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Antifungal activity of the extract of *Andrographis paniculata* and andrographolide

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Abstract
Antifungal activity assay of the methanol extract of the aerial parts of the plant *Andrographis paniculata* revealed that it exhibits mycelial growth inhibition of *Fusarium solani* and spore germination inhibition of *Alternaria solani.* Pure andrographolide present in the methanol extract also exhibited spore germination inhibition of *A. solani.* Quantitative estimation of andrographolide in the methanol extract by HPLC revealed that it had 6.82% andrographolide in it. This is probably the first report on the antifungal activity of andrographolide.

Keywords: Antifungal, *Andrographis paniculata,* andrographolide, *Fusarium solani, Alternaria solani*.

1. Introduction
Andrographis paniculata (Burm.f.) Wall ex Nees (Acanthaceae) is an annual herbaceous plant native to India and Sri Lanka. It is widely cultivated in Southern and South eastern Asia, where it has been traditionally used to treat infections and some diseases [1, 2]. Mostly the leaves and roots are used for medicinal purposes.
A. paniculata is an erect annual herb extremely bitter in taste in all parts of the plant body. The genus *Andrographis* consists of 28 species of small annual shrubs essentially distributed in tropical Asia. Only a few species are medicinal, of which *A. paniculata* is the most popular.
There are several reports on the antifungal properties of the crude extracts [3, 4, 5, 6], but there are fewer studies on the chemical nature of antifungal compounds present in the plant. Sule *et al.* [7] has reported the identification of 3-O-β-D-glucosyl-14-deoxyandrographolide, 14-deoxy andrographolide and 14-deoxy-11, 12-didehydro andrographolide as antifungal compounds in the plant, but antifungal activity of andrographolide was not reported by them. While Xu *et al.* [8] has reported no antibacterial property for andrographolide, Arifullah *et al.* [9] has reported antibacterial activity for the compound. In this paper, the antifungal activity of methanol extract of *A. paniculata* and andrographolide (Figure 1) is being reported.

2. Materials and methods
2.1. Plant material
The aerial parts of *A. paniculata* which were collected from the experimental farm of Indian Institute of Horticultural Research (IIHR), Hessaraghatta, Bangalore, India were dried at 60 ^{0}C and were powdered.

2.2. Preparation of the extracts
The plant material was extracted using a Soxhlet apparatus first with hexane, then with ethyl acetate, and finally with methanol. The respective extracts were obtained by completely distilling out the solvents on a water bath.

2.3. Reagents and chemicals
Pure andrographolide (98%) was obtained from Sigma-Aldrich, USA. Hexane, ethylacetate, methanol, dextrose, agar and HPLC grade acetonitrile were obtained from Merck India and KH_2PO_4 from Spectrochem Pvt. Ltd, Mumbai.

2.4. Antifungal activity assays
2.4.1. Mycelial growth inhibition activity
Antifungal activity of hexane, ethyl acetate, and methanol extracts was evaluated by poisoned-food technique [10]. Surfactant Tween-80 was added at a level of 0.3% to the media in both the control and the treated samples before plating. Observation on mycelial growth of *Fusarium*

solani was taken after 6 days of incubation at 27 ± 2 °C. The mycelial growth inhibition was calculated by the formula (C – T) /C × 100, where C is the mycelial diameter of the control and T is the mycelial diameter of the treated samples.

2.4.2. Spore germination inhibition study

This was done by hanging drop method [11]. Spores of *Alternaria solani* from infected tomato fruits collected from the IIHR experimental farm in Hessaraghatta, Bangalore, India were used. Spores were added to a solution of the compound in 3% *n*-propanol in water kept in cavity slides by the hanging drop method. Observation on spore germination was recorded after incubation for 3 hours using Carl Zeiss Axio Imager A1 microscope.

2.5. Estimation of andrographolide

With minor modification of the method given in literature [12], quantitative estimation of andrographolide was done by Shimadzu Nexera X_2 Ultra high-performance liquid chromatography (UHPLC) with the following conditions: column-Shim pack XR-ODS-III, 2 mm × 150 mm; detector–PDA detector set at 223 nm; time: 20 min; gradient method with mobile phase – potassium dihydrogen orthophosphate (KH_2PO_4) buffer and acetonitrile, flow rate: 0.4 mL/min, injection volume: 4 µL, Retention time of andrographolide: 9.0 to 9.1 min. Percentage of andrographolide was calculated from the peak response using the formula:

$$\frac{\text{Area of sample}}{\text{Area of standard}} \times \frac{\text{Standard weight}}{\text{Standard dilution}} \times \frac{\text{Sample dilution}}{\text{Sample weight}} \times \frac{\text{purity of Standard}}{100} \times 100$$

3. Results

The results of the bioassay of the extracts against the mycelial growth inhibition of *F. solani* on potato-dextrose-agar medium by poisoned food technique are presented in Table 1. It shows that only methanol extract showed the activity. Activity of pure andrographolide was not determined by this technique because of the paucity of the compound.

Table1: Antifungal activity of extracts of *Andrographis paniculata* against the mycelial growth of *Fusarium solani*

Extracts	Concentration (ppm)	Per cent inhibition
Hexane	2000	0.0
Ethyl acetate	2000	0.0
Methanol	2000	33.0 (±1.5)

Once it came to be known that the antifungal activity against the mycelial growth of *F. solani* is exhibited only by the methanol extract, further study of this extract and andrographolide (Figure 1) present in the extract was done on the spore germination of *A. solani*. Percent spore germination inhibition of the methanol extract, pure andrographolide and that of phenol (standard) are presented in Table 2. The values are the averages of 2 replications, standard deviations being given in parenthesis.

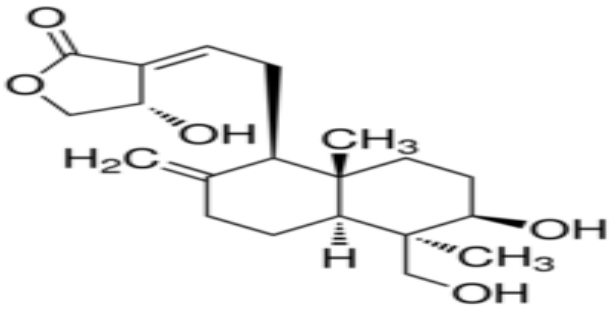

Fig 1: Chemical Structure of Andrographolide

From Table 2, it is clear that andrographolide at a concentration of 500 mg/L exhibits 64.8% spore germination inhibition of *A. solani*. This is probably the first report on the antifungal activity of andrographolide. Quantitative estimation of andrographolide in the methanol extract by HPLC revealed that it contains 6.82% andrographolide in it.

Table 2: Spore germination inhibition of *Alternaria solani* by methanol extract of *Andrographis paniculata* and andrographolide

Treatments	Per cent germination	Per cent inhibition w.r.t control
Control (3% n-propanol in water)	92.86 (± 0.6)	-
Methanol extract 5000 mg/L	33.85 (± 1.3)	63.54 (± 1.2)
Andrographolide 500 mg/L	32.66 (± 1.7)	64.82 (± 1.4)
Phenol (Standard) 500 mg/L	80.0 (± 0.6)	13.84 (± 0.7)

4. Conclusions

Andrographolide has been identified as one of the antifungal compounds present in the methanol extract of *A. paniculata*. The plant has potential to be used as a source of antifungal compounds. The plant can be considered in the disease management of other crops by crop rotation and intercropping. Synthesis of analogues of andrographolide and structure-activity relationship studies can lead to the development of new fungicides.

5. Conflict of interest

The authors declare that there is no conflict of interests.

6. References

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