 11 | 10 |
| *Microsporium gypsium* | 17 | 14 | 14 | 12 | 11 | 10 |
| *Trichophyton kannin* | 19 | 17 | 16 | 14 | 12 | 12 |
| *Chrysosporium zonatum* | 16 | 15 | 14 | 14 | 13 | 11 |

**Results are average of three trials**

present in various solvent extracts of root and leaf of *Chromolena odorata*. The results show the presence of more phytoconstituents in leaf extracts than root extracts. Ethanol and Chloroform extracts of root were found to contain more phytochemicals than petroleum ether extracts. TABLE 2 depicts antifungal activity of different solvent extracts of roots and leaves of *Chromolena odorata.* The results reveal maximum inhibitory activity in ethanol extract of leaves followed by chloroform and petroleum ether. Among fungi tested, *T.kannin* was found to be more inhibited followed by *T.equineum, M.gypsium* and *C.zonatum*. Antifungal activity of root solvent extracts was not found to possess activity comparable with that of leaf extracts. More inhibitory activity was noticed in *T.kannin* and *C.zonatum* followed by *T.equineum* and *M.gypsium*.

Phytoconstituents present in plants are producing exciting opportunity for the expansion of modern chemotherapies against wide range of microorganisms[5]. Several phytochemical surveys have been published, including the random sampling approach which involved some plant accessions collected from all parts of the world. The major chemical substances of interest in these surveys have been the alkaloids and steroidal sapogenins (saponins), however, other diverse groups of naturally occurring phytochemicals such as flavonoids, tannins, unsaturated sterols, triterpenoids, essential oils, etc. have also been reported[6]. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant[2].

## CONCLUSION

Infectious diseases still represent an important cause of mortality and morbidity among humans especially in developing countries. Interest in plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics. The most important bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds. In the present study, various phytochemical constituents have been found to be present in different solvent extracts. Marked antifungal activity of solvent extracts was observed in the study. The antifungal activity of the solvent extracts could be due to the presence of different phytoconstituents. Use of these phytoconstituents could confer protection against microbes including drug resistant microorganisms. The study was done *in vitro*. Further studies in animal models could possibly reveal the antifungal activity of this plant *in vivo*.

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