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

JAMUNA SENGUTTUVAN, SUBRAMANIAM PAULSAMY AND KARTHIKA KRISHNAMOORTHY

Department of Botany, Kongunadu Arts and Science College, Coimbatore, India. Email: paulsami@yahoo.com

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 Hypochaeris 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 500µg/mL and 200 and 600µg/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: Hypochaeris 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, Hypochaeris 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 Paeclomyces 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 2×10⁵ CFU/mL spores [12].

Antifungal activity

Antifungal activity was investigated by the disc diffusion method [13]. Fungal suspension (2×10⁵) 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 (1800µl) 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.

<table>
<thead>
<tr>
<th>Control/Extracts</th>
<th>Zone of inhibition (mm)</th>
<th>PL</th>
<th>Ms</th>
<th>TV</th>
<th>VL</th>
<th>CA</th>
<th>Fs</th>
<th>Ps</th>
<th>AF</th>
<th>AN</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC -</td>
<td>20.56 ± 0.51</td>
<td>24.66 ± 0.35</td>
<td>25.86 ± 0.15</td>
<td>16.73 ± 0.25</td>
<td>9.40 ± 0.36</td>
<td>37.23 ± 0.25</td>
<td>10.66 ± 0.50</td>
<td>13.50 ± 0.56</td>
<td>10.32 ± 0.54</td>
<td></td>
</tr>
<tr>
<td>PE -</td>
<td>8.76 ± 0.25</td>
<td>12.93 ± 0.15</td>
<td>7.86 ± 0.15</td>
<td>8.56 ± 0.60</td>
<td>-</td>
<td>7.83 ± 0.15</td>
<td>13.50 ± 0.50</td>
<td>12.73 ± 0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF -</td>
<td>9.93 ± 0.11</td>
<td>14.66 ± 0.61</td>
<td>14.76 ± 0.25</td>
<td>9.93 ± 0.11</td>
<td>9.83 ± 0.15</td>
<td>10.16 ± 0.15</td>
<td>8.83 ± 0.28</td>
<td>14.66 ± 0.28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EA -</td>
<td>14.60 ± 0.40</td>
<td>7.73 ± 0.25</td>
<td>7.60 ± 0.52</td>
<td>15.53 ± 0.47</td>
<td>7.73 ± 0.25</td>
<td>-</td>
<td>-</td>
<td>12.96 ± 0.57</td>
<td>19.96 ± 0.76</td>
<td></td>
</tr>
<tr>
<td>WA -</td>
<td>11.26 ± 0.25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15.56 ± 0.51</td>
<td></td>
</tr>
</tbody>
</table>

- ' indicates no activity.

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

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.

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

- ' indicates no activity.

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

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 Tricoderma viride (20mm) followed by the petroleum ether and water extracts. A 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 present 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 scleroderma, dermatomyositis, and allergic alveolitis are made [19, 20]. Rai 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 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, Duraipandian 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. alternate and Helminthosporium tetrameru 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., Tricoderma viride, Verticillium lecanii, Candida albicans, Penicillium sp., A. fumigates and A. niger and instead of Penicillium sp. V.fumigatus 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 least resistant organism was P. lilacinus.

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 Hypochaeris radicata.](image)

**DISCUSSION**

The results obtained from the present investigation revealed that the highest antifungal activity was exhibited by the ethyl acetate and chloroform extracts of root of H. radicata against the fungi Mucor sp. and Tricoderma viride (20mm) followed by the petroleum ether and water extracts. A 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 present 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 scleroderma, dermatomyositis, and allergic alveolitis are made [19, 20]. Rai 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 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, Duraipandian 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. alternate and Helminthosporium tetrameru 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., Tricoderma viride, Verticillium lecanii, Candida albicans, Penicillium sp., A. fumigates and A. niger and instead of Penicillium sp. V.fumigatus 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 least 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 Hypochaeris 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 600µg/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 Hypochaeris radicata of 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.

REFERENCES


