Journal of Medicinal Plants Studies
www.PlantsJournal.com

ISSN (E): 2320-3862
ISSN (P): 2394-0530
NAAS Rating 2017: 3.53
JMPS 2017; 5(4): 114-117

Received: 18-05-2017
Accepted: 19-06-2017

Prashith Kekuda TR
Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India

Vinayaka KS
Department of Botany, Kumadvathi First Grade College, Shimoga Road, Shikaripura, Karnataka, India

Correspondence
Prashith Kekuda T R
Department of Microbiology, S.R.N.M.N College of Applied Sciences, N.E.S Campus, Balraj Urs Road, Shivamogga-577201, Karnataka, India

Inhibitory activity of *Harpullia arborea* (Blanco) Radlk. and *Hydnocarpus pentandra* (Buch.-Ham.) Oken against seed-borne fungi

Prashith Kekuda TR and Vinayaka KS

Abstract
Seed-borne fungi are of much significance for crop production as they are known to reduce germination and cause huge crop loss. Botanicals are shown to be effective in the management of seed associated pathogenic fungi. The present study was performed to investigate antifungal potential of methanolic extract obtained from leaves of two plants viz. *Harpullia arborea* (Blanco) Radlk. (Sapindaceae) and *Hydnocarpus pentandra* (Buch.-Ham.) Oken (Achariaceae) against a total of 7 seed-borne fungi. Maceration process was used to obtain extracts from the shade dried and powdered leaf material of selected plants. Poisoned food technique was used to determine antifungal activity. Both extracts were effective in causing mycelial growth inhibition in a concentration dependent manner. Marked antifungal activity was displayed by *H. arborea* when compared to *H. pentandra*. Among fungi, *Aspergillus niger* and *Penicillium* sp. was inhibited to highest and least extent respectively by both extracts. The plants selected in this study are promising in terms of their potential to inhibit seed mycoflora. In suitable formulation, these plants can be used against mycoflora associated with seeds for the management of seed-borne fungal diseases of plants.

Keywords: *Harpullia arborea*, *Hydnocarpus pentandra*, Maceration, Seed-borne fungi, Poisoned food technique

1. Introduction
Seeds are the passive carriers of fungi and are considered as one of the most effective means of transmission of pathogenic fungi. Fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, *Alternaria*, *Curvularia*, *Helminthosporium*, *Sclerotium*, *Mucor*, *Cladosporium*, *Bipolaris*, *Cercospora*, *Rhizoctonia* and *Fusarium* are often found on seeds of many crops. Seed-borne fungi are one among the important factors that are involved in the spoilage of stored grains. Seed-borne fungi are found externally or internally and are known to cause seed abortion, seed rot, reduction of germination ability and seedling damage. Seed-borne fungi are known to suppress the seedling vigor and plant growth leading to marked reduction in crop yield. Many of seed associated fungi are capable of producing toxic substances, mycotoxins, which leads to adverse health effects on consumption [1-9]. Management of seed-borne fungi and other phytopathogenic fungi is carried out mainly through the use of synthetic chemicals. However, the unrestrained use of such chemicals is having environmental and health effects. Moreover, extensive use of these chemicals is resulting in the emergence of resistant pathogens which are difficult to control. Hence, there is an upsurge in demand for developing antifungal agents from alternate sources for controlling phytopathogenic fungi including seed-borne fungi. It is shown that plant extracts, plant based formulations and purified compounds from plants exhibit growth inhibitory activity against a range of phytopathogenic fungi [3, 10-13].
Harpullia arborea (Blanco) Radlk, a native to Indo-Malayan, belongs to the family Sapindaceae. It is a medium sized evergreen tree. The plant is used traditionally as leech repellent, to treat rheumatism, as an appertizer and to cure digestive problems [14-16]. The plant is shown to exhibit bioactivities such as antibacterial [15, 17], antifungal [18] and antimalarial activity [19]. *Hydnocarpus pentandra* (Buch.-Ham.) Oken. belonging to the family Achariaceae, is a large evergreen tree. The plant is common in forests. Seeds yield an oil (hydnocarpus oil, chaulmoogra oil) which is used in the treatment of skin diseases such as leprosy. The plant *H. pentandra* is a medicinal plant and is traditionally used for treatment of various ailments or diseases such as eczema, white patches, itching, leprosy, skin burn, tubercular laryngitis,

chronic ulcers, dyspepsia, flatulence and verminosis [20, 21]. The plant is reported to exhibit bioactivities such as larvicidal[22], antifungal [18], antioxidant [23], antibacterial [24] and anticancer [25] activity. The present study was undertaken to investigate the antifungal activity, in terms of inhibition of mycelial growth of seed-borne fungi, of leaf extract of *H. arborea* and *H. pentandra*.

Materials and Methods

Plant materials

The plants viz. *H. pentandra* and *H. arborea* were collected from outskirts of Shiralakoppa, Shivamogga district, Karnataka during February 2017. The plants were authenticated on the basis of their characteristics by referring flora [26].

Extraction

The leaves were separated from plants, cleaned to removed adhering matter, dried under shade and powdered. The leaf powders (10g) were extracted using methanol (100ml) by maceration process over a period of 48 hours [27]. The crude leaf extracts were stored in refrigerator.

Test fungi

Fungi viz. *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Rhizopus* sp., *Penicillium* sp., *Cladosporium* sp., and *Helminthosporium* sp., isolated previously from sorghum and paddy seeds, were tested for their susceptibility to leaf extract of *H. pentandra* and *H. arborea*. The pure cultures of test fungi were maintained on potato dextrose agar slants in refrigerator.

Antifungal activity of leaf extracts

Potato dextrose agar medium (HiMedia, Mumbai) was prepared, autoclaved and poisoned with different concentrations of leaf extracts (0.5 and 1.0mg extract/ml of medium). The control (lacking extract) and poisoned potato dextrose agar plates were inoculated aspetically with the well sporulated 7 days old cultures of test fungi. The plates were incubated at room temperature for 5 days and the diameter of colonies of test fungi on control and poisoned plates was measured in mutual perpendicular directions. Antifungal effect of leaf extracts, in terms of inhibition of mycelial growth of fungi, was determined by using the formula:

Antifungal activity (%) = (Dc – Dt / Dc) x 100, where 'Dc' and 'Dt' refers to colony diameter of fungi on control plates and poisoned plates respectively [13].

Statistical analysis

The experiment was carried in triplicate (n=3). Results are presented as Mean ± Standard deviation (S.D).

Results and Discussion

Several methods such as poisoned food technique, spore germination inhibition assay, agar overlay bioassay, disk diffusion method, well diffusion method and broth microdilution assay are used to determine antifungal activity. Among these, poisoned food technique is one of the most widely used *in vitro* antifungal assays and is widely used to determine antifungal potential of plant extracts and plant based formulations against a variety of phytopathogens including seed-borne fungi [28-32]. The present study evaluated antifungal potential of *H. arborea* and *H. pentandra* by poisoned food technique and the result is shown in Table 1 and Figure 1. Both extracts suppressed the mycelial growth to a considerable extent and in a dose dependent manner. Overall, the extracts exhibited highest inhibitory activity against *A. niger* while *Penicillium* sp. was shown to be affected by both extracts to least extent. At 1.0mg/ml concentration of *H. arborea* extract, the susceptibility of test fungi was in the order *A. niger* >*Rhizopus* sp. >*Helminthosporium* sp. >*A. flavus* >*A. fumigatus* >*Cladosporium* sp. >*Penicillium* sp. In case of 1.0mg/ml concentration of *H. pentandra* extract, the order to susceptibility of test fungi to extract was *A. niger* >*A. flavus* >*A. fumigatus* >*Rhizopus* sp. >*Helminthosporium* sp. >*Cladosporium* sp. >*Penicillium* sp. At 1mg/ml extract concentration, *H. arborea* inhibited all test fungi to >50%. However, at 1mg/ml concentration, extract of *H. pentandra* inhibited all test fungi except *Penicillium* sp. to >50%. The extent of susceptibility of *Aspergillus* species to extract of both plants was in the order *A. niger* >*A. flavus* >*A. fumigatus*. It was observed that the extract of *H. arborea* displayed marked antifungal potential when compared to extract of *H. pentandra*. In an earlier study, similar observation was made where aqueous extract of *H. arborea* leaf exhibited marked inhibition of seed-borne fungi viz. *Alternaria* sp., *Curvularia* sp. and *Fusarium* sp. when compared to aqueous extract of *H. pentandra* leaf [18].

Table 1: Antifungal activity of *H. arborea* and *H. pentandra*

Test fungi	Colony diameter of test fungi in cm (Mean±S.D)				
	Control	H. arborea		H. pentandra	
		0.5mg/ml	1.0mg/ml	0.5mg/ml	1.0mg/ml
A. niger	5.46±0.05	1.30±0.10	0.60±0.10	2.20±0.00	1.20±0.10
A. flavus	4.16±0.05	1.93±0.05	1.20±0.00	2.40±0.10	1.40±0.10
A. fumigatus	3.80±0.10	2.20±0.10	1.43±0.05	2.40±0.00	1.60±0.00
Penicillium sp.	3.46±0.05	2.46±0.05	1.50±0.10	2.83±0.05	1.76±0.05
Cladosporium sp.	2.80±0.00	1.80±0.00	1.10±0.00	2.20±0.10	1.30±0.00
Helminthosporium sp.	4.20±0.00	2.60±0.00	1.20±0.00	3.10±0.10	1.86±0.05
Rhizopus sp.	6.03±0.20	3.33±0.05	1.20±0.10	3.96±0.05	2.66±0.05

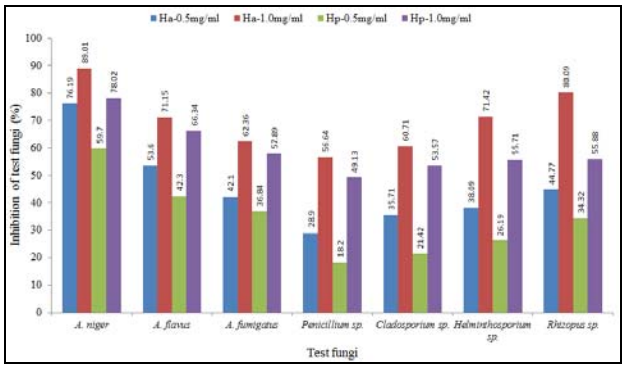

Fig 1: Inhibition of test fungi (%) by *H. arborea* (Ha) and *H. pentandra* (Hp)

Conclusion

Botanicals are shown to be promising alternatives for synthetic fungicides as they lack the drawbacks that are associated with the use of synthetic fungicides. Marked dose dependent mycelial growth inhibitory activity exhibited by leaf extract of *H. arborea* and *H. pentandra* indicates their possible utilization in the management of seed-borne fungi and the diseases caused by them.

References

1. Kawube G, Kanobe C, Edema R, Tusiime G, Mudingotto PJ, Adipala E. Efficacy of manual seed sorting methods in reduction of transmission of rice and cowpea seed-borne diseases. African Crop Science Conference Proceedings, 2005; 7:1363-1367.
2. Fakhrunnisa, Hashmi MH, Ghaffar A. Seed-borne mycoflora of wheat, sorghum and barley. Pakistan Journal of Botany, 2006; 38(1):185-192.
3. Satish S, Mohana DC, Ranhavendra MP, Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus* sp. Journal of Agricultural Technology, 2007; 3(1):109-119.
4. Elisabeth ZP, Paco S, Vibeke L, Philippe S, Irenee S, Adama N. Importance of seed-borne fungi of sorghum and pearl millet in Burkina Faso and their control using plant extracts. Pakistan Journal of Biological Sciences 2008; 11(3): 321-331.
5. Pande BJ, Gupta RC. Role of seed mycoflora on seed germination of Oroxylum indicum (L.) Vent. in Kumaun region of Indian Central Himalaya. International Journal of Biodiversity and Conservation, 2011; 3(13):715-720.
6. Maširević S, Sladjana M, Birvalski S. Mycoflora of maize seed. Research Journal of Agricultural Science 2012; 44(2):58-62.
7. El-Wakil DA. Seed-borne fungi of sunflower (*Helianthus annuus* L.) and their impact on oil quality. IOSR Journal of Agriculture and Veterinary Science, 2014; 6(6):38-44.
8. Fleurat-Lessard F. Integrated management of the risks of stored grain spoilage by seedborne fungi and contamination by storage mould mycotoxins - An update. Journal of Stored Products Research, 2017; 71:22-40.
9. Mohana DC, Thippeswamy S, Abhishek RU, Shobha B, Mamatha MG. Studies on seed-borne mycoflora and aflatoxin B1 contaminations in food based seed samples: Molecular detection of mycotoxigenic Aspergillus flavus and their management. International Food Research Journal, 2017; 24(1):422-427.
10. Tagoe DNA, Nyarko HD, Akpaka R. A comparison of the antifungal properties of onion (*Allium cepa*), ginger (*Zingiber officinale*) and garlic (*Allium sativum*) against Aspergillus flavus, Aspergillus niger and Cladosporium herbarum. Research Journal of Medicinal Plants 2011; 5(3): 281-287.
11. Kumari A, Kumar R, Maurya S, Choudhary JS, Kumar S. Antifungal efficacy of aqueous extracts of neem cake, karanj cake and vermicompost against some phytopathogenic fungi. The Bioscan, 2013; 8(2):671-674.
12. Neela FA, Sonia IA, Shamsi S. Antifungal activity of selected medicinal plant extract on Fusarium oxysporum Schlechtthe causal agent of fusarium wilt disease in tomato. American Journal of Plant Sciences, 2014; 5:2665-2671.
13. Kekuda PTR, Raghavendra HL. Antifungal activity of Helichrysum buddleioides DC. Against seed borne fungi. EC Microbiology, 2017; 6(2):54-59.
14. Karuppusamy S, Rajasekaran KM, Karmegam N. Evaluation of phytomedicines from street vendors in Tamil Nadu, South India. Indian Journal of Traditional Knowledge, 2002; 1(1):26-39.
15. Gowri SS, Vasantha K. Solvent based effectiveness of antibacterial and phytochemical derivatized from the seeds of Harpullia arborea (Blanco) Radlk. (Sapindaceae). Journal of Applied Sciences and Environmental Management, 2009; 13(4):99-101.
16. Sharma PP, Sawant RJ. Indigenous traditional practices for eco-friendly management of insect/pest in Maharashtra, India. Recent Research in Science and Technology 2012; 4(10):21-24.
17. Chung PY, Chung LY, Ngeow YF, Goh SH, Imiyabir Z. Antimicrobial activities of Malaysian plant species. Pharmaceutical Biology, 2004; 42(4-5):292-300.
18. Karthik KN, Ankith GN, Avinash HC, Rajesh MR, Kekuda PTR, Raghavendra HL. Antifungal activity of some botanicals against seed-borne fungi. Journal of Agriculture, Food and Natural Resources, 2017; 1(1):40-43.
19. Widyawaruyanti A, Devi AP, Fatria N, Tumewu L, Tantular IS, Hafid AF. *In vitro* antimalarial activity screening of several Indonesian plants using HRP2 assay. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(6):125-128.
20. Zahid IH, Bawazir AS, Naser R. Plant based native therapy for skin problems in Aurangabad district (M.S.). Journal of Pharmacognosy and Phytochemistry, 2013; 2(1):241-244.
21. Deepa MR, Dharmapal SP, Udayan PS. Floristic diversities and medicinal importance of selected sacred groves in Thrissur district, Kerala. Tropical Plant Research, 2016; 3(1):230-242.
22. Sivaraman G, Paulraj GM, Gandhi RM, Reegan DA, Ignacimuthu S. Larvicidal potential of *Hydnocarpus pentandra* (Buch.- Ham.) Oken seed extracts against *Aedes aegypti* Linn. and Culex quinquefasciatus Say (Diptera: Culicidae). International Journal of Pure and Applied Zoology, 2014; 2(2):109-112.
23. Krishnan SM, Dhanalakshmi P, Sudhalakshmi YG, Gopalakrishnan S, Manimaran A, Sindhu S. Evaluation of phytochemical constituents and antioxidant activity of Indian medicinal plant Hydnocarpus pentandra. International Journal of Pharmacy and Pharmaceutical Sciences, 2013; 5(2):453-458.
24. Shirona TK, Sruthy KB, Rajendran N. Antibacterial and antioxidant properties of two medicinal plants from Kerala, India. International Journal of Chemical and Pharmaceutical Sciences, 2014; 5(1):68-72.

25. Rengaraju S, Gurunagarajan S. *In vitro* and *in vivo* anticancer activity of aqueous extract of Hydnocarpus pentandra (Buch.-Ham.) oken. Against Ehrlich ascites carcinoma cell lines. International Journal of Pharma Bio Sciences, 2017; 8(2):964-972.
26. Bhat GK. Flora of South Kanara. Akriti Prints, Mangalore, India, 2014.
27. Agbor AM. Methanol extracts of medicinal plants used for oral healthcare in Cameroon. Biochemistry and harmacology, 2015; 4:164.
28. Magaldi S, Mata-Essayag S, de Capriles HC, Perez C, Colella MT, Olaizola C. Well diffusion for antifungal susceptibility testing. International Journal of Infectious Diseases, 2004; 8:39-45.
29. Mohana DC, Raveesha KA. Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. Journal of Agricultural Technology, 2007; 4(1):119-137.
30. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. Journal of Medicinal Plants Research, 2010; 4(2):104-111.
31. Harishekar MB, Kiran B, Lalitha V, Raveesha KA. Investigation of antifungal activity of Cicer arietinum L. leaves against important seed borne fungi. Journal of Drug Delivery and Therapeutics, 2012; 2(3):164-166.
32. Balouiri M, Sadiki M, Ibnsouda SK. Methods for *in vitro* evaluating antimicrobial activity: A review. Journal of Pharmaceutical Analysis, 2016; 6:71-79.