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Antibacterial and antifungal activity of *Andrographis echiodes*

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Abstract

Objective: To investigate the antibacterial and antifungal activity of chloroform, acetone, methanol and aqueous extracts of whole plant *Andrographis echiodes.* Materials and methods: Antimicrobial activity was studied at different concentrations of the extract dissolved in dimethyl sulfoxide against seven strains of bacteria, three strains of fungi and one yeast by disc diffusion method. The minimum inhibitory concentration of the extracts were also determined. Results: All the extracts except aqueous posses antimicrobial activity against most of the organisms tested. The minimum inhibitory concentrations ranges from 0.05 mg/ml to 5 mg/ml on tested bacteria. Conclusion: The activities of *Andrographis echiodes* confirms with its folk use as remedy for fever. In addition to this use it may also be used topically for treatment of bacterial and fungal infections.

Key words: *Andrographis echiodes,* Antibacterial and antifungal activity, bacteria. fungi, yeast.

1. Introduction

Andrographis echiodes Nees (Acanthaceae) is an erect, hairy, annual herb up to 6cm in height, with oblong or subelliptic obtuse leaves and numerous flowers [1]. The plant is widely distributed in the dry districts of tropical India and Sri Lanka [2]. It was reported to be used as a remedy for fever [3]. The whole plant collected from Madras, found to contain echioidinin (0.017%) and echiodin [4]. In the present study the whole plant extracts were investigated for antibacterial and antifungal activities.

2. Materials and methods

2.1. Plant materials

The whole plant materials of *Andrographis echiodes* (Acanthaceae), as authenticated by Dr.V.Chelladurai, Survey of Medicinal Plants Unit (CCRAS), Palayamkottai and Dr. R. Sampath Kumar, Professor of Botany, Annamalai University, were collected from different localities of Sankaran Kovil, Tamilnadu. A voucher specimen of the plant has been deposited in the Department of Pharmacy, Annamalai University, India.

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2.2 Preparation of extract

75 g of sun dried plant material coarsely powdered, was successively extracted with 1 lt of n-hexane (40-60°C) , petroleum ether (35-45°C), chloroform (60-70°C), acetone (50-60°C), methanol (70-80°C) and water (100°C) for 6 h in a soxhlet apparatus [5]. The extracts were dried under vacuum. The dry drug extracts were dissolved in dimethyl sulfoxide (DMSO) and tested for antibacterial and antifungal activities.

2.3 Microorganisms

The following bacteria obtained from National Chemical Laboratory, Pune, India, were used to study antibacterial activity of the extracts: *Staphylococcus aureus* (NCIM 2079), *Bacillus subtilis* (NCIM 2063), *Micrococcus luteus* (NCIM 2103), *Klebsiella pneumonia* (NCIM 2957), *Escherichia coli* (NCIM 2065), *Pseudomonas aureginosa* (NCIM 2036), *Bacillus pumilus* (NCIM 2327).

The fungal species obtained from Department of Medical Microbiology, Raja Muthiah Medical College Hospital, Annamalai University, were used and they include one yeast *Candida albicans* and three moulds *Aspergillus niger*, *Microsporum gypseum* and *Trichophyton tonsurans*. The bacterial strains were grown and maintained on nutrient agar slants while yeast and fungi on yeast nitrogen base and sabouraud dextrose agar media respectively. The inoculated agar slants were incubated at 37°C for bacteria and 30°C for yeast and fungi.

2.4 Evaluation of antibacterial activity

2.4.1 Disc diffusion method [6]

Plates were prepared by pouring 10ml of sterile nutrient agar (Himedia) into sterile petridishes and were inoculated with a loopful broth culture of each organism. Sterile filter paper discs (whatman no.3, 6 mm diameter) impregnated with 10 µl quantity of dimethyl sulfoxide solution of extract were air dried and placed on the agar plates. The plates were incubated at 37°C for 24 h. Control studies with erythromycin 100 µg / ml and the solvent (DMSO) were done concurrently.

2.4.2 Minimum inhibitory concentration determination

An appropriate amount of each drug extract was aseptically mixed with the sterile medium (Mueller - Hinton) to get final concentrations of 10.0 - 0.05 mg/ml and poured into sterile petridishes. Plates (containing various concentration of the drug extracts) were inoculated with a loop of each bacterial culture and incubated at 37°C for 24 h. The solvent (DMSO) was used as control. The minimum inhibitory concentration was defined as the lowest concentration of extract that did not show any growth of the tested microorganism.

2.5 Antifungal activity

2.5.1 Disc diffusion method

Sterile yeast nitrogen base (Himedia) with 2% agar was inoculated by a rotating swab (soaked in standard inoculum suspension) over the surface of the media. Extract impregnated discs at the concentration of 100.0 –1.0 mg/ml were placed on the agar and incubated at 37°C for 18 h. The solvent (DMSO) was used as control. Presence of clear zone of inhibition is indicative of antifungal activities.

2.5.2 Minimum inhibitory concentration

Using serial dilution technique [7] various concentration of plant extracts in sabouraud dextrose broth were prepared. 50 µl of the standard fungal broth cultures were added to each tube and incubated at 25°C for 7 days. A positive control containing each organism with sterile medium was maintained. The minimum inhibitory concentration was regarded as the lowest concentration of extract that did not show visible growth.

Table 1
Antibacterial efficacy of various solvent extracts of *Andrographis echiodes*, compared to the reference standard Erythromycin (100µg/ml).

Sl. No.	Extracts	zone of inhibition (mm)*						
		Staphylococcus aureus	***Bacillus subtilis***	***Bacillus pumilus***	***Micrococcus luteus***	***Klebsiella pneumonia***	***Escherichia coli***	***Pseudomonas auregnosa***
1.	Chloroform extract (100 mg/m1)	8 ± 0.57	8 ± 1	7 ± 1.5	9 ± 0.5	9 ± 1.5	-	9 ± 1
	Erythromycin	12 ± 0.5	22 ± 0.57	15 ± 0	11 ± 1.5	19 ± 0.5	19 ± 0	17 ± 1
2.	Acetone extract (100 mg/ml)	-	9 ± 1	9 ± 0.5	15 ± 1	9 ± 0	9 ± 1	10 ± 0.5
	Erythromycin	12 ± 1	24 ± 0.5	19 ± 0.5	20 ± 0.5	20 ± 1.5	19 ± 2	15 ± 0.5
3.	Methanol extract (100 mg/m1)	-	7 ± 1	-	10 ± 1	8 ± 1	-	-
	Erythromycin	15 ± 1	24 ± 0.5	20 ± 1.5	25 ± 0.5	20 ± 1	19 ± 0	18 ± 1
4.	Water extract (100 mg/m1)	-	-	20 ± 0.5	-	-	-	-
	Erythromycin	15 ± 0.5	17 ± 1	20 ± 0.5	25 ± 0.5	20 ± 0.5	19 ± 0.5	20 ± 1.5

* Zones are mean ± SD for N = 3 ; - : No zone of inhibition

Table 2
Antifungal efficacy of various solvent extracts of *Andrographis echiodes,* compared to the reference standard Clotrimazole (100µg/disc).

Sl. No.	Extracts	zone of inhibition (mm)*			
		Candida albicans	***Microsporum gypseum***	***Trichophyton tonusurans***	***Aspergillus niger***
1	Chloroform extract (10 mg/m1)	10 ± 0.5	-	-	-
	Clotrimazole	16 ± 0.57	15 ± 1	15 ± 0	17 ± 0.5
2	Acetone extract (10 mg/ml)	8 ± 1	-	-	-
	Clotrimazole	20 ± 1.5	17 ± 0.5	18± 0.57	16 ± 0
3	Methanol extract (10 mg/m1)	7 ± 0	-	-	-
	Clotrimazole	15 ± 0.5	17 ± 1.5	18 ± 0.57	16 ± 0
4	Water extract (10 mg/m1)	-	-	-	-
	Clotrimazole	18 ± 0.5	15 ± 0.5	19 ± 1	20 ± 0.5

* Zones are mean ± SD for N = 3; - : No zone of inhibition

3. Results and discussion

Chloroform, acetone, methanol and aqueous extracts of *Andrographis echiodes* were tested for antibacterial and antifungal activities. The zones of inhibition with reference to a standard drug erythromycin / clotrimazole are presented in Table 1 and Table 2 for bacteria and fungi respectively. The minimum inhibitory concentration of extracts and positive control are given in Table 3.

Table 3
Minimum inhibitory concentration value of *Andrographis echiodes* for various microorganism

Test Organism	Chloroform extract mg/ml	Acetone extract mg/ml	Methanol extract mg/ml	Water extract mg/ml	Erythromycin (µg/ml)
Bacteria					
Staphylococcus aureus	1	5	5	-	0.2
Bacillus subtilis	0.1	0.1	1	-	0.3
Bacillus pumilus	0.07	1	1	-	0.3
Micrococcus luteus	1	1	0.05	-	-
Klebsiella pneumonia	0.05	0.1	0.1	-	0.2
Escherichia coli	0.07	0.7	-	-	0.3
Fungi					not tested
Microsporum gypseum	12.5	50	12.5	-	
Trichophyton tonusurans	6.25	12.5	25	25	

- : No activity

The analysis of the study showed that all the extracts tested except aqueous, possess antibacterial activity against 50% or more of the organisms tested. The minimum inhibitory concentration of the extracts ranges from 0.05 mg/ml to 5 mg/ml on tested bacteria. The tested plant extracts except aqueous one showed antifungal activities against *Candida albicans* only out of four fungi used.

The acetone extract showed better activity against tested bacteria and fungi than the chloroform, methanol and aqueous extracts. The acetone extract showed activity even against gram negative bacteria, *Escherichia coli*, while other extracts showed activity only against gram positive bacteria.

Presence of flavonoids in the extracts as reported earlier [8] are likely responsible for antibacterial and antifungal activity. The activities of *Andrographis echiodes* confirms with its reported folk use an remedy for fever.

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