

IN VITRO ANTIFUNGAL ACTIVITY OF LEAF AND ROOT EXTRACTS OF THE MEDICINAL PLANT, *HYPOCHAERIS RADICATA* L.

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Received: 27 Apr 2013, Revised and Accepted: 16 Jun 2013

ABSTRACT

Objective: To assess the antifungal activity of the successive extracts (petroleum ether, chloroform, ethyl acetate, methanol and water) of leaf and root parts of *Hypochoeris radicata* and to determine the minimum inhibitory (MIC) concentration against nine human pathogenic fungi.

Methods: The antifungal activities of the leaf and root extracts against nine pathogenic fungi were tested by using disc diffusion method. Tetracycline was used as positive control. MIC for the methanolic leaf and root extracts of this species was assessed for antifungal susceptibility using broth micro dilution method.

Results: From the evaluation it was found that ethyl acetate extracts inhibited the fungal growth effectively than the other solvent extracts. The fungal species, *Aspergillus niger* and *Mucor* sp. were very sensitive to the ethyl acetate extract of both leaf (24.83 ± 0.28) and root (20.96 ± 0.25). Among the two parts studied, the root part showed higher antifungal activity than the leaf part. MIC for the methanolic leaf and root extracts of this species was ranging between 200 and 500 μ g/mL and 200 and 600 μ g/mL respectively.

Conclusion: The obtained results provide a support for the use of this plant in traditional medicine and it is a potential antiseptic source for the prevention and treatment of fungal infections.

Keywords: *Hypochoeris radicata*, Antifungal activity, Disc diffusion, MIC.

INTRODUCTION

Generally, the fungal infections are the most common cause of many skin diseases in developing countries [1]. Opportunistic fungal infections, mainly resulting from the species of *Candida*, *Cryptococcus* and *Aspergillus* are life-threatening in immunocompromised patients (with AIDS, cancer, or organ transplant) [2]. Due to the increasing number of individuals of this category, fungal infections have increased in the last two decades, affecting millions of people worldwide [3]. Using of synthetic chemicals for controlling these skin diseases is not ecofriendly and they are not providing environmental security. Therefore, it is necessary to search for more effective and less toxic novel antifungal agents that would overcome these disadvantages. Interestingly, plants are widely employed in folk medicine, mainly in communities with inadequate conditions of public health and sanitation. The antimicrobial compounds produced by plants are active against plant and human pathogenic microorganisms [4]. Several medicinal plants have been extensively studied in order to find more effective and less toxic compounds [5].

The species, *Hypochoeris radicata* (Asteraceae) commonly known as cat's-ear, is native to Europe also distributed in high hills of Nilgiris, the Western Ghats, India (above 2200m msl). It has been extensively used in traditional medicine for its anticancer, anti-inflammatory, anti-diuretic and hepatoprotective activities and to treat kidney problems. The leaves and roots of this species also possess antioxidant property [6] and antibacterial activity [7] also. The milky sap is bitter and the plant is suspected by some of being unwholesome as fodder [8]. It is high in protein and low in fibre. The calcium content is exceptionally high and it is rich in copper, sulphur and chloride. The seed is an important constituent in the diet of many farmland birds including linnets (*Carduelis cannabina*) [9]. The objective of the present study was to evaluate the antifungal activity and minimum inhibitory concentration of leaf and root extracts of *H. radicata* against certain pathogenic fungal species.

MATERIALS AND METHODS

Plant collection and identification

The fresh plant material was collected from Kattabettu, Nilgiris, the Western Ghats, India (above 2200m above msl). The authenticity of the plant was confirmed in Botanical Survey of India Southern Circle,

Coimbatore by referring the deposited specimen. The voucher number of the specimen is BSI/SRC/5/23/2010-11/Tech.153.

Preparation of plant extracts

Fresh plant material of leaf and root parts were washed under running tap water, shade dried and then homogenized to fine powder and stored in airtight bottles. About 50g of coarsely powdered leaves and roots (50g/250mL) were extracted separately in a soxhlet extractor for 8 to 10 hours (50-85°C) sequentially with petroleum ether, chloroform, ethyl acetate, methanol and water separately in order to extract non-polar and polar compounds [10].

Preparation of inoculums

The nine fungal cultures obtained from TNAU, Coimbatore such as *Paecilomyces lilacinus*, *Mucor* sp., *Trichoderma viride*, *Verticillium lecanii*, *Candida albicans*, *Fusarium* sp., *Penicillium* sp., *Aspergillus fumigatus* and *A. niger* were grown at 27°C on potato dextrose agar (PDA) medium. Spores of the each fungus species was collected from cultures on agar plates after 7 days [11]. PDA broth prepared by transferring a loop full of cells from the stock cultures was diluted with fresh potato dextrose broth. The sporangial suspension concentration was adjusted to 2×10^5 (CFU/mL) spores [12].

Antifungal activity

Antifungal activity was investigated by the disc diffusion method [13]. Fungal suspension (2×10^5) was streaked on the potato dextrose agar (PDA) medium containing Petri plates. Then, sterile discs (made from Whatman filter paper) each about 5mm diameter impregnated with the leaf and root extracts separately were placed on the inoculated plates. Similarly, each plate was placed with a sterile disc, tetracycline as positive control. All the plates were incubated at 28°C for 24-48 hours. The zones of growth inhibition around the disc were measured after 48 hours. The sensitivity of the fungal species to the plant extracts was determined by measuring the sizes of inhibition zones (diameter of the zone) on the agar surface around the disc.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined through the broth dilution method [14]. The fungal inoculum (10^5 dilution) was taken in test tubes with (1800 μ l) nutrient broth

supplemented with eight different concentrations of both leaf and root extracts 100-800µl/mL separately. The results of the extracts were compared with a standard, positive control (tetracycline 100µg/mL) and negative control (methanol 100µg/mL). All the test tubes were incubated at 35°C for 24-48 hours. The tubes were examined for visual turbidity. The MIC values were taken as the lowest concentration that inhibited the visual growth of the tested organisms [15, 16].

Statistical analysis

The antibacterial activity of *H. radicata* leaf and root extracts was indicated by clear zones of growth inhibition. All experiments were

performed in triplicates and the results are presented as mean ± SD (Standard Deviation) according to New Duncan's Multiple Range Test [17].

RESULTS

Exploitation of the evaluation of antifungal activity of the present study revealed that the *H. radicata* possess potential antifungal activity against nine pathogenic fungal species. From the evaluation it is found that ethyl acetate extract inhibited the growth of the colonies of large number of fungal species than the other solvent extracts studied.

Table 1: Antifungal activity of various alcoholic and aqueous leaf extracts of *Hypochaeris radicata*.

Control/ Extracts	Zone of inhibition (mm)								
	PL	Ms	TV	VL	CA	Fs	Ps	AF	AN
PC	-	20.56 ± 0.51 ^a	24.66 ± 0.35 ^a	25.86 ± 0.15 ^a	16.73 ± 0.25 ^b	9.40 ± 0.36 ^c	37.23 ± 0.25 ^d	33.50 ± 0.50 ^d	31.36 ± 0.56 ^d
PE	-	8.76 ± 0.25 ^a	-	-	-	-	-	8.83 ± 0.15 ^a	10.23 ± 0.40 ^b
CF	-	12.96 ± 0.57 ^a	12.83 ± 0.15 ^a	7.86 ± 0.15 ^b	8.56 ± 0.60 ^b	-	7.83 ± 0.15 ^b	13.50 ± 0.50 ^a	12.73 ± 0.25 ^a
EA	9.93 ± 0.11 ^a	14.66 ± 0.61 ^b	14.76 ± 0.25 ^b	9.93 ± 0.11 ^a	9.83 ± 0.15 ^a	10.16 ± 0.15 ^{ab}	8.83 ± 0.28 ^a	14.66 ± 0.30 ^b	24.83 ± 0.28 ^c
ME	-	14.60 ± 0.40 ^a	7.73 ± 0.25 ^b	7.60 ± 0.52 ^b	15.53 ± 0.47 ^a	7.73 ± 0.25 ^b	-	12.96 ± 0.57 ^a	19.96 ± 0.57 ^c
WA	-	-	-	-	11.26 ± 0.25 ^a	-	-	-	15.56 ± 0.51 ^b

'-' indicates no activity.

PC = Positive control (Ampicillin), PE = Petroleum ether, CF = Chloroform, EA = Ethyl acetate, ME = Methanol, WA = Water.

PL = *Paecilomyces lilacinus*, Ms = *Mucor* sp., TV = *Trichoderma viride*, VL = *Verticillium lecanii*, CA = *Candida albicans*,

Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus*, AN = *Aspergillus niger*.

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscript in a column are significantly different (p<0.05).

Table 2: Antifungal activity of various alcoholic and aqueous root extracts of *Hypochaeris radicata*.

Control/ Extracts	Zone of inhibition (mm)								
	PL	Ms	TV	VL	CA	Fs	Ps	AF	AN
PC	-	20.56 ± 0.51 ^a	32.16 ± 0.76 ^b	24.40 ± 0.52 ^a	19.66 ± 0.25 ^a	10.23 ± 0.25 ^c	34.63 ± 0.40 ^b	34.46 ± 0.47 ^b	34.66 ± 0.51 ^b
PE	-	7.73 ± 0.25 ^a	9.66 ± 0.30 ^b	8.56 ± 0.51 ^{ab}	-	-	7.86 ± 0.75 ^a	10.76 ± 0.20 ^c	10.66 ± 0.50 ^c
CF	6.66 ± 0.57 ^a	19.50 ± 0.50 ^b	20.90 ± 0.36 ^b	12.76 ± 0.25 ^{ab}	13.73 ± 0.25 ^{ab}	9.30 ± 0.60 ^a	11.66 ± 0.30 ^{ab}	13.66 ± 0.54 ^{ab}	17.66 ± 0.23 ^b
EA	7.50 ± 0.50 ^a	20.96 ± 0.25 ^b	12.80 ± 0.26 ^{ab}	8.23 ± 0.68 ^a	11.73 ± 0.25 ^{ab}	14.83 ± 0.15 ^{ab}	15.46 ± 0.56 ^{ab}	15.30 ± 0.60 ^{ab}	15.66 ± 0.15 ^{ab}
ME	-	9.73 ± 0.25 ^a	14.83 ± 0.15 ^b	9.73 ± 0.25 ^b	9.73 ± 0.25 ^a	6.66 ± 0.57 ^c	7.16 ± 0.15 ^{ca}	11.66 ± 0.57 ^{ab}	14.65 ± 0.12 ^b
WA	-	-	10.73 ± 0.64 ^a	-	8.93 ± 0.83 ^b	-	-	12.56 ± 0.51 ^c	10.50 ± 0.50 ^a

'-' indicates no activity.

PC = Positive control (Ampicillin), PE = Petroleum ether, CF = Chloroform, EA = Ethyl acetate, ME = Methanol, WA = Water.

PL = *Paecilomyces lilacinus*, Ms = *Mucor* sp., TV = *Trichoderma viride*, VL = *Verticillium lecanii*, CA = *Candida albicans*,

Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus*, AN = *Aspergillus niger*.

Values were performed in triplicates and represented as mean ± SD.

Mean values followed by different superscript in a column are significantly different (p<0.05).

Effect of leaf extract

Among the five solvents attempted, the ethyl acetate extract showed higher inhibitory activity (24mm) followed by methanol extract (19mm) and chloroform extract (12mm) against the fungus,

Aspergillus niger. The chloroform and methanol extracts showed significant activity against all the tested fungal species which was ranging between 7mm and 13mm, and 6mm and 19mm respectively. However, the petroleum ether and water extracts showed moderate activity against the fungal species viz., *Mucor* sp.,

A. fumigatus, *A. niger* and *Candida albicans*, and *A. niger* respectively (Table 1).

Effect of root extract

The greater zone of inhibition was produced by ethyl acetate and chloroform extracts of root of *H. radicata* against the fungi, *Mucor* sp. and *Trichoderma viride* (20mm) followed by methanol, water and petroleum ether extracts (Table 2). Methanol extract showed highest activity against *T. viride* and *Aspergillus niger* (14mm) and minimum

activity against the fungus, *Fusarium* sp. The activity of petroleum ether and water extracts the fungal growth was not noteworthy.

Minimum inhibitory concentration

Table 3 presents the data on minimum inhibitory concentration (MIC) of methanolic leaf and root extracts of *H. radicata*. The leaf and root extracts exhibited remarkable antifungal activity which was ranging between 200 and 500µg/mL and 200 and 600µg/mL respectively.

Table 3: Minimum inhibitory concentration (MIC) of methanolic leaf and root extracts of *Hypochoeris radicata*.

Plant parts	Minimum inhibitory concentration (µg/ml)							
	PL	TV	VL	CA	Fs	Ps	AF	AN
Leaf	300	400	300	400	200	300	200	300
Root	300	400	300	400	200	600	300	200

PL = *Paecilomyces lilacinus*, Ms = *Mucor* sp., TV = *Trichoderma viride*, VL = *Verticillium lecanii*, CA = *Candida albicans*, Fs = *Fusarium* sp., Ps = *Penicillium* sp., AF = *Aspergillus fumigatus*, AN = *Aspergillus niger*.

DISCUSSION

The results obtained from the present investigation revealed that the highest antifungal activity was exhibited by the ethyl acetate extract and the least by the petroleum ether and water extracts. The basis of varying degree of sensitivity of test organisms of fungi may be due to the intrinsic tolerance of microorganisms and the nature and combinations of phytochemicals presents in the crude extracts.

The antifungal activity of ethyl acetate extract of *H. radicata* leaf showed highest inhibitory activity against the fungus, *Aspergillus niger*. It is a filamentous ascomycete fungus that is ubiquitous in the environment and has been implicated in opportunistic infections of humans [18]. It causes various diseases in plants and animals. In plants it causes black mould and rot diseases and in human beings it causes aspergillosis by which pulmonary allergy, bronchopulmonary aspergillosis and pulmonary aspergilloma are made [19, 20]. Raji and Raveendran [21] reported that water extracts of Asteraceae members showed strongest effect on reduction in growth of *A. niger* than the species of certain other families. They explained that certain specific compounds of unknown functional group may present in Asteraceae member which may played role in the inhibition of fungal colonies. This fact perhaps be a reason for the fungal inhibitory property of the study species of Asteraceae family, *H. radicata*. As in the present, Duraipandiyan and Ignacimuthu [22] reported that in majority of the species, out of 45 plants studied, ethyl acetate extract exhibited more pronounced antifungal activity than the other solvents. Similarly, Saheb et al.[23] assayed various extracts like aqueous, alcoholic and ethyl acetate extracts of leaves of five *Terminalia* species against five plant pathogenic fungi like *A. flavus*, *A. niger*, *Alternaria brassicicola*, *A. alternata* and *Helminthosporium tetramera* and found that the ethyl acetate extract showed better inhibitory effect against all the fungi tested. In the present study, chloroform and methanol extracts of leaf showed significant antifungal activity against seven fungi viz., *Mucor* sp., *Trichoderma viride*, *Verticillium lecanii*, *Candida albicans*, *Penicillium* sp., *A. fumigates* and *A. niger* and instead of *Penicillium* sp. the *Fusarium* sp. was inhibited by methanol extract. Water and petroleum ether extracts of leaf inhibited less number of two to three fungi only (*C. albicans* and *A. niger*, and *Mucor* sp., *A. fumigates* and *A. niger*). This could be due to the lack of specific active compounds in the extracts. However, all the extracts of *H. radicata* leaf inhibited the growth of *A. niger*.

The ethyl acetate and chloroform extracts of root of *H. radicata* exhibited greater zone of inhibition against the fungi *Mucor* sp. and *Trichoderma viride* which are causing many infectious diseases in human beings. On the other hand, the methanol extract generally inhibited the growth of all fungal species except *Paecilomyces lilacinus*. Petroleum ether and water extracts inhibited only few fungal species (*Mucor* sp., *T. viride*, *Verticillium lecanii* *Penicillium* sp., *Aspergillus fumigates* and *A. niger* and *T. viride*, *Candida albicans*, *A.*

fumigates and *A. niger*). The most susceptible organisms to root extracts of the study species were determined to be *A. fumigatus*, *A. niger* and *T. viride*, and the most resistant organism was *P. lilacinus*.

The present results showed that the ethyl acetate extracts of leaf and root were more effective than the other extracts tested. In another Asteraceae member, *Stevia rebaudiana* also the ethyl acetate extract has reported to have higher antifungal activity than that of the other alcoholic solvent extracts [24]. Among the two parts studied, the root part showed higher antifungal activity than the leaf part. In earlier report also it has been known that the root part of *Hypochoeris radicata* displayed prodigious antibacterial activity [7]. This may be attributed to the presence of variety of flavonoids and phenolic compounds in the roots [25] which may have the capacity to rupture the cytoplasmic membrane of the fungal cells and damage the intracellular compounds [26] or they may interact with lipid bilayers or inhibit the protein and nucleic acid synthesis of the fungal cell [27]. Various publications have documented the effective antifungal activity of Asteraceae members [28, 29, 30, 31]. It was further observed that the inhibitory activities of ethyl acetate extract of leaf against *Fusarium* sp. and ethyl acetate extract of root against *Mucor* sp. and *Fusarium* sp. were significantly greater than that of the standard drug, tetracycline, which indicates the effectiveness and specific inhibitory function of ethyl acetate solvent by deriving specific compounds against these fungi.

Minimum inhibitory concentration (MIC) of methanolic leaf extract was ranging between 200 and 400µg/mL. The most susceptible species for this extracts were *Aspergillus niger* and *Fusarium* sp. (200µg/mL) and most resistant species were *Trichoderma viride* and *Candida albicans* (400µg/mL). MIC of methanolic root extract of the study species was ranging between 200 (against *Fusarium* sp. and *A. niger*) and 600µg/mL (against *Paecilomyces lilacinus*). From the above results it is known that *Fusarium* sp. and *Aspergillus* sp. were susceptible to both the leaf and root extracts.

CONCLUSION

The present study revealed that the leaf and root extracts *Hypochoeris radicata* possess significant antifungal activity and it leads to discover novel antifungal drugs. It is interesting to note that the ethyl acetate extract of this species, could be used mainly against the two fungi, *Aspergillus niger* and *Mucor* sp. to control the infectious diseases aspergillosis and zygomycosis respectively in an effective manner.

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