

ANTIFUNGAL POTENTIALS OF *DERRIS INDICA* (LAM.) BENNET EXTRACTIVES

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Abstract: Antifungal activity of the *D. indica* (Lam.) Bennet. extractives collected in CHCl₃ and methanol were tested against seven pathogenic fungi *F. vasinfectum*, *A. fumigatus*, *A. niger*, *A. flavus*, *Mucor* sp., *C. albicans* and *P. notatum* at concentrations of 50 and 200 µg/disc along with a standard Nystatin (50 µg/disc). The fruit shell extract showed activity index against *C. albicans* and *P. notatum*. The leaf and the root bark extracts were responsive on *A. fumigatus*, *C. albicans*, *P. notatum* and *F. vasinfectum*. For the root wood extract *F. vasinfectum*, *A. fumigatus*, *C. albicans* were responsive. For the seed, stem bark and stem wood extract showed activity index against *A. flavus*, *C. albicans*, *A. fumigatus*, *P. notatum* and *Mucor* sp. According to the intensity of activity indices *D. indica* extracts (CHCl₃) could be arranged in a descending order of fruit shell > leaf > root bark > root wood > seed > stem wood > stem bark extract. For the MeOH extracts the fruit shell, leaf, root bark and root wood extracts showed activity indices against *A. fumigatus*, *C. albicans*, *P. notatum*, *Mucor* sp., *F. vasinfectum*, and *A. flavus*. In case of the seed, stem bark and stem wood extracts *A. flavus*, *C. albicans*, *A. fumigatus*, *P. notatum* and *Mucor* sp. were responsive. According to the intensity of activity indices *D. indica* extracts (MeOH) could be arranged in a descending order of fruit shell > leaf > root bark > root wood > seed > stem bark > stem wood extract.

Key words: Chloroform and methanol extract, *Derris indica*, antifungal activity.

সারাংশ: *Derris indica* (Lam.) Bennet. (করঞ্জা)-এর ফলের খোসা, পাতা, মূলের বাকল, মূলের কাঠ, বীজ, কাণ্ডের বাকল ও কাণ্ডের কাঠের ক্লোরোফর্ম ও মিথানল নির্যাসের উপর সাত ধরনের রোগ সৃষ্টিকারী ছত্রাকের কার্যকারিতা পর্যবেক্ষণ করা হয়। নির্যাসগুলোর কার্যকারিতা তুলনা করার জন্য Nystatin (50 µg/disc) ব্যবহার করা হয়। ফলের খোসার ক্লোরোফর্ম নির্যাস *C. albicans* এবং *P. notatum* এবং মিথানল নির্যাস *A. fumigatus*, *C. albicans* and *P. notatum* -এর বিপরীতে কার্যকারিতা প্রদর্শন করে। পাতার ক্লোরোফর্ম নির্যাস *A. fumigatus*, *C. albicans* এবং *P. notatum* এবং মিথানল নির্যাস *A. fumigatus*, *Mucor* sp., *C. albicans* এবং *P. notatum* এর বিপরীতে কার্যকারিতা প্রদর্শন করে। মূলের বাকলের ক্লোরোফর্ম এবং মিথানল নির্যাস উভয়ই *F. vasinfectum*, *C. albicans* এবং *P. notatum*- এর বিপরীতে কার্যকারিতা প্রদর্শন করে। মূলের কাঠের ক্লোরোফর্ম নির্যাস *F. vasinfectum*, *A. fumigatus*, *C. albicans* এবং মিথানল নির্যাস *F. vasinfectum*, *A. fumigatus*, *A. flavus* এবং *C. albicans* -এর বিপরীতে কার্যকারিতা প্রদর্শন করে। বীজের ক্লোরোফর্ম এবং মিথানল নির্যাস শুধুমাত্র *A. flavus*, *C. albicans* and *P. notatum*- এর বিপরীতে কার্যকারিতা প্রদর্শন করে। কাণ্ডের বাকলের ক্লোরোফর্ম এবং মিথানল নির্যাস শুধুমাত্র *A. fumigatus* এবং *P. notatum*- এর বিপরীতে কার্যকারিতা প্রদর্শন করে। কাণ্ডের কাঠের ক্লোরোফর্ম ও মিথানল নির্যাস *A. fumigatus*, *Mucor* sp. এবং *P. notatum* -এর বিপরীতে কার্যকারিতা প্রদর্শন করে। কার্যকারিতার মাত্রা অনুসারে ক্লোরোফর্ম নির্যাসগুলিকে যথাক্রমে ফলের খোসা > পাতা > মূলের বাকল > মূলের কাঠ > বীজ > কাণ্ডের কাঠ > কাণ্ডের বাকল এবং মিথানল নির্যাসগুলিকে ফলের খোসা > পাতা > মূলের বাকল > মূলের কাঠ > বীজ > কাণ্ডের বাকল > কাণ্ডের কাঠ ক্রমানুসারে সাজানো যায়।

Introduction

The well-known medicinal plant *Derris indica* (Lam.) Bennet (Class- Magnoliopsida: Family- Fabaceae) has a number of synonyms of which *Pongamia pinnata* (L.) Pierre, *P. glabra* Vent., *Galedupa indica* Lam., *G. pinnata* (L.) Taub., *M. novo-guineensis* Kane. & Hat. and *Milletia pinnata* (L.) Panigrahi are often cited in literature. It is a leguminous tree that has attracted much attention to the botanists, biochemists, pharmaceutical analysts and traditional medicine practitioners (Mathur et al. 2007; Wagh et al. 2007; Arote et al. 2009). Moreover, this is a preferred species for controlling soil erosion and binding sand dunes because of its dense network lateral roots (Sangwan et al. 2010).

Almost all parts of *D. indica* have been used as crude drug for the treatment of piles, tumours, skin diseases, wound and ulcers. For example, root bark, leaves, flowers and seeds have medicinal properties and so this is traditionally used as a medicinal plant (Sangwan et al. 2010). Antifungal activities of the plant have been

demonstrated by a number of recent workers (Biswal et al 2011; Johnson et al. 2011; Sharma et al. 2011a,b) whereas antibacterial properties of *D. indica* were tested among others by Wagh et al. (2007), Arote et al. (2009), Kogithoju et al. (2012) and Sajid et al. (2012). In addition, antihelmintic (Biswal et al. 2011), anti-diabetic (Jagadeesha 2011) and anti-dermatophytic properties (Sharma et al. 2012) of the plant have recently been reported.

Since mycoses or fungal diseases are very common in plants, animals and human beings, and various parts of *D. indica* extractives, either crude or in aqueous and/or organic solvents have shown to be effective against such diseases as bronchitis, whooping cough, diarrhea, skin itch, rheumatic arthritis and bleeding piles (Sangwan et al. 2010; Biswal et al. 2011; Sajid et al. 2012), the present investigation was aimed at evaluating chloroform and methanol extracts of some selected parts of *D. indica* against a number of pathogenic fungi using disc diffusion method.

Materials and Methods

The test fungi and their culture: Pure cultures of seven species of fungi viz., *Fusarium vasinfectum*, *Aspergillus fumigatus*, *Aspergillus niger*, *Aspergillus flavus*, *Mucor* sp., *Candida albicans* and *Penicillium notatum* were collected from the Department of Pharmacy, Rajshahi University (RU), and were maintained in the Molecular Biology Laboratory, IBSc, RU. Potato dextrose agar (PDA) media, consisting of 20g potato, 2g dextrose, 1.5g agar and 100ml distilled water, were used to perform the antifungal activity tests and for the maintenance of the subcultures of the test organisms. The constituents of the media were accurately weighed and dispersed in a conical flask with distilled water. It was heated in water bath to dissolve the ingredients until a transparent solution was obtained. The pH of the medium was adjusted to 5.6. The volume was adjusted by adding distilled water and sterilized in an autoclave at 121° C and 15 lb. inch⁻² pressures for 15 min.

Plant parts and their extraction: The fresh leaves, fruit shell, seeds, stem bark, stem wood, root bark and root wood of *D. indica* were collected from the RU Campus. After drying under shade, the plant materials were powdered in a grinder separately avoiding excess heat during grinding. The ground dried plant materials were extracted with sufficient amount of chloroform and methanol (500g × 1500ml × 3 times) for each of the items. Separate extracts were collected by the cool method after 72 hours of plunging for each of the materials. Extracts thus obtained through filtration and evaporation of the solvent as residue were kept in a refrigerator until used.

Tests for antifungal activity: The agar diffusion technique (Vander and Vlietinck 1991) was employed to conduct antifungal screening while PDA medium was used for determining antifungal activity (Bauer et al. 1966) of the plant extractives where standard antibiotic discs of Nystatin (50 µg/disc) were used for comparison.

Results and Discussion

D. indica extracts in chloroform: Compared to inhibition zones 28mm and 31mm by the standard control Nystatin (50 µg/disc), the activity indices of the fruit shell extracts were recorded 0.71mm, 0.58 mm, 0.36mm and 0.26 mm respectively for 200 and 50 µg/disc application against two fungal species *C. albicans*, *P. notatum*. The leaf extract showed 0.64mm, 0.71mm, 0.55mm and 0.32mm, 0.36mm and 0.29 mm activity indices for 200 and 50 µg/disc application against three fungal species *A. fumigatus*, *C. albicans* and *P.*

notatum, while the inhibition zones for the standard Nystatin 50 µg/disc were 28 mm and 31mm. The root bark extract showed activity indices 0.57mm, 0.64mm, 0.48 mm and 0.30mm, 0.36mm and 0.26 mm for 200 and 50 µg/disc application against *F. vasinfectum*, *C. albicans* and *P. notatum*, while the inhibition zones for the standard Nystatin 50 µg/disc were 30mm, 28mm and 31mm. In case of the root wood extract *F. vasinfectum*, *A. fumigatus*, *C. albicans* showed activity indices 0.60mm, 0.54mm, 0.64 mm and 0.30mm, 0.29mm and 0.36 mm for 200 and 50 µg/disc application, while the inhibition zones for the standard Nystatin 50 µg/disc were 30mm and 28mm. For the seed extract *A. flavus*, *C. albicans* and *P. notatum* showed activity indices 0.60mm, 0.54mm, 0.48 mm and 0.27mm, 0.25mm and 0.26mm for 200 and 50 µg/disc application, while the inhibition zones for the standard Nystatin 50 µg/disc were 30mm, 28mm and 31mm. In case of the stem bark extract only *A. fumigatus* and *P. notatum* showed activity indices 0.57mm, 0.42 mm, 0.29mm and 0.23 mm for 200 and 50 µg/disc application, while the inhibition zones for the standard Nystatin 50 µg/disc were 30mm and 31mm. For the stem wood extract *A. fumigatus*, *Mucor* sp. and *P. notatum* showed activity indices 0.61mm, 0.58mm, 0.52 mm and 0.32mm, 0.26mm, 0.26 mm for 200 and 50 µg/disc application, while the inhibition zones for the standard Nystatin 50 µg/disc were 28mm and 31mm respectively and results are mentioned in the Table 1.

D indica extracts in methanol: The activity indices of the fruit shell extracts were recorded 0.54mm, 0.57mm, 0.39mm and 0.21mm, 0.25mm, 0.13 mm against three fungal species *A. fumigatus*, *C. albicans* and *P. notatum* whereas the standard Nystatin yielded 28mm and 31mm inhibition zones respectively. For the leaf extract *A. fumigatus*, *Mucor* sp., *C. albicans*, and *P. notatum* showed activity indices 0.43mm, 0.23mm, 0.50mm, 0.39 mm, 0.21mm, 0.13mm, 0.25mm and 0.16 mm for 200 and 50 µg/disc application, whereas the standard Nystatin had 28mm and 31mm inhibition zones respectively. For the root bark extract *F. vasinfectum*, *C. albicans* and *P. notatum* showed activity indices 0.30mm, 0.43mm, 0.32 mm, 0.17mm, 0.21mm and 0.16 mm, while the inhibition zones for the standard Nystatin 50 µg/disc were 30 mm, 28mm and 31mm for the above mentioned test agents respectively. For the root wood extract *F. vasinfectum*, *A. fumigatus*, *A. flavus* and *C. albicans* showed 0.37mm, 0.32mm, 0.27mm and 0.39 mm and 0.20mm, 0.18mm, 0.17mm and 0.21 mm activity indices, while the inhibition zones for the standard Nystatin 50 µg/disc were 30 mm and 28mm respectively. For the seed

extract *A. flavus*, *C. albicans* and *P. notatum* showed activity indices 0.33mm, 0.32mm, 0.35 mm, 0.17mm, 0.18mm and 0.19 mm, while the inhibition zones for the standard Nystatin 50 µg/disc were 30mm, 28mm and 31mm respectively. For the stem bark extract *A. fumigatus* and *P. notatum* showed activity indices 0.36mm, 0.29 mm, 0.21mm and 0.13mm, while the inhibition zones for the standard Nystatin 50 µg/disc

were 28mm and 31mm respectively. For the stem wood extract *A. fumigatus*, *Mucor* sp. and *P. notatum* showed activity indices 0.39mm, 0.26mm, 0.29 mm, 0.18mm, 0.13mm and 0.13 mm, while the inhibition zones for the standard Nystatin 50 µg/disc were 28mm and 31mm for the above mentioned test agents respectively and results are mentioned in the Table 2.

Table 1 Antifungal activity of the chloroform extracts of the different parts of *D. indica* in comparison with the standard antibiotics

Test organisms	Activity index (diameter in mm) with the plant extracts														Standard
	Fruit shell		Leaf		Root bark		Root wood		Seed		Stem bark		Stem wood		
	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
<i>F. vasinfecum</i>	-	-	-	-	0.30	0.57	0.30	0.60	-	-	-	-	-	-	30
<i>A. fumigatus</i>	-	-	0.32	0.64	-	-	0.29	0.54	-	-	0.29	0.57	0.32	0.61	28
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30
<i>A. flavus</i>	-	-	-	-	-	-	-	-	0.27	0.60	-	-	-	-	30
<i>Mucor</i> sp.	-	-	-	-	-	-	-	-	-	-	-	-	0.26	0.58	31
<i>C. albicans</i>	0.36	0.71	0.36	0.71	0.36	0.64	0.36	0.64	0.25	0.54	-	-	-	-	28
<i>P. notatum</i>	0.26	0.58	0.29	0.55	0.26	0.48	-	-	0.26	0.48	0.23	0.42	0.26	0.52	31

50 and 200 refer to doses in µg/disc; standard= Nystatin 50 µg/disc

Table 2 Antifungal activity of the methanol extracts of the different parts of *D. indica* in comparison with the standard antibiotics

Test organisms	Activity index (diameter in mm) with the plant extracts														standard
	Fruit shell		Leaf		Root bark		Root wood		Seed		Stem bark		Stem wood		
	50	200	50	200	50	200	50	200	50	200	50	200	50	200	
<i>F. vasinfecum</i>	-	-	-	-	0.17	0.30	0.20	0.37	-	-	-	-	-	-	30
<i>A. fumigatus</i>	0.21	0.54	0.21	0.43	-	-	0.18	0.32	-	-	0.21	0.36	0.18	0.39	28
<i>A. niger</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30
<i>A. flavus</i>	-	-	-	-	-	-	0.17	0.27	0.17	0.33	-	-	-	-	30
<i>Mucor</i> sp.	-	-	0.13	0.23	-	-	-	-	-	-	-	-	0.13	0.26	31
<i>C. albicans</i>	0.25	0.57	0.25	0.50	0.21	0.43	0.21	0.39	0.18	0.32	-	-	-	-	28
<i>P. notatum</i>	0.13	0.39	0.16	0.39	0.16	0.32	-	-	0.19	0.35	0.13	0.29	0.13	0.29	31

50 and 200 refer to doses in µg/disc; standard= Nystatin 50 µg/disc

Comparison of the chloroform and methanol extractives: The results showed that increasing concentrations of extracts increased the activities in all of the microorganisms. The chloroform extracts of plant parts showed significant antifungal activity. The leaf extract was found to have maximum activity indices against *C. albicans* and *A. fumigatus* when tested by the disc diffusion method. *C. albicans* showed significant activity against root bark extract.

The present results clearly demonstrated that both chloroform and methanol extractives from various parts of *D. indica* have significant antifungal properties. This could be due to the active compounds like rotenoid,

karanjin and flavonol present in the extracts as confirmed earlier by 2D NMR (nuclear magnetic resonance) and spectral analysis (Simin et al. 2002). The present findings also fit well with those of Mathur et al. (2007) who found rotenoids and flavonoids isolated from stem and leaves of *D. indica* effective against *Aspergillus flavus* and *Penicillium tubesulum*. But in contrast, however, Khan et al. (2006) did not find any antifungal activity of various parts of *D. indica*, *D. elliptica* and *D. trifoliata* in petrol, dichloromethane, ethyl acetate, butanol and methanol. The antifungal activity of the plant extracts was further shown against important seed borne pathogens of *Aspergillus* species (Satish et al. 2007) whereas Wagh

et al. (2007) observed that oils and fatty acids obtained from *P. pinnata* had high degree antifungal activity against *A. niger* and *A. fumigatus* by MIC determination and dry-weight method. Leaf, flower, pods, seeds, root and bark extracts of *Pongamia pinnata* possessed significant anti-fungal, anti-diarrhoeal, anti-plasmodial, anti-ulcerogenic, anti-inflammatory and analgesic activities (Sangwan et al. 2010).

The present data on the antifungal efficacy of the experimental plant are supported by a number of recent works. Badole et al. (2011) reported that the stem bark extracts of *P. pinnata* having concentration range of 10-100 µg/mL exhibit broad-spectrum activity against bacteria and strong activity against yeast type of fungi. Besides, the leaf extract of *Derris indica* in petroleum ether and chloroform showed the presence of phytosterols and saponins, that in chloroform and ethanol had flavonoids and fixed oils, and that in ethanol and aqueous had carbohydrates (Biswal et al. 2011). The chloroform extracts had significant antifungal activity against *A. niger* and *C. albicans*. Johnson et al. (2011) have demonstrated effectiveness of the aqueous and alcoholic extracts of *P. pinnata* against *Aspergillus* using the minimum inhibitory concentration (MIC) method followed by antifungal activity by spore germination method. The flavonoids from leaf extracts of *P. pinnata* were tested against *C. albicans* and *Trichoderma viride* adopting disc diffusion method and the results in terms of activity index (AI) were compared with the zone of inhibition (ZI) produced by commercially available standard antibiotics, 25µg each of Amphotericin B and Fluconazole (Sharma et al. 2011a). Also the flavonoids of the plant possessed antibacterial and antifungal activity (Sharma et al. 2011b). In very recent studies, Sharma et al. (2012) used chloroform, methanol and water extracts of *P. pinnata* against three species of *Trichophyton* and two species of *Microsporum* where chloroform extracts (1.25-10.00 MIC) were more effective than the methanol extracts. Methanol, ethanol, acetone (both absolute and aqueous) and de-ionized water extracts of bark, leaf and seeds of the plant were evaluated where the bark extracts against a set of bacterial and fungal strains revealed strongest antimicrobial activity with the largest ZI and lowest MIC values (Sajid et al. 2012). These findings corroborate to those reported here using *D. indica* extractives.

Conclusion

The present extractives represent novel leads and so future studies may be undertaken for the development of a pharmacologically acceptable antimicrobial agent or class of agents. Our findings suggest the possibility

of using the extractives in the treatment of various fungal infections and of discovering new bioactive compounds which can also be used for prophylactic treatments. Especially the bark of the plant has strong potential for the isolation of antioxidant and antimicrobial agents for functional pharmaceutical uses.

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