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Research Article

ANTIFUNGAL ACTIVITY OF ANDROGRAPHOLIDE COMPOUND EXTRACTED FROM *Andrographis paniculata*

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ABSTRACT

Antifungal activity of methanol extract of shoot part of the plant *Andrographis paniculata* revealed that the plant extract exhibits best mycelial growth inhibition against some fungal pathogens isolated from solanaceous vegetable fruit. Among the isolated fungal pathogens *Fusarium oxysporum* exhibits 17.24mm of growth inhibition followed by *Aspergillus fumigatus* 15.98mm, *Aspergillus flavus* 10.62mm, *Alternaria alternata* 9.02mm and there was no inhibition found in *Rhizopus stolonifer*. Phytochemical analysis of shoot part of *Andrographis paniculata* revealed the occurrence of glycosoides, flavonoids, alkaloids, steroids, phenols tannins and saponins. Quantitative estimation of andrographolide compound in methanol extract by HPLC method revealed that it constitutes 1.07 % of andrographolide.

Keywords: Antifungal activity, *Andrographis paniculata*, Andrographolide, Solanaceous vegetables & fungal pathogens.

INTRODUCTION

Andrographis paniculata an annual herbaceous plant native to India and other countries viz., Srilanka, China, Thailand and it is widely cultivated in Southern and South eastern Asia, where it is to be used to treat many infectious diseases [2,9]. It is found in any in wild throughout the plains of India especially in Maharashtra, Orissa and Uttar Pradesh, Tamil Nadu, Karnataka, Maharashtra. Some recent researcher found that these plants are having antihypertensive, antipyretic, antithrombotic, antidote antidiarrheal, anti-inflammatory, choleretic, antimalarial, and hepatoprotective. *Andrographis paniculata* was used in the era of 175 BC in Charaka Samhita for treatment of jaundice and in combination with other for plant preparation [11].

Fungal pathogens cause immense losses to many valuable vegetable crops and plants. Pathogenic fungi are the main pathogens responsible for the alteration during developmental stages including post harvest. In fruit and vegetables, many fungal pathogens were responsible for causing quality problems related to nutritional, organoleptic and decrease shelf life [1]. In addition, in some cases fungi are indirectly responsible for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Extensive research prevailed that *Andrographis paniculata* has broad range of pharmacologic effects such anti-inflammatory [12,13], antimalarial[10], Cardiovascular and anti-inflammatory activities[7,3].

MATERIALS AND METHODS

Plant material

The aerial parts of *Andrographis paniculata* were collected from Balaghat region of Jabalpur division MP and were dried at $60C^0$ and were then grinded to make fine powder.

Preparation of the extracts

The plant material was extracted by using Soxhlet apparatus so that constituents of plant material were fully extracted. The plant material was first extracted with hexane solvent then was extracted with ethyl acetate and last with methanol solvent. The respective extracts were obtained by completely distilling out the solvents on a water bath.

HPLC Instrumentation

HPLC instrument that was used for the estimation of andrographolide were of the following features, HPLC- grade waters, Pump - 515 Isocratic pump, Injector - Rheodyne injector with a 20-microlitre loop, Detector - UV Vis detector, Software - Data ace software, Column - Thermo C-18 column (4.6 x 250mm, 5µ particle size), sample size (20µl). Isocratic elution was carried out with methanol at a flow rate (1ml/min). The detection was performed with wavelength (230 nm) and column temperature was ambient (30^0C). Class VP software was used for integration and calibration. Evaluation was via peak areas with linear regression. Inject the solution of *Andrographis paniculata* in HPLC with the help of 20 µl fixed loop injector and percent content of andrographolide were estimated by counting the area of andrographolide peak in HPLC chromatogram in all sample.

Antifungal activity

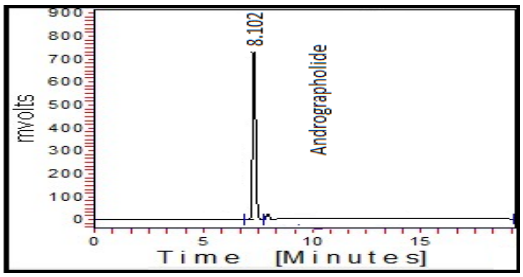
Antifungal activity was screened by agar well diffusion method. The methanol and Hexane solvent extracts of different plants were tested against fungal pathogens isolated from infected solanaceous vegetable fruits. The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton

swabs. Then wells (6 mm) were made and in some petriplates 6mm discs were used in the medium. Wells were prepared by using sterile cork borer. 25, 50 and 75µl of different concentration extracts were transferred in to the separate wells. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the well and around the discs which indicated the presence of antifungal activity. The plates were done in triplicates. The zone of inhibition was calculated with standard Himedia scale. The antimicrobial activity was taken on the basis of diameter of zone of inhibition, which was measured after 7 days of incubation and the mean of three readings is presented. The presence of inhibition of the treated fungus was calculated using DMSO as standard.

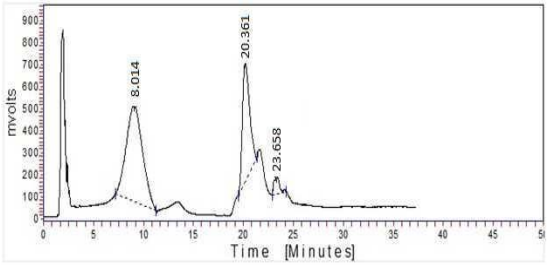
RESULTS

Table 1: Antifungal activity of the shoot extract of *Andrographis paniculate*

Fungal pathogens	Mycelial growth inhibition in mm at 75µl concentration		
	Andrographis paniculata extract		Solvents as negative control.
	Methanol solvent	Hexane solvent	Solvents as negative control
Aspergillus fumigates	15.98 ± 1.71	13.11± 1.09	0.00
Fusarium oxysporum	17.24 ± 1.42	10.09 ± 0.92	0.00
Alternaria alternate	9.02 ±0 .89	9.28 ± 1.24	0.00
Rhizopus stolonifer	0.00	0.00	0.00
Aspergillus niger	16.62 ± 0.23	11.26 ± 0.33	0.00
Aspergillus flavus	13.18 ± 0.24	12.24 ± 0.81	0.00
Rhizoctonia solani	10.12 ± 0.42	8.23 ± 0.95	0.00

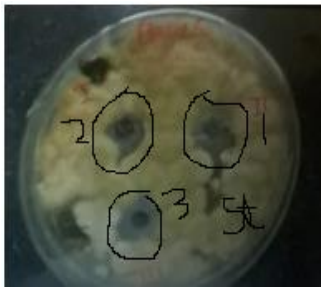


Graph 1: Standard of andrographolide prepared by HPLC method



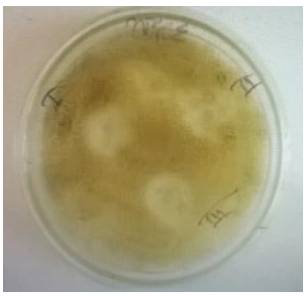
Graph 3: Andrographolide peak of *Andrographis paniculata* by HPLC method

Andrographis paniculata			
Peak Name	Retention Time	Peak area	% of andrographolide
Andrographolide	8.014	5359.873	1.07

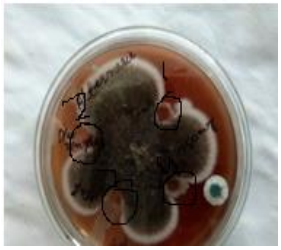


Aspergillus fumigatus

Rhizoctonia solani



Aspergillus flavus



Alternaria alternata

Fusarium oxysporum

Rhizopus stolonifer

I Extract in methanol at 75µl 2, extract in hexane solvent, 4 Solvent as negative control

Petriplates 1: Petriplates shows antifungal activity of leaf extract of *Andrographis paniculata* in methanol and Hexane solvent system

DISCUSSION

Highest zone of inhibition was shown by *Fusarium oxysporum* (17.24mm), Followed by *Aspergillus fumigatus* (15.98mm) *A.niger* (16.62mm), *Alternaria alternata* (9.02mm) in methanolic solvent and no zone of inhibition was found in *Rhizopus nigricans*. In Hexane extract of *Andrographis paniculata*, zone of inhibition was (13.11mm) in *Aspergillus fumigatus*, while in *Alternaria alternata*, zone of inhibition was (9.28mm) and no zone of inhibition in *Rhizopus nigricans***.** it was concluded that the observed antimicrobial activity was done to other active principle present in the extracts used in [14]. *Andrographis paniculata* extracted components like neoandrographolide [8], andrographolide [5] and andrograpanin [6] that are reported to have medicinal usages. Antifungal activity of *Andrographis paniculata* against *Aspergillus flavus* and *Aspergillus fumigatus* were also reported by Gurupriya and Cathrine 2016[4] .

CONCLUSION

Andrographis panuculata shows best inhibition against some fungal pathogens isolated from solanaceous vegetables and the solvent system to be used were methanol and hexane. *Andrographis paniculata* shoot extract after analysis by HPLC shows the presence of antifungal compound andrographolide**.**

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