

# ANTIFUNGAL ACTIVITY OF THREE FERN EXTRACTS ON CAUSATIVE AGENTS OF GROUNDNUT EARLY LEAF SPOT AND RUST DISEASES

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**Abstract:** Antimycotic activity of three fern extracts from *Hemionitis arifolia* (Burm.f.) Moore., *Pteridium aquilinum* (Linn.) Kuhn. and *Christella parasitica* (Linn.) H. Lev. were evaluated against groundnut early leaf spot and rust disease causative agents *viz.*, *Puccinia arachidis* Speg. and *Phaeoisariopsis personata* (Berk & M.A. Cuortis) Vanarx., respectively by mycelial dry weight method. Both the fungi were sensitive to all the three fern extracts tested. Among the extracts, chloroform extract of *H. arifolia* was found to have maximum antifungal activity against both fungi. Between these two fungi, *P. personata* was found to be more sensitive to the tested plant extracts than *P. arachidis*. From this study it is concluded that chloroform extract from *H. arifolia* can be utilized for managing rust and leaf spots diseases on groundnut.

**Key words:** antimycotic activity, *Christella parasitica*, *Hemionitis arifolia*, *Phaeoisariopsis personata*, *Pteridium aquilinum*, *Puccinia arachidis*

## INTRODUCTION

Groundnut is an important oil seed crop particularly for small-scale farmers in semi-arid regions of India (FAO 2006). In India, it is grown annually and about 80% is cultivated under rain fed conditions and the remaining 20% is grown under protected irrigated conditions during summer season (Dwivedi and Verma 2002) with an annual production of about 8.3 million tons. This is contributing about 26.7% to the world groundnut production (Jayaraj 2005). The state of Tamil Nadu has only 14.1% share in area under groundnut cultivation. However, Tamil Nadu produces 23.4% of the total production of the country and the average yield is about 1000 kg/ha (Krishnamurthy and Umamaheswari 2004). An important factor contributing to the low groundnut production is diseases attack. The early leaf spot and rust diseases caused by *Phaeoisariopsis personata* (Berk & M.A. Cuortis) Vanarx, and *Puccinia arachidis* Speg, respectively. These diseases are the most destructive diseases, present worldwide causing substantial losses in crop yield in many groundnut-growing regions (Valand *et al.* 1997; Grichar *et al.* 1998; Culbreath *et al.* 2002).

The magnitude of yield losses and percentage of foliation caused by these diseases are very high all over the world, but vary considerably from place to place and between seasons. Subramanyan *et al.* (1985) and Grichar *et al.* (1998) reported that applications of fungicide were inevitable to control the groundnut fungal pathogens. Synthetic fungicides give immediate remedy and the farmers use them indiscriminately in field to safeguard their crops it has led to the adverse effects such as pol-

lution and health hazards to the farmers and also the consumers. These effects lead the scientists to look for less persistent and biodegradable alternatives to manage these diseases. In order to reach environmentally safe and economically payable alternative methods, scientist considers the plant products as an alternative to the synthetic fungicides. Few data were available about the fungicidal activity of ferns (Manikam and Irudayaraj 1992; Parihar and Bohra 2002; Singh 2003). However, no serious attempts have been made to use fern extracts against the groundnut diseases management. We evaluated the fungicidal activity of *Hemionitis arifolia* (Brum.) T., *Pteridium aquilinum* (L.) Khun. and *Christella parasitica* (L.) H. Lev. extracts on *P. personata* and *P. arachidis* under laboratory condition by mycelial dry weight method.

## MATERIALS AND METHODS

### Collection of ferns

*Hemionitis arifolia* (Burm. f.) Moore. (*Heminiotidaceae*), *Pteridium aquilinum* (Linn.) Kuhn (*Dennstaedtiaceae*) and *Christella parasitica* (Linn.) H. Lev. (*Thelypteridaceae*) were collected from Kothiyar hills, Tirunelveli, Tamil Nadu, India. The samples were authenticated by Centre for Biodiversity and Biotechnology, St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India were the voucher specimens are preserved.

### Preparation of ferns extracts

Collected ferns *H. arifolia*, *P. aquilinum* and *C. parasitica* were washed with sterile distilled water, shade dried for

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two weeks, whole plant were powdered in a domestic grinder and stored in refrigerator for further use. Known weight (150g) of crushed fern material was extracted in a Soxhlet apparatus using chloroform (40–60°C), hexane and water subsequently. The last trace of solvent was removed under reduced pressure. Then distilled and the crude extract was dried in a vacuum desiccator and the final residue was collected, stored in refrigerator at 4°C for further use.

### Isolation of fungal pathogen

The early leaf spot and rust diseased leaves caused by *P. personata* and *P. arachidis*, respectively were collected from the groundnut field at Sivanthipatti and Kaval Kinaru, Tirunelveli District, Tamil Nadu, India and brought to laboratory in a sterile container and used for further isolation of fungal pathogens. The diseased leaves were cut into small pieces (0.5 x 0.5 cm), transferred into the Petri plates contained 1.1% HgCl<sub>2</sub> solution and kept for 5 min. Then they were rinsed twice with distilled water and impregnated into the sterile potato dextrose agar (PDA) plates and incubated at room temperature. After

72 hours the grown pathogens were stored in PDA slants for further analysis.

### Antimycotic activity (Modified method described by Ghahfraokhi *et al.* 2003)

For each treatment, 0.1% of respective plant extracts in 10 ml of sterile potato dextrose broth was inoculated with a 0.3 x 0.3 cm piece of potato dextrose agar colonized aided by pathogen. Both positive (potato dextrose broth alone) and negative (potato dextrose broth with respective solvents) controls were also maintained for comparison. After 7 days of incubation the pathogen primarily mycelial dry (Primarily mycelium) weight for each treatment was determined separately. The fungus was filtered into 45 mm diameter filter paper (Whatman No. 1) *in vacuo* and the medium discarded. Prior to their use the filter paper were held 24 h at 70°C and then weighed. After filtration, the filter paper with mycelium was held again at 70°C for 24 hours and weighed. Activity index and percentage of inhibition of the fern extracts were calculated by using the following formula:

$$\text{Activity index} = \frac{\text{Mycelial dry weight of control} - \text{mycelial dry weight of treatment}}{\text{Mycelial dry weight of treatment}}$$

$$\text{Percentage of inhibition} = \left( \frac{\text{Mycelial dry weight of treatment}}{\text{Mycelial dry weight of control}} \times 100 \right) - 100$$

### Statistical analysis

Mycelial dry weight of the negative control was compared with plant extracts statistically using Duncan's Multiple Range Test (DMRT) and their significance was expressed at 5% level.

## RESULTS

*P. arachidis* and *P. personata* were isolated from rust and leaf spot diseased leaves of groundnut. The isolates were identified based on colonial morphology and microscopic appearance. The effect of aqueous, hexane and chloroform *H. arifolia*, *P. aquilinum* and *C. parasitica* extracts on fungal growth was studied by mycelial dry weight method. The result showed that *P. arachidis* growth was significantly inhibited by chloroform extract of *H. arifolia* followed by hexane extract of *P. aquilinum* and water extract of *H. arifolia* when compared with positive and negative control ( $p < 0.05$ ) (Table 1). The result further indicated that *P. personata* growth was significantly limited by chloroform extract of *H. arifolia* followed by aqueous extract of *P. aquilinum* and hexane extract of *P. aquilinum* when compare with positive and negative control ( $p < 0.05$ ) (Table 1). It is noteworthy that all the aqueous fern extracts inhibited the fungus growth significantly when compare to the control. The inhibition of *P. arachidis* and *P. personata* fungal growth were insignificant when we compare *P. aquilinum* with *C. parasitica*. However two fungal pathogens growth inhibition activity was significant between *H. arifolia* with *C. parasitica*.

The invitro inhibition of *P. arachidis*, *P. personata* uredinospores by fern extracts ranged from 35.77 to 63.35% and 38.25 to 75.28%, respectively (Fig. 1). The plant extracts of *H. arifolia* showed maximum (75.28%) inhibition followed by *P. aquilinum* (73.98%) and *C. parasitica* (72.86%) for of *P. personata*. Similarly, *H. arifolia* exhibited highest (65.35%) inhibition followed by *P. aquilinum* (60.07%) and *C. parasitica* (58.31%) on *P. arachidis*. Mycelial dry weight in table 1 showed that the extracts of all the plants was found to be effective in controlling the rust and leaf spot diseases under laboratory conditions. The activity index in figure 1 shows the maximum activity by *H. arifolia*: 1.56 and 2.49 in the growth of *P. arachidis* and *P. personata*, respectively.

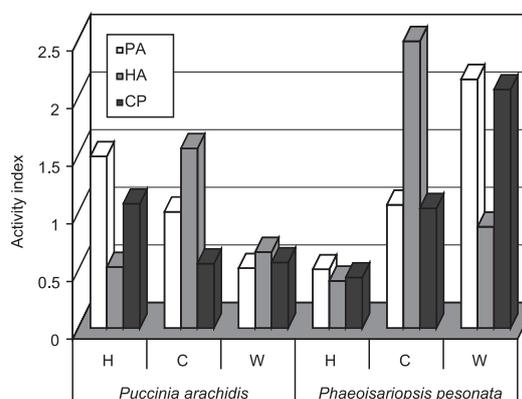


Fig. 1. Activity index of hexane (H), chloroform (C) and water (W) extracts of *H. arifolia* (HA), *P. aquilinum* (PA) and *Ch. parasitica* (CP) on *P. arachidis* and *P. personata*

Table 1. Effect of *H. arifolia*, *P. aquilinum* and *Ch. parasitica* extracts on *P. arachidis* and *P. personata* mycelial dry weight [in mg]

Ferns	Extracts		
	hexane	chloroform	water
<i>Puccinia arachidis</i>			
<i>P. aquilinum</i>	124.14* (60.07)	157.35* (55.77)	228.21* (35.77)
<i>H. arifolia</i>	202.23* (43.66)	123.01* (65.35)	208.03* (41.41)
<i>C. parasitica</i>	148.11* (58.31)	201.02* (43.38)	221.12* (37.75)
Negative Control	309.04 (12.96)	315.20 (11.27)	346.14 (2.54)
Positive Control	355.06 (0.00)		
<i>Phaeoisariopsis personata</i>			
<i>P. aquilinum</i>	308.15* (42.75)	224.08* (58.36)	142.01* (73.98)
<i>H. arifolia</i>	329.57* (38.85)	133.19* (75.28)	238.12* (55.76)
<i>C. parasitica</i>	324.05* (39.78)	227.21* (57.81)	146.33* (72.86)
Negative Control	465.22 (13.57)	464.09 (13.75)	449.26* (16.54)
Positive Control	538.13 (0.00)		

Values in parentheses indicate the percentage of growth inhibition

\* denotes statistical significance at 5% level by DMRT

## DISCUSSION

*P. arachidis* and *P. personata* causing rust and leaf spot diseases, respectively in groundnut ecosystem. The antimycotic properties of pteridophytes are widely known (Sen and Nandi 1951; Hansraj 1996). In the present study, the inhibitory effects of *H. arifolia*, *P. aquilinum* and *C. parasitica* against *P. arachidis* and *P. personata* were established. *C. parasitica* was known to possess antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Manikam *et al.* 2005). Antibacterial activity was also reported in *H. arifolia* (Singh 2003). The obtained results were suitably correlated with results from other workers on rust disease control (Sharma *et al.* 1980; Usman *et al.* 1991; Sunkad *et al.* 2005). Change in hyphal structure and disarrangement in cellular compartments may be responsible for inhibitory effects of fern extract on growth of these two important groundnut fungi. Further studies are needed for confirming this hypothesis. Antifungal activities might be due to the presence of  $p$ -coumaric, caffeic, ferulic,  $p$ -hydroxybenzoic, protocatechuic and vanillic acid in *H. arifolia*; phenolic acids, ponasteroside-A, procyanidin, pteridine, ptersinns, quercetin, thaminas-I, tiliroside and xylose (Singh 2003),  $\alpha$  and  $\beta$ -ecdysones in *P. aquilinum* (Selvaraj *et al.* 2005) and kaempferol-3-O- $\beta$ -D-glucoside (astragaloside), kaempferol-3-O-rutinoside in *C. parasitica* (Britto *et al.* 1996). In addition it was reported that fungicidal activity might be due to the presence of phenolic compounds present in the studied ferns (Singh 1999). More studies are needed to identify the active principles of the tested ferns, their efficacy in the field level and fungal growth inhibiting mechanism(s) of these ferns. Now phytochemical study is in progress in our centre.

## CONCLUSIONS

Among the examined ferns, chloroform extracts of *H. arifolia* was found to be the most antimycotic activity and can be used for the control of the groundnut rust and leaf spot diseases.

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## REFERENCES

- Britto A.J.D., Manikam V.S., Tanaka., Gopalkrishnan S. 1996. Flavanoid glycosides of *Christella parasitica* (L.) H. Lev. of western ghats. J. Indian Chem. Sci. 73: 285–286.
- Culbreath A.K., Stevenson K.L., Brenneman T.B. 2002. Management of late leaf spot of peanut with benomyl and chlorothalonil: A study in preserving fungicide utility. Plant Dis. 86: 349–355.
- Dwivedi S.L., Verma T.S. 2002. Molecular diversity among groundnut varieties differing in drought tolerance traits. Int. Arach. Newslet. 22: 34–36.
- FAO 2006. Results from the FAO biotechnology forum by Ruane J., Sonnino A. Food and Agriculture Organization Research and Technology paper No. 11. Rome, Italy.
- Ghahfraokhi M.S., Razafsha M., Allamesh A., Abyanesh M.R. 2003. Inhibitory effects of aqueous onion and garlic extracts on growth and keratinase activity in *Trichophyton mentagrophytes*. Iranian Biomed. J. 7 (3): 113–118.

- Grichar W.J., Besler B.A., Jaks A.J. 1998. Peanut (*Arachis hypogaea* L.) cultivar response to leaf spot disease development under four disease management programs. *Peanut Sci.* 25: 35–39.
- Hansraj H. 1996. Medicinal value of *Adiantum*. *Indian Drugs* 34: 36–35.
- Krishnamurthy S.K., Umamaheswari P. 2004. Improved groundnut production technology. *Agrobios Newslet.* 3 (4): 36–39.
- Manikam V.S., Benniamin A., Irudayaraj V. 2005. Antibacterial activity of leaf extracts of *Christella paracitica* (L.) Lev. *Indian Fern J.*: 87–88.
- Manikam V.S., Irudayaraj V. 1992. Pteridophyte Flora of Western Ghats. New Delhi: B. I. Publication Pvt. Ltd., 400 pp.
- Jayaraj S. 2005. Botanicals and biological control of major pests of groundnut. p. 70–80. In: "Green Pesticides for Insect Pest Management" (S. Ignachimuthu, S Jayaraj, eds.). Narosa Publishing House, New Delhi.
- Parihar P., Bohra A. 2002. Antifungal efficacy of various pteridophytic plant parts extracts: a study in vitro. *Ad. Plant Sci.* 15 (1): 35–38.
- Selvaraj P., Britto A.J.D., Sahayaraj K. 2005. Phytoecdysone of *Pteridium aquilinum* (L) Kuhn (*Deunstaedtiaceae*) and its pesticidal property on two major pests. *Arch. Phytopathol. Plant Prot.* 38 (2): 99–105.
- Sen S., Nandi P. 1951. Antibiotics from pteridophytes. *Sci. Cult.* 16: 328–329.
- Sharma N.D., Vyas S.C., Jain A.C. 1980. *Eudarlucia caricis* (Fr.) O. Erikias a hyperparasite on *Puccinia arachidis* Speg. the incitant of groundnut rust. *Sci. Cult.* 46: 50–51.
- Singh H.B. 2003. Pteridology in the New Mellenium. (S. Chandra, M. Srivastava eds). Kluwer Academic Publication, London: 421–426.
- Singh H.B. 1999. Potential medicinal Pteridophytes of India and other chemical constituents. *J. Econ. Taxon. Botany* 23 (1): 63–78.
- Sunkad G., Kulkari S., Benagi V.I. 2005. Effectiveness of neem seed kernel extract in combination with selected fungicides for groundnut rust management. *Int. Arach. Newsletter* 25: 39–41.
- Usman M.R., Jaganathan R., Dinakaran D. 1991. Plant disease management if groundnut with naturally occurring plant products. *Madras Agr. J.* 78: 152–153.
- Subramanyan P., Reddy L.J., Gibsons R.W., Me Donald D. 1985. Peanut rust a major threat to peanut production in the semi-arid tropics. *Plant Dis.* 69: 813–819.
- Valand G.B., Patel H.R., Patel J.G., Sheikh A.M. 1997. Impact of late leaf spot disease (*Phaeoisariopsis personata*) on growth performance of common varieties of groundnut (*Arachis hypogaea*). *Indian J. Agric. Sci.* 67(8): 319–321.

## POLISH SUMMARY

### AKTYWNOŚĆ PRZECIWRZYBOWA TRZECH EKSTRAKTÓW Z PAPROCI W STOSUNKU DO SPRAWCÓW PŁAMISTOŚCI I RDZY LIŚCI ORZECHA ZIEMNEGO

Oceniano aktywność przeciwgrzybową trzech ekstraktów z paproci otrzymanych z gatunków *Hemionitis arifolia* (Burm. f.) Moore., *Pteridium aquilium* (Linn.) Kuhn. i *Christella parasitica* (Linn.) H. Lev. w stosunku do sprawców płamistości i rdzy liści orzecha ziemnego, a mianowicie *Puccinia arachidis* Speg. oraz *Phaeoisariopsis personata* (Berk & M.A. Curtis) Vanarx., stosując metodę „mycelial dry weight”. Obydwa grzyby były wrażliwe na wszystkie trzy ekstrakty z paproci. Spośród ekstraktów, chloroformowy z *H. arifolia* posiadał najwyższą aktywność przeciw obydwu grzybom. Z kolei *P. personata* był wrażliwszy w stosunku do badanych ekstraktów roślinnych niż *P. arachidis*. Z powyższych badań wynika, że chloroformowy ekstrakt z *H. arifolia* może być wykorzystany w zwalczaniu płamistości i rdzy liści orzecha ziemnego.