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Antifungal Activity of Methanolic of *Centella asiatica* and *Andrographis panicuiata*

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The antifungal activity of methanolic extracts of *Centella asiatica* and *Andrographis* paniculata leaves was observed against fourteen fungi, viz., *Alternaria alternata, A. brassicae, A. brassicicola, A. solani, A. tenuissima, Cercospora blumae, Curvularia lunata, C. penniseti, and Drechslera monoceras, D. oryzae, D. turitica, Fusarium albizziae and F. udum*. Different concentrations of the methanolic extract (1000, 2000, 3000, 5000, 7000, 10000ppm) were used. The effect of mixed leaf extract (1500 ppm of C. *asiatica* + 1500 ppm of A. *paniculata*) and its l:2 ad 1:4 dilutions were also studied. The individual extracts of both the plants showed significant inhibitory effect on spore germination of all the fungi tested. *F. udum, F. albizzae, D. oryzae, D. turtica*, and *D. monoceras* were particularly sensitive to these extracts. In general, the extract of C. *asiatica* showed a higher inhibitory effect in all concentrations against all the fungi as compared to A. *paniculata,* except for A. brassicae *A. solani, D. oryzae, D. penniseti and Curvularia* sp. The inhibitory effect of extracts increased when they were used in combination with or without dilutions against A. *brassicicola, A. solani A. brassicae, A. alternata, A. tenussima, C. blumae, C. lunata, C. penniseti and Curvularia* species. Higher efficacy of active ingredient of these extracts under field condition is envisaged against plant pathogens.

KEYWORDS: *Andrographis paniculata, Centella asiatica*, **Methanolic leaf extract, Spore germination**

Centella asiatica (L.) Urban, Syn *Hydrocotyle asiatica* L. is named in Hindi (an Indian language) as "Brahmi". It belongs to the family Apiaceae (Umbelliferae). The plant is native to India, China, Indonesia, Sri Lanka, Australia, Medagascar, Southern and Central Africa (Satyavati, 1976). It is a small perennial, prostrate, faintly aromatic herb, occurring along river and canal sides, sending large runners which produce leaves, roots, flowers and fruits at the nodes. It is being extensively used in various parts of India as a cure for various ailments, viz., headache, blood. dysentery, body ache, cholera, stomachache, diabetes and bone fracture. The plant extract is also used as tonic in Ayurvedic (Ancient Indian Medicine) formulations. Glycosides of the plant have been specifically used for their antistress (Sharma *et al.*, 1996), antitumour (Babu *et al.*, 1995), antifilarial (Chakraborty *et al.*, 1996) and antibacterial properties (Srivastava *et al.*, 1997).

Andrographis paniculata (Burm. F.) Wall ex Nees. vernacularly known as Kalmegh, is an important medicinal plant. It is an annual bitter herb, belonging to the family Acanthaceae. The Plant is indigenous to India, grows in moist shady areas in the Himalayan foothills, in the Indo-Gangetic plains as well as in humid tracts of peninsular India. The fresh and dried leaves as well as extracts from the plants used to cure several diseases, viz., diarrhoea, dysentery, fever, malaria, liver disorders, skin infections and worm infestation. The plant has been reported to possess anti-ulcerogenic (Madav *et al.*, 1995), antidiarrhoeal (Gupta *et al.*, 1990), antiinflammatory (Tajuddin and Tariq, 1983), antimalarial (Misra *et al.*, 1992), antipyretic (Vadavathy and Rao, 1991), antihelminthic and hepatoprotective activities (Rana and Avadhoot, 1991). Recently, the altibacterial property of *C. asiatica* (Srivastava *et al.*, 1997) and antifungal property of aqueous leaf extracts of both the plants, i.e., *C. asiatica* and *A. paniculata* (Singh *et al.*, 1999) have been reported. The present study deals with the efficacy of methanolic leaf extracts of both the plats singly as well as in combination on the spore germination of some fungi.

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Materials and Methods

Preparation of methanolic leaf extract

Two kg of healthy leaf from each species were collected separately from their natural populations occurring on the campus of the Banaras Hindu University (2518N lat. and 831E lat. at an altitude of 79.1 meterabove the sea level). The leaves were air-dried at room temperature and powdered. The powder was soaked in methanol for 10 days and stirred three to five times a day. After 10 days, the mixture (methanol + powder) was filtered and the filtrate was dried on a water bath at 80. The resultant material (methanolic extract) was stored at room temperature for antifungal assay. The experiment was conducted with different concentrations of methanolic extract. In another experiment the extracts of the two plants were mixed together as: (A) equal amounts of methanolic extract of both the plants (l500 ppm extract of *C. asiatica* + 1500 ppm extract of *A. paniculata*) (B) equal amounts of 1:2 aqueous dilution of the above methanolic

extract, i.e. the extract at (A), (C) equal amounts of 1:4 aqueous dilution of the mixed extract at (A). A control for each fungus was concurrently run in sterilized distilled water.

The test fungi

The fungi included in the present study were *Alternaria alternata*, *A. brassicae*, *A.brassicicola*, *A. solani*, *A. tenuissima*, *Cercospora blumae*, *Curvularia lunata*, *C. Penniseti, and Curvularia* sp., *Drechslera. monoceras*, *D. oryzae*, *D. turtica*, *Fusariunm albizziae* and *F. udum*. Spores of *C. blumeae* (causing leaf spots on *Blumea* species)were directly removed from the infected leaves and mixed in a drop of extract on glass slides.

The mixing of spores was done in all the concentrations of both the extracts separately on separate glass slides. The other fungi were isolated on potato dcxtrose agar (PDA) medium (potato 250 g + dextrose 20 g + agar 20 g + distilled water 1000 ml), and later purified by single spore isolation technique. Spores of these fungi were picked up from fresh and sporulating cultures by an inoculating needle aseptically, and about ten slides of each fungus were prepared for the respective concentrations of the extracts.

All the slides were examined under the microscope to assess the number of spores in each slide. A minimum of thee slides, each containing 200-300 spores for each fungus per treatment were selected. All the slides were kept in moist chambers prepared in petri-dishes by putting a filter paper at the bottom of the patri-dish as well as on the inner surface of the upper lid. The filter papers were moistened with Sterilized distilled water.

All the petri-dishes were incubated at 25 ± 2°C for 24 h. At the end of the incubation period, a drop of cotton blue was mixed with the spore suspension and covered with a cover glass. The spore germination was observed under a Nikon binocular research microscope. All the experiments were conducted in triplicate. Data were subjected to one-way ANOVA for statistical significance (Snedecor and Cochran, 1989). Test fungi and their plant hosts are given in Table 1.

Results and Discussion

Methanolic leaf extract of both plants affected spore germination of all the fungi. The sensitivity of different fungi varied significantly. Methanolic extract of C. asiatica, completely inhibited the spore germination of *F. udum*, and *D. monoceras* at 5000 ppm or higher, and up to 97~99% in *F. albizziae*, *D. oryzae*, *D. turtica* and *C. blumae* at the same concentration. Spore germination of *C. lunata*, *C. penniseti*, *Curvularia* sp. was also significantly affected (by 93~98%) at 7000 ppm while lower efficacy (66~88%) was seen against *A. brassicicola*, *A. brassicae*, *A. solani*, *A. alternata and A. tenuissima* at 10000ppm (Table 2). Statistical analysis showed significant differences in per cent spore germination due to extract concentration for all fungi (Table 3). In these analyses control was considered zero concentration.

Spore germination of *F. udmn* was completely inhibited by the extract of *A. paniculata* at 5000 ppm or higher. More than 95% inhibition was recorded in *F. albizziae*, *D. oryzae*, *D. turtiica*, and *D. monoceras* at this concentration Slightly higher concentration (7000 ppm) was highly effective (96-99%) against *C. blumae*, *C. lunata*, *C. penniseti*, and *Curvularia* sp. Extract was less effective (64-83%) against all *Alternaria* species at 10000 ppm (Table 4). One-way analysis of variance indicated significant differences in per cent spore germination among the extract concentrations for all fungi (Table 3).

In general, the inhibitory effect of mixture (A) as compared to individual extract (3000 ppm) of both the plants, was higher against *C. blumae*, *A. brassicicola*, *A. tenuissima*, *A. solani*, *A. brassicae*, *A. alternata* followed by *Cur-*

Table 1. Test fungi and their host plants

Fungus	Host
Alternaria alternata (Fr.) Keissler	Azadirachta indica L.
Alternaria brassicae (Berk.) Sacc.	Brassica oleracea L. var. capitata
Alternaria brassicicola (Schw.) Wiltshire	Brassica compestris L.
Alternaria solani (E. & Mart.)	Solanum tuberosum L.
Alternaria tenuissima	Abelmoschus esculentus (L.) Moench
Cercospora blumeae Thirum	Blumea erantha DC.
Curvularia lunata (Wakker) Boedijn	Oryza sativa L.
Curvularia penniseti	Pennisetum typhoides (Burm. F.) Stapf. & Hubb.
Curvularia sp	Linum usitassimum L.
Drechslera monoceras Drechsl	Echinochloa colonum (L) Link.
Drechslera oryzae (Breda de Hann) Subram. and Jain	Oryza sativa L.
Drechslera turtica (pass.) Subram. & Jain	Pennisetum typhoides (Burm. F.) Stapf. & Hubb.
Fusarium albezzae Bagchee	Saprophytic on dead pods of Albizia lebbeck (L.) Benth
Fusarium udum	Cajanus cajan (L.) Millsp.

Table 2. Effect of methanolic leaf extract of *Centella asiatica* on spore germination of 14 fungi

Fungus	Concentration (ppm)						
	Control	1000	2000	3000	5000	7000	10000
	Percent germination						
Alternaria alternater	98.77 ± 0.91*	95.83 ± 1.28	76.16 ± 0.92	64.54 ± 1.91	43.39 ± 2.03	20.74 ± 0.98	12.20 ± 1.52
Alternaria brassicae.	93.11 ± 0.91	90.11 ± 1.32	84.55 ± 1.73	73.11 ± 0.70	58.75 ± 1.14	45.11 ± 1.16	29.29 ± 0.98
Alternaria brassicicola	96.80 ± 0.67	85.78 ± 1.19	79.66 ± 0.55	66.11 ± 1.34	54.90 ± 0.52	38.83 ± 0.76	25.30 ± 0.79
Alternaria solani	96.59 ± 1.06	94.34 ± 1.05	82.00 ± 1.11	71.32 ± 1.00	62.61 ± 1.07	51.64 ± 2.47	32.44 ± 1.98
Alternaria tenuissima	99.05 ± 0.58	88.37 ± 2.31	81.22 ± 1.67	71.63 ± 1.07	65.49 ± 1.36	44.39 ± 1.76	32.98 ± 1.28
Cercospora blumeae	95.68 ± 0.77	75.16 ± 1.16	48.93 ± 0.80	19.72 ± 1.66	3.34 ± 0.51	0.00	0.00
Curvularia lunata	92.93 ± 1.38	83.08 ± 1.29	71.76 ± 1.39	58.40 ± 2.19	27.28 ± 1.17	1.54 ± 0.44	0.00
Curvularia penniseti	95.18 ± 0.75	90.05 ± 1.64	76.71 ± 0.81	54.31 ± 1.58	17.46 ± 0.33	3.13 ± 1.17	0.00
Curvularia sp	98.01 ± 1.82	77.37 ± 2.22	67.85 ± 1.21	50.85 ± 2.21	26.76 ± 1.78	2.00 ± 1.21	0.00
Drechslera monoceras	99.30 ± 2.10	56.68 ± 1.16	43.57 ± 0.80	3.27 ± 0.80	0.00	0.00	0.00
Drechslera oryzae	99.10 ± 0.52	54.51 ± 0.97	42.55 ± 1.67	17.48 ± 0.80	1.09 ± 0.31	0.00	0.00
Drechslera turtica	95.67 ± 1.41	34.05 ± 1.05	21.86 ± 1.25	6.30 ± 0.87	1.10 ± 0.51	0.00	0.00
Fusarium albezzae	95.11 ± 0.79	46.03 ± 0.96	33.86 ± 1.44	16.33 ± 0.93	1.30 ± 0.20	0.00	0.00
Fusarium udum	97.11 ± 2.00	22.76 ± 1.03	6.10 ± 1.22	1.46 ± 2.12	0.00	0.00	0.00

*Mean ± SE.

Table 3. Summary of ANOVA for the effect of different concentrations of leaf extracts of *Centella asiatica* and *Andrographis paniculata,* and of different dilutions of the mixed extract. All F values are significant at P<0.001.

Fungus	Methanolic extract of *C. asiatica*		Methanolic extract of *A. paniculata*		Mixture of C. asiatica and A. paniculata	
	d.f	F	d.f	F	d.f	F
Alternaria alternata	6	863.29	6	551.64	3	54.67
Alternaria brassicae	6	379.79	6	681.99	3	514.29
Alternaria brassicicola	6	323.36	6	95.54	3	808.00
Alternaria solani	6	389.58	6	345.91	3	783.93
Alternaria tenuissma	6	311.98	6	197.88	3	1438.19
Cercospora blumeae	6	2745.39	6	1617.29	3	488.93
Curvularia lunata	6	1246.45	6	867.00	3	371.60
Curvularia penniseti	6	1498.41	6	1285.66	3	183.29
Curvularia sp	6	947.53	6	550.62	3	577.37
Drechslera monoceras	6	3511.30	6	1886.92	3	162.09
Drechslera oryzae	6	1983.61	6	1997.66	3	248.45
Drechslera turtica	6	785.56	6	1601.66	3	179.30
Fusarium albezzae	6	1023.28	6	1914.50	3	878.94
Fusarium udum	6	1384.86	6	1335.56	3	521.37
Residual	14		14		8	

vulaia spp., *C. penniseti* and *C. lunata.* The spore germination of the remaining fungi was less suppressed by mixture (A) (Table 5). Mixture (B) was effective against most of the fungi, but efficacy was lower than mixture (A). Mixture (C) inhibited spore germination of all fungi, but efficacy was lower than mixtures (A) and (B). Thus the efficacy of mixtures decreased with increasing dilution. One-way analysis of variance indicated significant differences in per cent spore germination among dilutions of the mixture for all fungi (Table 3).

Plant diseases have been controlled by synthetic fungicides since the very beginning of their-appearance (Cafè Filhe *et al.*, 1968). However, due to increasing awareness of the ill effects of synthetic chemicals on human being, animals, and also on the agroecosystems, recent research efforts concentrate on the disease control through ecofriendly methods such a biological control, induced resistance by biotic ad abiotic means (Lyon *et al.*, 1995) and use of biodegradable natural products, especially from medicinal plants (Prithviraj and Singh, 1995; Suhelyal *et al.*, 1996; Abbas *et al.*, 1992). May Works have tested crude plant extracts against several plant pathogens (Chakravorty and Priya, 1977; Chaturvedi *et al.*, 1987; Singh *et al.*, 1990; Singh *et al.*, 1999) as well as methanolic extract (Prithiviraj *et al.*, 1996; Mukherjee *et al.*, 1995; Kobayashi *et al.*, 1987; Millard *et al.*, 1987; Osswald *et al.*, 1987). Neem (Azadirachta

Table 4. Effect of methanolic leaf extract of *Andrographis paniculata* on spore germination of fourteen fungi

Fungus	Concentration (ppm)						
	Control	1000	2000	3000	5000	7000	10000
	Percent germination						
Alternaria alternata	98.77 ± 0.91*	94.69 ± 1.28	73.02 ± 0.92	45.44 ± 1.91	38.71 ± 2.03	21.62 ± 0.98	16.31 ± 1.52
Alternaria brassicae	95.11 ± 0.91	94.49 ± 1.32	87.03 ± 1.73	77.26 ± 0.70	47.84 ± 1.14	32.88 ± 1.16	22.06 ± 0.98
Alternaria brassicicola	98.66 ± 0.67	86.97 ± 1.19	64.73 ± 0.55	56.86 ± 1.34	44.35 ± 0.52	42.16 ± 6.45	28.50 ± 0.79
Alternaria solani	96.59 ± 1.07	95.79 ± 1.05	84.86 ± 1.11	76.07 ± 1.00	59.08 ± 1.07	38.45 ± 2.47	26.22 ± 1.98
Alternaria tenuissma	99.05 ± 0.58	89.00 ± 2.31	80.65 ± 1.67	72.80 ± 1.07	66.99 ± 1.36	64.70 ± 1.76	35.56 ± 1.28
Cercospora blumeae	98.68 ± 0.77	81.11 ± 1.16	65.63 ± 0.80	32.38 ± 1.66	15.12 ± 1.10	2.31 ± 0.51	0.0
Curvularia lunata	92.93 ± 1.38	84.63 ± 1.29	69.36 ± 1.40	54.11 ± 2.19	25.45 ± 1.17	1.33 ± 0.44	0.0
Curvularia penniseti	95.19 ± 0.75	88.26 ± 1.64	74.51 ± 0.81	54.18 ± 1.58	15.74 ± 1.10	4.17 ± 1.17	0.0
Curvularia sp	98.01 ± 1.83	78.81 ± 2.22	67.19 ± 1.21	44.88 ± 2.21	14.16 ± 1.78	4.16 ± 1.21	0.0
Drechslera monoceras	99.30 ± 0.54	56.29 ± 1.00	37.08 ± 1.48	11.04 ± 0.64	3.26 ± 1.05	0.0	0.0
Drechslera oryzae	99.08 ± 0.52	48.93 ± 0.97	34.30 ± 1.68	7.79 ± 0.80	2.80 ± 0.31	0.0	0.0
Drechslera turtica	95.67 ± 1.41	57.00 ± 1.05	31.67 ± 1.25	11.33 ± 0.87	4.67 ± 0.50	0.0	0.0
Fusarium albezzae	95.10 ± 0.78	48.62 ± 0.96	34.02 ± 1.43	16.48 ± 0.93	0.61 ± 0.35	0.0	0.0
Fusarium udum	97.18 ± 2.00	25.08 ± 1.01	14.37 ± 1.22	2.12 ± 0.29	0.0	0.0	0.0

Table 5. Effect of the mixture of methanolic leaf extract of Centella asiatica and Andrographis paniculata on spore germination of fourteen fungi

Fungus	Concentration (ppm)			
	Control	Mixture (A)	Mixture (b)	Mixture (c)
	Percent germination			
Alternaria alternata	93.00 ± 0.58*	35.60 ± 0.84	64.39 ± 2.27	79.09 ± 1.41
Alternaria brassicae	95.74 ± 0.58	33.49 ± 1.55	56.74 ± 1.27	76.93 ± 1.10
Alternaria brassicicola	94.59 ± 0.96	23.07 ± 1.46	38.01 ± 0.95	73.48 ± 1.14
Alternaria solani	96.48 ± 0.70	27.44 ± 1.24	56.26 ± 0.94	76.38 ± 1.24
Alternaria tenuissma	98.93 ± 0.68	26.48 ± 0.33	45.80 ± 1.16	77.93 ± 0.99
Cercospora blumeae	90.77 ± 1.24	18.52 ± 0.78	52.52 ± 2.27	75.08 ± 0.84
Curvularia lunata	87.37 ± 1.08	45.41 ± 1.09	64.65 ± 0.91	78.31 ± 0.64
Curvularia penniseti	92.51 ± 2.59	42.08 ± 1.61	69.44 ± 0.80	84.04 ± 0.86
Curvularia sp	98.33 ± 0.88	43.18 ± 1.22	69.78 ± 0.80	82.51 ± 0.93
Drechslera monoceras	85.99 ± 0.84	52.74 ± 0.64	75.14 ± 2.04	85.47 ± 0.84
Drechslera oryzae	96.74 ± 0.94	49.11 ± 1.11	67.59 ± 0.77	83.44 ± 2.02
Drechslera turtica	95.52 ± 1.38	48.59 ± 0.70	67.85 ± 0.79	84.04 ± 2.13
Fusarium albezzae	98.63 ± 0.69	19.78 ± 0.75	47.99 ± 1.59	73.20 ± 1.28
Fusarium udum	91.33 ± 1.86	15.01 ± 1.07	43.18 ± 3.08	59.37 ± 0.54

Mixture (A): equal amounts of methanolic extract of both the plants (1500 ppm extract of *C. asiatica* + 1500 ppm extract of A. paniculata
Mixture (B): Equal amounts of 1:2 aqueous dilution of extract at A
Mixture (C): equal amounts of 1:4 aqueous dilution of the methanolic extract at B
*Mean ± SE.

indica) extract has shown inhibitory effect against several fungi in vitro (Singh *et al.*, 1980) and control of pea powdery mildew with ginger (Zingiber, officinale) extract under field conditions has also been successfully achieved (Singh *et al.*, 1991).

The results of the present experiment on the efficacy of methanolic leaf extracts of two plant, viz., *C. asiatica* and *A. paniculata*, against fourteen fungal species, some of which incite serious diseases affecting the economic yields of the crops, reveal that *F. udum*, *F. albizziae*, *D. oryzae*, *D. turtica*, *D. monoceras* and *C. blumae* are highly sensitive to both the extract of 5000 ppm (Tables 2, 4). In general, efficacy of *C. asianca* extract in all concentrations was greater against most of the fungi than that of. *A. paniculata* except for *A. brassicae*, *A. solani*, *D. oryzae*, *D. turtica* and *Curvularia* sp. which were relatively resistant. However, the efficacy of extracts increased when they were used in combination, with or without dilution against *Alternaria* species, *Cercospora blumae* and *Curvularia* species (Table 4). Similarly, the increased efficacy of crude aqueous extract was recorded when they were used in combination with or without dilution against *Cerospora* species and *Curvularia* species

(Singh *et al.*, 1999). Maximum inhibitory effect of mixture (A) as compared to individual leaf extracts (3000 ppm) of both the plants, may be because of the increased efficacy of active principles in the mixture but this needs further investigation. Comparing the results of individual extracts as well as their mixture, it is suggested that they can be used to control some fungal diseases under field conditions. It is further suggested that fractionation of these plant materials with nonpolar solvents as well as the active ingredients may be more effective in plant disease control. The efficacy of methanolic leaf extracts of *C. asiatica* and *A. paniculata* is being reported here for the first time.

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