COMPARATIVE ANTIMICROBIAL EFFICACY OF ETHYL ACETATE AND ETHANOL LEAF EXTRACTS OF ARISTOLOCHIA BRACATEATA RETZ

NAGARAJAN, K.1, DUTTA, S.1, SINGHAL, S.1, RUCKMANI, K.2, VALARMATHI, R.3, UMADEVI, G.3

1Division of Bio-Medicinal Chemistry R and D Laboratory, Department of Pharmacy, IIMT College of Medical Sciences, ‘O’ Pocket, Ganga Nagar, Mawana Road, Meerut 250001, 2Division of Pharmaceutics Research Laboratory, Anna University, Tiruchirapalli -620021, 3Division of Microbiology Research Laboratory, Periyar College of Pharmaceutical Sciences, Tiruchirapalli-620021. E. mail: nagarajan_mph@yahoo.co.in

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Abstract: The present study was carried out to compare the antimicrobial activities of leaf extracts of Aristolochia bracteata Retz. prepared in two different solvents. The coarse dried leaf powder of Aristolochia bracteata was successively extracted with ethyl acetate and ethanol using Soxhlet apparatus. Both the extracts were screened for their antibacterial and antifungal properties using Kirby-Bauer disc diffusion method. The microorganisms used for antibacterial and antifungal studies were Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans. Ciprofloxacin (1µg/mL) and Clotrimazole (5µg/mL) were used as reference standard. Leaf extracts prepared in both the solvents show antimicrobial activity. Further, ethyl acetate extract was found to be much effective against bacterial and fungal strains as compared to ethanol extract.

Key words: Aristolochia bracteata, Antimicrobial, Clotrimazole, Ciprofloxacin

INTRODUCTION

Aristolochia bracteata Retz. (Aristolochiaceae) is commonly called as ‘Aadutheendaapaalai’ in Tamil. It is a shrub widely distributed in India. In the indigenous system of medicine, it is reported that, the decoction of the leaves were used for treating skin diseases, rheumatism and as analgesic [2]. Aristolochia bracteata Retz. is bitter, purgative, anthelmintic; useful in “vata”, “kapha”, fevers, painful joints; applied to sores to kill maggots. The leaves are applied to the navel to move the bowels of children and are also given internally in combination with castor oil as a remedy for colic. Bruised leaf mixed with castor oil is applied externally in obstinate cases of eczema of the legs of children [3]. The plant has been used for the treatment of round worm infection [4]. The root and leaves are bitter, acrid, thermogenic, cathartic, anti-inflammatory, antiperiodic and useful in validated conditions of constipation, foul ulcers, syphilis, gonorrhoea, dyspepsia, skin disorder, eczema and intermittent fevers [5,6].

The ethyl acetate extract of the roots of Aristolochia bracteata was found to have effective antibacterial activity [7]. Antimicrobial activity of Aristolochia bracteata methanolic extract fruits was already tested and found to be effective against S. aureus [8]. The leaves of Aristolochia bracteata Retz were extracted with petroleum ether, chloroform and alcohol and has been already tested against Bacillus subtilis, Lactobacillus plantarum, Escherichia coli, Staphylococcus aureus, Streptococcus faecalis and Pseudomonas aeruginosa in which the alcoholic extract showed significant antibacterial activity as compared to that of other extracts [9].

Our objective is to assess the comparative antimic-
robial efficacy of moderately polar ethyl acetate extract with polar ethanolic leaf extract of this plant against various bacterial strains, including the opportunistic gram negative pathogen *Pseudomonas aeruginosa* as well as fungal strains also.

**MATERIALS AND METHODS**

**Plant material:** The leaves of *Aristolochia bracteata* were collected from the local areas of Tiruchirappalli. It was authenticated by the botanist and a voucher specimen was deposited to Mr. Dhiravia Das, Research Dept. of Botany, Bharathidasan University, Trichy.

**Source of microorganisms and antibiotics:** The organisms used were *Bacillus subtilis* (ATCC 55422), *Staphylococcus aureus* (ATCC 103207), *Escherichia coli* (ATCC 10412), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 10231). The organisms were obtained from the National Chemical Laboratory, Pune, and were maintained on agar slants at 12-18°C. Standard antibiotics ciprofloxacin and clotrimazole were procured from Cadilla Pharmaceuticals, Gujarat and Glenmark Pharmaceuticals, Mumbai.

**Standardization of microorganisms:** Exactly 0.2ml of overnight cultures of each organism was dispensed into 20 ml of sterile nutrient broth and incubated for 3–5 h to standardize the culture to 10^6 cfu/ml. A loopful of the standard cultures was used for the antimicrobial assay [10].

**Preparation of plant extracts:** Fresh leaves were dried under shadow at room temperature and ground into fine powder. The leaf powder (100 g) was extracted separately with 500 ml of ethyl acetate and ethanol using Soxhlet apparatus until the solvent became colorless. The extracts were filtered and evaporated to dryness by rotary vacuum evaporator (Buchi Rota vapour, Switzerland) at 30 °C. The air dried extract was stored for 48 h in sterile universal bottles at room temperature. The sterility of the extract was tested before use [9]. Both the extracts were purified by preparative TLC using 60 % ethylacetate-n-hexane as mobile phase.

**Preliminary phytochemical screening:** All the extracts were subjected to preliminary phytochemical qualitative screening for the presence or absence of various primary or secondary metabolites following standard procedures [11,12].

**Antimicrobial screening:** The antibacterial and antifungal activity was carried out by employing 24 h cultures of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. Activity of the above mentioned extracts was tested separately in vitro by Kirby-Bauer disc diffusion method [13,14]. The disc diffusion method is highly effective for rapidly growing microorganism and the activities of the test compounds are expressed by measuring the diameter of the zone of inhibition [15]. The formation of inhibition zones represents the dynamic interaction between antibiotic diffusion and bacterial growth. The method is essentially a qualitative or semi quantitative test, indicating sensitivity or resistance of microorganisms to the test materials as well as bacteriostatic or bactericidal activity of a compound [16,17].

The medium was sterilized by autoclaving at 120 °C (15 lb/in^2). About 30 ml of the medium (Nutrient Agar Medium and Sabouraud Dextrose Agar Media) with the respective strains of bacteria and fungi was transferred aseptically into each sterilized Petri plate. The Plates were left at room temperature for solidification. The extracts were tested at concentrations of 50mg/mL. The samples and the control (0.1mL) were placed in 6-mm diameter disc. Antibacterial assay plates were incubated at 37 ± 2 °C for 24 h, antifungal assay plates were incubated at 28 ± 2 °C for 48 h. For each bacterial and fungal strain, a negative solvent control was maintained without extract. Standard disc (6 mm diameter) with Ciprofloxacin (1μg/mL) was used as a positive control for antibacterial activity, whereas Clotrimazole (5μg/ml) was used as a positive control for antifungal activity. Each experiment was carried out in triplicates, and diameter of the zone of inhibition was measured. Observations and results are shown in Table 1.

**RESULTS AND DISCUSSION**

The results of the comparative antimicrobial efficacy of ethyl acetate extracts and ethanol extract of the plant part (leaves) of *Aristolochia bracteata* are expressed in table 1. Among the two different extracts tested with Gram positive *Bacillus subtilis* and *Staphylococcus aureus*, we found that the ethyl acetate extract was highly sensitive (20 mm; 28 mm) in comparison with reference standard ciprofloxacin.
In analyzing the effectiveness, the ethyl acetate extract showed more potency against *Staphylococcus aureus* when compared with standard. Meanwhile the ethanol extract was found to be moderately sensitive against the Gram positive *Bacillus subtilis* and *Staphylococcus aureus* with a zonal inhibition diameter of 16 mm and 13 mm respectively.

As far as the Gram negative organisms are concerned, the ethyl acetate extract showed better antimicrobial potency against *Escherichia coli* (22 mm) and *Pseudomonous aeruginosa* (21 mm) in comparison with standard ciprofloxacin (20 mm; 22 mm). The ethanol extract was found to be moderately effective only against the Gram negative *Escherichia coli* with a zonal inhibition diameter of 16 mm, whereas the same extract does not show any significant zonal inhibition against the opportunistic pathogen *Pseudomonous aeruginosa*.

In analyzing the antifungal efficiency of both the extracts tested, we found that the ethanol extract as well as the ethyl acetate extract are equipotent in their antifungal efficiency with a zonal inhibition diameter of 20 mm each. Both the extracts proved to be better fungicidal than the standard clotrimazole (18 mm).

When comparing the antimicrobial efficiency among the two extracts studied, we found that the ethyl acetate extract was very effective against all the bacterial and fungal strains investigated. This may be due to the presence of more bioactive constituents such as terpenoids and alkaloids in the ethyl acetate extract. The probable antimicrobial effect is due to apoptosis of the bacterial cell with the presence of diterpene aristolochic acid in the leaves of *Aristolochia bracteata* [18,19]. This opens a new vista for the upcoming herbal researchers to concentrate the plants active constituents other than the conventional highly polar solvents.

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