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The effect of leaf extracts of *Centella asiatica* and *Andrographis paniculata* on spore germination of some fungi

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Abstract: The antifungal activity of leaf extracts of *Centella asiatica* and *Andrographis paniculata* was observed against 11 fungi, viz., *Alternaria alternata, A. brassicae, A. brassicicola, Cercospora cajani* and *C. blumeae, Colletotrichum capsici, Curvularia lunata, Erysiphe pisi, Fusarium* spp., *Drechslera oryzae* and *Ustilago cynodontis.* Undiluted leaf extract (100%) and three aqueous dilutions (1:1, 1:2 and 1:4) were used. The effect of mixed leaf extracts of the two plants was also seen. Leaf extracts of both the plants showed significant inhibitory effect on the spore germination of all the fungi tested. *U. cynodontis, D. oryzae, C. lunata, E. pisi, C. cajani* were found highly sensitive to the individual leaf extract of both plants. The inhibitory effect of extracts increased when they were used in combination with or without dilutions against *Fusarium* spp., *C. cajani* and *C. lunata.* Isolation of active ingradients with a possibility of their increased efficacy in vitro as well as in vivo is envisaged.

Key words: Leaf extracts, *Centella asiatica, Andrographis paniculata,* fungal spore germination

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INTRODUCTION

Centella asiatica (L.) Urban known as 'Brahmi-manduki' in Hindi, belongs to the family Apiaceae (Umbelliferae). It is a prostrate, perennial, faintly aromatic herb, commonly found on river and canal sides. The plant forms large runners which produce roots, leaves, flowers and fruits at the nodes. According to Satyavati (1976), the plant is native to parts of India, China, Sri Lanka, Australia, Madagascar and southern and central Africa. It is reported to have antileprotic (Bailey, 1945), antitumerogenic (Babu *et al.,* 1995), antistress (Sharma *et al.,* 1996), wound healing (Yoshinori *et al.,* 1982), antifilarial (Chakraborty *et al.,* 1996), antifeedent and antibacterial (Srivastava *et al.,* 1997) properties. The plant extract is also used as tonic in Ayurvedic formulations.

Andrographis paniculata Nees. vernacularly known as "Kalmegh", is an important medicinal plant belonging to the family Acanthaceae. The plant is indigenous to India. It grows in moist shady areas in the Himalayan foothills as well as in the Indo-Gangetic plains and humid tracts of peninsular India. The fresh and dried leaves as well as extracts from the plant are used to combat several diseases. The plant has been specifically used as an antiulcerogenic (Madav *et al.,* 1995), antidiarrhoeal (Gupta *et al.,* 1990), antiinflammatory (Tajuddin *et al.,* 1985), antimalarial (Mishra *et al.,* 1992), antipyretic (Vedavathy and Rao, 1991), antihelminthic and

Table 1. The fungi and their host plants

Fungus species	Host plants
Ustilago cynodontis P. Henn.	*Cynodon dactylon* Pers.
Alternaria brassicicola (Schw.) Wiltshire	*Brassica campestris* L.
Drechslera oryzae (Breda de Hann) Subram. & Jain	*Oryza sativa* L.
Fusarium spp.	Dead pods of *Albizzia lebbek* (L.) Benth.
Alternaria brassicae (Berk.) Sacc.	*Brassica oleracea* L. var. *capitata*
Alternaria alternata (Fr.) Keissler	*Albizzia lebbek* (L.) Benth.
Curvularia lunata (Wakker) Boedijn	*Oryza sativa* L.
Cercospora blumeae Thuem.	*Blumea* species
Erysiphe pisi DC.	*Pisum sativum* L.
Colletotrichum capsici (Syd.) Butler and Bisby	*Capsicum annuum* L.
Cercospora cajani P. Henn.	*Cajanus cajan* (L.) Millsp.

hepatoprotective (Rana and Avadhoot, 1991) agent.

Although the antibacterial property of *C. asiatica* has been recently reported (Srivastava *et al.*, 1997), neither of the above two plants has been tested for antifungal activity. The present study deals with the effect of aqueous extracts of the leaves of both plants singly as well as in combination on the spore germination of some fungi.

MATERIALS AND METHODS

Preparation of leaf extracts of *Centella asiatica* and *Andrographis paniculata*

Two kg of healthy leaves from each species were collected separately from the Ayurvedic garden of the Banaras Hindu University (25°.18' North latitude and 83°.1' East latitude at an altitude of approximately 79.1 ms above the sea level). The freshly collected leaves, being succulent in nature, were directly crushed in an electric blender and then the material was taken out and squeezed through muslin cloth into a beaker. The extracts thus obtained were filtered through bacteria-free filter into a conical flask, aseptically. The extract was prepared from both the plants in the same way and the individual filtrates served as 100% stock solution. The experiment was conducted with the pure extract as well as with three different aqueous dilutions (1:1; 1:2 and 1:4). In another experiment the extracts of the two plants were mixed together as follows: (A) equal amounts of pure extract of both the plants, (B) equal amounts of 1:2 diluted extracts of both the plants, (C) equal amounts of 1:4 diluted extracts of both the plants.

The test fungi

Fungi belonging to different genera were selected for this study. These were *Ustilago cynodontis, Alternaria brassicae, A brassicicola, A. alternata, Drechslera oryzae, Fusarium* spp., *Curvularia lunata, Erysiphe pisi, Colletotrichum capsici, Cercospora cajani* and *C. blumeae*. The chlamydospores of *U. cynodontis* (causing smut on *Cynodon dactylon*) and spores of *E.pisi* (causing powdery

Table 2. Effect of leaf extracts of *Centella asiatica* on spore germination of eleven fungi

Fungus species	Extract concentration		Dilution factor (Extract: Water)		
	Control (Water only)	100%	1:1	1:2	1:4
	Percent germination				
Ustilago cynodontis	99.16	0.66**	2.33**	5.50**	9.50**
Alternaria brassicae	99.60	55.83*	90.16**	93.00	98.30
Drechslera oryzae	99.83	2.16**	4.66**	8.16**	16.66**
Fusarium spp.	99.83	5.83**	22.33**	33.00**	38.83**
Alternaria brassicicola	94.83	44.16**	65.16**	69.83**	81.66
Alternaria alternata	96.83	67.83**	79.83	87.66	96.33
Curvularia lunata	94.00	0.50**	2.00**	5.83**	17.33**
Cercospora blumeae	99.16	2.50**	12.33**	23.50**	35.50**
Erysiphe pisi	67.00	1.16**	5.83**	23.83**	43.83
Colletotrichum capsici	63.16	5.50**	15.33**	32.00**	51.50
Cercospora cajani	94.83	2.16**	9.33**	30.83**	40.00**

Values suffixed with double asterisk are significantly different from corresponding control values at $P \leq 0.01$ based on the Student *t*-test.

mildew on pea), *C. cajani* (causing leaf spot on *Cajanus cajan*), *C. blumeae* (causing leaf spots on *Blumea* species) (Table 1) were directly removed from the infected plant leaves and mixed in a drop of plant extract kept on a glass slide. The mixing of spores was done in all the dilutions of both the extracts separately on separate glass slides. The other fungi, viz. *A. brassicae, A. alternata, A. brassicicola, D. oryzae, Fusarium* spp., *C. lunata, C. capsici* were isolated on potato dextrose agar (PDA) (Potato 250 g + Dextrose 20 g + Agar 20 g + Distilled water 1000 ml) medium and purified by single spore isolation technique (Singh *et al.*,1990). Spores of these fungi were picked up from fresh and sporulating cultures by an inoculating needle aseptically and about ten slides of each fungus was prepared for the respective concentrations of the extracts.

All slides prepared as above were examined under the microscope to assess the number of spores in each slide. A minimum of three slides, each containing about 200-300 spores for each fungus per treatment were selected. Each slide was kept in moist chambers prepared in Petri dishes by putting the filter paper inside the Petri dishes as well as on the lower surface of the upper lid of Petri dishes. The filter papers were moistened by sterilized distilled water. A control for each fungus was concurrently run in sterilized distilled water only. All the Petri dishes were incubated at 25 ± 2 °C for 24 h. After incubation the spores were killed by mixing with a drop of cotton blue and finally covered with a cover glass. The spore germination (germ tube > half the length of spore/chlamydospore) was observed under the Nikon binocular research microscope. All the experiments were conducted in triplicate.

Table 3 Effect of leaf extracts of *Andrographis paniculata* on spore germination of eleven fungi

Fungus	Extract concentration		Dilution factor		
	Control (Water only)	100%	1:1	1:2	1:4
	Per cent Germination				
Ustilago cynodontis	99.16	0.83**	2.16**	3.83**	6.50**
Alternaria brassicae	99.60	55.00**	89.50**	98.30	99.83
Drechslera oryzae	99.83	0.83**	2.33**	7.00**	87.33**
Fusarium spp.	99.83	20.16**	25.33**	36.00**	60.16**
Alternaria brassicicola	94.83	52.33**	61.00**	71.00**	78.16**
Alternaria alternata	96.83	15.33**	27.16**	41.50**	54.33**
Curvularia lunata	94.00	0.66**	1.83**	5.33**	31.50**
Cercospora blumeae	99.16	0.66**	2.16**	15.66**	22.66**
Erysiphe pisi	67.00	3.66**	9.16**	26.66**	38.00**
Colletotrichum capsici	63.16	8.33**	18.18**	29.16**	48.16**
Cercospora cajani	94.83	3.16**	8.16**	9.33**	17.66**

Values suffixed with double asterisk are significantly different from corresponding control values at $P \leq 0.01$ based on the Student *t*-test.

RESULTS AND DISCUSSION

The pure extract (100%) of *C. asiatica* inhibited the spore germination of *U. cynodontis* and *C. lunata* by more than 99%. Spore germination was suppressed by 97-98% in *D. oryzae*, *C. blumeae*, *C. cajani* and *E. pisi*. Other fungi were also significantly affected and the inhibition ranged from 29-91%. Aqueous dilutions were similarly effective against the respective fungi, except that 1:2 and 1:4 for *A. brassicae*, 1:4 for *A. brassicicola*, *E. pisi* and *C. capsici* and 1:1, 1:2 and 1:4 dilutions for *A. alternata* were not inhibitory (Table 2).

The pure extract of *A. paniculata* was highly effective against *U. cynodontis*, *D. oryzae*, *C. blumeae* and *C. lunata* and suppressed spore germination by more than 99%. It was also significantly effective against *E. pisi*, *C. cajani* and *C. capsici* inhibiting the spore germination by 87-94%. The spore germination of the remaining fungi was also affected. A similar trend of efficacy was seen for 1:1, 1:2 and 1:4 dilutions except for the 1:2 and 1:4 dilutions which were not effective against *A. brassicae*. Interestingly, *U. cynodontis* showed only 6.5% germination in 1:4 dilution while germination was higher for other fungi at this dilution (Table 3).

The inhibitory effect was maximum in mixture (A) of the leaf extracts of *C. asiatica* and *A. paniculata* against *U. cynodontis*, *Fusarium* spp., *C. lunata*, *C. cajani* followed by *D. oryzae* and *C. capsici*. Almost similar inhibitory effect was also seen against *C. blumeae* and *E. pisi* followed by *A. alternata*. The spore germination of the remaining two fungi was also significantly reduced. While mixture (B) was effective against most of the

Table 4. Effect of the mixture of leaf extracts of *Centella asiatica* and *Andrographis paniculata* on spore germination of eleven fungi

Fungus	Extract concentration			
	Control (Water Only)	Mix. (A)	Mix. (B)	Mix. (C)
	Per cent Germination			
Ustilago cynodontis	99.16	1.33**	4.16**	5.20**
Alternaria brassicae	99.60	54.83**	83.66	90.33
Drechslera oryzae	99.83	3.83**	17.33**	29.00**
Fusarium spp.	99.83	0.66**	3.33**	24.50**
Alternaria brassicicola	94.83	35.83**	55.16**	66.33
Alternaria alternata	96.83	11.33**	56.33**	75.33**
Curvularia lunata	94.00	0.50**	1.33**	3.60**
Cercospora blumeae	99.16	7.66**	34.33**	44.00**
Erysiphe pisi	67.00	5.83**	19.50**	34.83**
Colletotrichum capsici	63.16	4.16**	25.16**	42.33
Cercospora cajani	94.83	0.83**	3.00**	8.33**

Mix. (A) = equal amounts of pure extracts, Mix. (B) = equal amounts of 1:2 dilutions of both extracts, Mix. (C) = equal amounts of 1:4 dilutions of both extracts.
Values suffixed with double asterisk are significantly different from corresponding control values at $P \leq 0.01$ based on the Student *t*-test.

fungi except *A. brassicae*, the mixture (C) inhibited spore germination of most of the fungi except *A. brassicae*, *A. brassicicola* and *C. capsici* (Table 4). The inhibitory effect of the mixture was significantly more on *Fusarium* spp., *C. cajani* and *C. lunata* as compared to the individual extract.

Plant diseases have been controlled by synthetic fungicides since the very beginning of their appearance (Cafe Filhe *et al.*, 1968). However, due to increasing awareness of the ill effects of synthetic chemicals on human beings and animals, and also on the agro-ecosystems, research efforts on alternative and more environment friendly methods of controlling plant diseases have proliferated (Lyon *et al.*, 1995). Several workers have screened extracts from higher plants for antifungal activity (Kobayashi *et al.*, 1987; Millard *et al.*, 1987; Osswald *et al.*, 1987; Singh *et al.*, 1980, 1990). Extracts of *Reynoutria sachalinensis* have been found to induce resistance in plants, specially against powdery mildews and to some extent against other pathogens (Herger *et al.*, 1989). Similarly, neem (*Azadirachta indica* syn: *Melia indica*) extract has also shown inhibitory effect against several fungi (Singh *et al.*, 1980). Interestingly, Singh *et al.* (1991) reported control of pea powdery mildew with ginger (*Zingiber officinale*) extract under field conditions. The results of the present experiment revealed that some of the fungal species, namely *U. cynodontis, D. oryzae, C. lunata, E. pisi* and *C. cajani* were highly sensitive to the leaf extracts of both the plants, when tested as in pure extracts as well as in their aqueous dilutions (Tables 2 and 3). Further, the

efficacy of the extracts increased when they were used in combinations, with or without dilutions against *Fusarium* spp., *C. cajani* and *C. lunata*. Slight increase in spore germination of *U. cynodontis, H. oryzae* and *E. pisi* in mixture (A) as compared to pure extracts may be because of the reduction of the efficacy of effective compound(s) in the mixture but this needs further investigation. Comparing the results of individual extracts as well as their mixtures, it is suggested that they can be used to control the pathogens caused by the fungi included in this study under field conditions. The latter aspect will investigated in the future.

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