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ANTIMICROBIAL ACTIVITY OF JUSTICIA ADHATODA

Alka Sharma[1] and Ashwani Kumar*[2]

[1]Department of Botany University of Rajasthan, Jaipur 302004.
[2]Former Head of Department of Botany, University of Rajasthan, Jaipur 302004.

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***Corresponding Author**
Ashwani Kumar
Former Head of Department of Botany, University of Rajasthan, Jaipur 302004.

ABSTRACT

The presence of antimicrobial substances in higher plants is well established. Antimicrobial potential of leaf extract of *Justicia adhatoda* Nees. (Family Acanthaceae) alone has significant contribution. The effectiveness of a particular antimicrobial agent results in the production of growth inhibition zone appearing as clear area around the source agent. And this can be considered as a relative potential of its efficiency. During the present investigation, the antimicrobial activity of methanol and leaf extracts of *Justicia adhatoda* was tested on *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Penicillium digitatum* by the disc diffusion method (Atlas *et al.,* 1995). Antibiotic was used as positive control.

KEYWORDS: Acanthaceae, Inhibition zone, Justicia adhatoda, Staphylococcusaureus, Proteus vulgaris.

1. INTRODUCTION

The presence of antimicrobial substances in the higher plants is well established. Several plants have been reported to have antimicrobial activity, some of these include *Plumbago zeylanica* (Belachew, 1993), *Ipomea fistulosa* (Reza *et al.*, 1994), *Diospyrus mespiliferms* (Lajubutu *et al.*, 1995), *Polyalthia longifolia* var. *pandula* (Rashid *et al.*, 1996), *Tagetis minuta* (Tereschuck *et al.*, 1997), *Drosera pellata* (Didry *et al.*, 1998), *Tagetes erecta, T. patula, Cyathocline purpurea, Blumea* molis, *Ageratus conzoides and Chrysanthemum indicum* (Rai and Acharya, 2000), *Asparagus racemosus* (Mandal *et al.,* 2000), *Ocimum sanctum* (Geetha *et al.*, 2001; Shokeen *et al.,* 2005; Singh *et al.*, 2005; Junaid *et al.*, 2006; Rahman *et al.*, 2010; Mishra and Mishra, 2011 and Prasannabalaji *et al.*, 2012), *Thespesia*

populnea (Nagappa and Cheriyan, 2001), *Lawsonia innermis* (Bhuvaneswari *et al*., 2002), *Taverniera cuneifolia* (Zore *et al.,* 2003), *Cassia alata, C. fistula, and C. tora* (Phongpaichit *et al.,* 2004), *Cynodon dactylon* (Premkumar and Shyamsunder, 2004), *Datura innoxia, D. stramonium* (Eftekhar *et al.,* 2005), *Pinus densiflora* (Kim and Shin, 2005), *Moringa oleifera, Azadirachta indica, Mirabilis jalapa* and *Chromoleana oborata* (Maji *et al.,* 2005), *Gunnera perpensa, Harpephyllum lucens, Ledebouria ovatifolia caffrum, Bersama* (Buwa and van Staden, 2006), *Combretum imberbe* (Angeh *et al*., 2007), *Dracocephalum foetidum* (Lee *et al.*,2007), *Centaurea aladagensis* (Bulent Kose *et al*., 2007), *Heracleum maximum* (Webster *et al.,* 2008), *Adhatoda vasica* (Karthikeyan *et al.,* 2010; Thokchom *et al.*, 2011 and Sheeba and Mohan, 2012).

A review of literature reveals that a significant contribution has been made on antimicrobial potential of family Acanthaceae (Grine, 1979; Forero Pinto, 1980; Serifert *et al.*, 1982; Koblikova *et al.,* 1983; Ferdous *et al.,* 1990; Chiappeta and DeMello, 1994; Merurer-Grimes *et al.,* 1996 and Maji *et al*., 2005; Karthekeyan, 2010; Thokchom *et al.,* 2011 and Sheeba and Mohan, 2012). Three important folk medicinal plants of India viz., *A. zeylanica Medic, Andrographis paniculata* Nees. and *Holarrhena antidysenterica* (L.) Wall have been found very effective against *Pseudocercospora mori* and *Peridiospora mori* (Maji *et al.,* 2005).

Antimicrobial activity of leaf extract of *Adhatoda vasica* Nees. alone as well as in combination of extract of different plants has been studied by various workers. Crude leaf extract of the plant exhibited significant inhibition of bean mosaic virus (Tripathi *et al*., 1981), chloroform and ethanol extracts of leaves of the plant showed in vitro antifungal activity against systemic fungal pathogens (Malik *et al.,* 1991), aqueous and organic solvent extract of *A. vasica, Allium sativum, Azadirachta indica, Embelica officinalis, Euphorbia pilulifera, Ocimum sanctum, Solanum trilobatum* and *Withania somnifera* showed bactericidal activity against *Mycobacterium tuberculosis in vitro* (Saroja *et al.,* 1997).

Recently antimicrobial activities of *Adhatoda vasica* Nees. has been reported by Panthi and Chakraborty (2006), Sarkar *et al.* (2009), Karthikeyan *et al.* (2010), Thokchom *et al.* (2011), Josephin and Mohan (2012), Kaur *et al*. (2012) and Sheeba and Mohan (2012). It also posses antibacterial potential against *Mycobacterium tuberculosis* causing tuberculosis (Sunita and Singh, 2010; Kumar *et al.,* 2010; Victoria, 2010; Jha *et al*., 2012; and Shobana *et al.,* 2014).

The effectiveness of a particular antimicrobial agent results in the production of growth-inhibition zones that appear as clear areas surrounding the disc from which the agent diffused. The diameter of the zones can be measured and the results of such an experiment are represented as zone of inhibition in mm (Atlas *et al.,* 1995).

The main objective of this research was to evaluate the potential of *Justicia adhatoda* extracts on antibiotic resistant bacteria and fungi and their comparison to the effect of the antibiotics upon bacterial and fungal growth. During the present investigation, the antimicrobial activity of methanol and leaf extracts of *Justicia adhatoda* was tested on *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum* and *Penicillium digitatum* by the disc diffusion method (Atlas *et al.,* 1995).

MATERIALS AND METHODS

Extract and disc preparation

The leaves of *Justicia adhatoda*, after identification were collected from Galtaji, Jaipur, Rajasthan in the month of March and April.

Leaf Extract

The shade dried leaves were coarsely powdered and extracted in Soxhlet apparatus using Whatman no. 1 filter paper thimbles. The powder was extracted with methanol (10g/100 ml) for 36h. The solvent was replaced two times with fresh solvent, remaining in contact with the plant material. Separated extract was concentrated under low pressure at 40°C and evaporated to dryness and stored at 4°C in air tight bottle. Finally, the per cent yield for extract was determined.

Methanol Extract

Fifty gram of dried leaf powder was taken in a separate container. To this 250 ml of methanol was added and kept for 24 h with periodic shaking, then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of methanol. The filtrates were pooled.

After 4 hour of incubation, filter paper discs of 6 mm diameter, impregnated with different concentration (200, 150, 100 and 50 mg/ml) were placed by using forceps aseptically. One disc of known concentration was loaded in a Petri plate. Discs are arranged in approximately

centre of microbe-seeded agar plates. Positive control disc were prepared by using standard antibiotic, Streptomycin (10µ/ml). Solvent as negative control and antibiotic streptomycin (10µg/ml) as positive controls were used. The extract loaded and control loaded discs were incubated at 37°C for 24 h in an incubator. After incubation period the plates were observed and the diameters of the zone of inhibition were measured and recorded. The inhibition zones produced due to the inhibitory activity of the extract is the total inhibition zone excluding the 6 mm diameter of filter paper disc.

Source of bacterial strains used for assay

Pure bacterial cultures of *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeroginosa and Proteus vulgaris,* (all clinical isolates) were obtained from the Pathology Department, SMS Medical College, Jaipur.

Maintenance

Nutrient agar for bacterial strains was prepared by autoclaving them at 121°C at 15 lbs/sq-inch presence for 30 minutes. The medium was poured in Petri plates and allowed to solidify. Microbial culture dilution was prepared by taking a loop full of microbial culture and mixing it with distilled water, for uniform distribution of microorganism in Petri plate.

Procedure

The bacterial cultures were maintained on nutrient broth and stored at 4°C. Cultures were reactivated by streaking culture on a nutrient agar plate before every test. A loop full of broth containing microbes was picked from this broth tube, transferred to nutrient agar plate and incubated for 16-18 h at 37°C prior to the test.

RESULTS

The results of antibacterial activity of methanolic and leaf extracts of *J. adhatoda*, methanol and antibiotic are shown in Table-1.1 Maximum inhibition zone (22 mm and 24 mm) was for *S. aureus* at 200 mg/ml methanolic and leaf extracts respectively followed by *P. vulgaris* (18 mm and 20 mm), *B. subtilis* (17mm and 20 mm) and *P. aeroginosa* (16mm and 18 mm). The leaf extract has the better antibacterial activity. As the concentration of extracts was reduced the antibacterial activity was equally reduced.

Table-1.1: Antibacterial activity of *Justicia adhatoda*

Bacterial strains	Mean Zone Diameter of Inhibition (mm)						
	Methanol Extract (mg/ml)				Negative control methanol	Leaf extract 200 mg/ml	Positive control Streptomycin 10µg/ml
	0	100	150	200			
Staphylococcus aureus	3	10	15	22	0	24	30
Bacillus subtilis	2	9	13	17	0	20	28
Pseudomonas aeroginosa	1	8	12	16	0	18	25
Proteus vulgaris	2	9	14	18	0	20	27

Antifungal Activity

The present study was undertaken to evaluate the antifungal activity of methanolic and leaf extracts of *J. adhatoda*, solvent and antibiotic against four fungi namely *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Penicillium digitatum.* Methanol as negative and tetramycin (30µg/l) as positive controls were tested.

Source of fungal strains used for the assay

The test fungi were obtained from the Microbiology laboratory, Department of Botany, University of Rajasthan, Jaipur.

Maintenance

Potato Dextrose Agar Medium was used as the medium for the antifungal assay by the disc diffusion method. Spread plates were prepared with the proper concentration of inocula. For inoculation, dried spores of *Aspergillus* spp. and *Pencillium digitatum* were distributed uniformly on the surface of agar plates with the help of a sterile cotton swab. *Fusarium oxysporum* was inoculated by taking a piece of fungal colony on a sterile cotton swab and gently swabbing on the surface uniformly. A known concentration of the extract was added on each disc. Tetracycline (30µg/ml) was used as a positive control and solvent as negative.

Procedure

The stock solutions of the extracts were diluted in the range of 50-200 mg/ml. A final inoculum of 105 cell/ml for all the fungi was spouted on top of the solidified PDA medium with a loop calibrated to deliver 0.10 ml of fungal suspension. Simultaneously sterilized paper discs were prepared in different concentrations of extract solution by keeping them in solution for overnight. These paper discs were inoculated in the centre of the test fungi in Petri dishes. All the experiments were performed in triplicates.

All the inoculated plates were incubated at 30°±2°C in the growth chamber. The fungal growth was examined after 7 days. Inhibition was recorded by measuring the zone of inhibition.

RESULTS

In the present study, methanolic and leaf extracts of *J. adhatoda,* methanol as negative control and antibiotic as positive control were analyzed against *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum and Penicillium digitatum.* Antifungal activity of methanolic and leaf extracts, methanol and antibiotic are given in Table-1.2. Methanolic and leaf extract were most effective against *Fusarium oxysporum* (20 mm and 22 mm), followed by *Penicillium digitatum* (18 mm and 21 mm), *Aspergillus niger* (15 mm and 16mm) and *Aspergillus flavus* (13 mm and 14 mm) respectively. With decreasing concentration of the extract the antifungal activity also decreased. Leaves exhibited better antifungal activity.

Table 1.2: Antifungal activity of *Justicia adhatoda*

Fungal strain	Mean Zone Diameter of Inhibition Zone (mm)						
	Methanol Extract (mg/ml)				Negative control methanol	Leaf extract 200 mg/ml	Positive control tetracycline 30µg/ml
	50	100	150	200			
Aspergillus niger	7	7	13	15	0	16	24
Aspergillus flavus	8	10	12	13	0	14	22
Fusarium oxysporum	11	16	18	20	0	22	26
Penicillium digitatum	3	10	14	18	0	21	26

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