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Antimicrobial Activity of *Justicia adhatoda* L. Leaf Extracts (မုယားကြီး)

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Justicia adhatoda L. is a well-known medicinal plant and widely distributed in Myanmar. In this study, antimicrobial activity of different extracts (95% ethanol and methanol) of *Justicia adhatoda* L. leaf was studied on *Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli.* Ceftriaxone was used as positive control. Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. Ethanolic and methanolic extracts showed zone of inhibition, i.e. 10 mm for *Escheriachia coli*. Broth dilution method was used for determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf. MIC of ethanolic and methanolic extracts were observed in 200 mg/ml and MBC of both was observed in 200 mg/ml. Phytochemical analysis of *Justicia adhatoda* L. leaf was also carried out and alkaloids, glycosides, steroids, phenolic compounds, amino acids, starch, flavonoids, proteins, resins, phenols, tannin and carbohydrates were detected. The presence of phenolic compound seemed to be exert antimicrobial activity. So, this study provided referential information about the antimicrobial activity of different extracts of *Justicia adhatoda* L. leaf.

Key words: Leaves extracts

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs.[1] It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin. In recent years, pharmaceutical companies have spent a lot of time and money to develop natural products extracted from plants and to produce more cost effective remedies that are affordable to the population.[2]

Medicinal plants used traditionally produce a variety of compounds which have known therapeutic properties. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants.[3]

Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity of *J. adhatoda*. *Justicia adhatoda* L. (Family Acanthaceae) is a shrub, widespread throughout the tropical regions of Southeast Asia.[4] Moreover, it is widely used as a medicinal plant and extensively grown in American, India, Nepal

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and Pakistan. It is commonly known as Vasaka or Malabar nut. It is a perennial, evergreen and highly branched shrub (1.0 m to 2.5 m height) with unpleasant smell and bitter taste. It has opposite ascending branches with white, pink or purple flowers. It is a highly valuable Ayurvedic medicinal plant used to treat cold, cough, asthma and tuberculosis.[5] Its main action is expectorant and anti-spasmodic (bronchodilator).[6] Moreover, the importance of Vasaka plant in the treatment of respiratory disorders can be understood from the ancient Indian saying, "No man suffering from phthisis needs despair as long as the Vasaka plant exists."

Thus, the frequent use of *J. adhatoda* has resulted in its inclusion in the WHO manual "The Use of Traditional Medicine in Primary Health Care" which is intended for health workers in Southeast Asia to keep them informed of the restorative utility of their surrounding flora.[5] The major alkaloids of the plant, vasicine and vasicinone, have been found to be biologically active and are the area under discussion of many chemical compounds and pharmacological studies. The source of the drug 'Vasaka' is well-known in the indigenous system of medicine for its beneficial effects, particularly in bronchitis.[7]

The present study was aimed to determine the antimicrobial activity of different extracts of *Justicia adhatoda* L. on *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus.*

MATERIALS AND MOTHODS

Place of study

Justicia adhatoda L. leaves were collected from Mandalay Region. Identification of plant was performed at Department of Botany, University of Mandalay by using The Flora of Ceylon (Dassanayake, 1998).[8] The study was conducted at Pharmacology Research Division and Bacteriology Research Division, Department of Medical Research (Pyin Oo Lwin Branch).

Phytochemical analysis

Phytochemical analysis was also done by phytochemical techniques.[9]

Extraction of leaves

Justicia adhatoda L. leaves were collected and thoroughly washed with water and then air-dried for about two weeks. The dried leaves were powdered and extracted by percolation method.[10] The powdered leaves of 70 g were percolated with 600 ml of 95% ethanol for a week in a percolator. The liquid extract containing plant constituents was filtered and evaporated on the water bath at 50°C until to get constant weight and stored in the desiccators. Methanolic extract of *Justicia adhatoda* L. leaves was obtained by the same procedure.

Determination of antimicrobial activity of different extracts of Justicia adhatoda L. leaves

Antimicrobial activity of different extracts of *Justicia adhatoda* L. leaves was determined by agar disc diffusion technique according to modified Kirby and Bauer method.[11]

- Preparation of medium
  Muller-Hinton agar plate was prepared and sterilized by moist heat at 121°C for 15 minutes. After autoclaving, 25 ml of the media was poured into 9 cm diameter petridishes and allowed to set at room temperature. It was prepared freshly before use. When the agar had solidified, the plates were dried at 50°C by placing them with the upright position in the incubator with the lids tilted. The plates were then labeled.
- Preparation of bacterial suspension
  A few colonies of organisms from the sub-culture to be tested were picked with a wire loop and introduced into test tube containing peptone solution. These tubes were incubated at 37°C for 3-4 hours to produce the growth turbidity.
- Preparation of impregnated disc of different plant extracts
  The sterile discs, 6 mm in diameter, were spread out separately in petridishes so that

each disc was not less than 2 mm from its neighbours. They were sterilized by dry heat at 160°C for 1 hour. Ethanolic extracts of *Justicia adhatoda* L. leaf (6.25 mg, 12.5 mg, 25 mg, 50 mg, 100 mg, 200 mg etc.,) were dissolved in each of 1 ml of 95% ethanol. From the different stock solutions, 20 µl of each solution was impregnated to discs, respectively and dried in the incubator at 37°C to evaporate the solvent.

Discs for methanol extract of *Justicia adhatoda* L. leaves were done by the same procedure. Cefriaxone (30 µg) was used as positive control reference standard. Disc as negative control was prepared using the same solvent employed to dissolve the plant extract.

- Antimicrobial susceptibility test

Antimicrobial susceptibility test was determined by a standard disc diffusion technique using Muller-Hinton agar according to the recommendations of Clinical and Laboratory Standards Institute (CLSI).

A sterile cotton swab was dipped into bacterial suspension (1% turbidity of Mac Ferland tubes). Freshly grown liquid cultures of the test pathogens were seeded over the Muller-Hinton agar (MHA) plates with a sterile cotton swab. The swab was streaked in at least three directions through the angle of 60° over the surface of the Muller-Hinton agar to obtain uniform growth. A final sweep was made around the edge of the agar surface.

After the inoculum has dried for a few minutes, the sterile filter paper discs impregnated with plant extracts were placed on the seeded MHA plates at equidistance with a sterile forceps and gently pressed down to ensure contact with the medium. The plates were incubated at 37°C for 24 hours. Following overnight incubation, a zone of inhibition occurred around the discs in the plates. The inhibition zones were recorded as millimeters (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum Bactericidal Concentration (MBC) is defined as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media.

The determination of the MIC involves a semi-quantitative test procedure which gives an approximation to the least concentration of an antimicrobial needed to prevent microbial growth. MIC/MBC values can be determined by a number of standard test procedures. The most commonly used methods are the tube dilution method and agar dilution method. Serial dilutions are made of the products in bacterial growth media. The test organisms were then added to the dilution of the products, incubated and scored for growth.

Determination of MIC and MBC of Ethanolic and Methanolic extracts of Justicia adhatoda L. leaf

The ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf were proceeded for Minimum Inhibitory Concentration by broth dilution method.[12]

Different concentrations of ethanolic and methanolic extracts of *Justicia adhatoda* L. leaf ranging from 3.125 mg/ml to 200 mg/ml were tested for different test organisms. A series of ten tubes for each test organisms were prepared. Each tube contained 20 µl of test organisms in 1 ml of Muller-Hinton broth. The different dilution of 1 ml of ethanolic extract of *Justicia adhatoda* L. leaf was added to the tubes. The eighth tube was used as control tube which contained Muller-Hinton broth, 95% ethanol with test organisms. The ninth tube was used as test extract control and the tenth tube, test organisms only. Then, the different dilution of methanolic extract was

also done and incubated at 37°C for 24 hours. After incubation, MIC was recorded as tube with lowest concentration at which no visible turbidity was observed. For determination of MBC, one loopful from each tube of above dilutions was streaked on Muller-Hinton agar plate and incubated at 37°C for 24 hours.

RESULTS

The plant was identified as *Justicia adhatoda* L. belonging to the family Acanthaceae. The yield percentage of plant extracts of *Justicia adhatoda* L. leaf are shown in Table 1.

Table 1. Determination of yield of different extracts of *Justicia adhatoda* L. leaves

Solvent	Yield (%)
95% Ethanol	53.9
Methanol	63.44

Phytochemical analysis

The results of qualitative tests of leaves are shown in Table 2.

Table 2. Results of phytochemical test on *Justicia adhatoda* L. leaves

Type of compound	Results
Alkaloid	(+)
Carbohydrates	(+)
Glycosides	(+)
Phenols	(+)
Amino Acids	(+)
Saponin	(-)
Starch	(+)
Tannins	(+)
Flavonoids	(+)
Steroids	(+)
Cyanogenic substance	(-)
Proteins	(+)
Resin	(-)
Cardiac glycosides	(+)

(+) Detected, (-) Not detected

Antibacterial activity of different extracts of Justicia adhatoda L. leaves

Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. Both 95% ethanolic extract and methanolic extract showed zone of inhibition, i.e. 10 mm for *Escherichia coli.* These results are shown in Table 3.

Table 3. Antibacterial activities of different extracts of *Justicia adhatoda* L. leaves

Test organism	Diameter of inhibition zone of different extracts of studied leaves	
	95% Ethanolic	Methanolic
Escherichia coli.	10 mm	10 mm
Staphylococcus aureus	<8 mm	<8 mm
Pseudomonas aeruginosa	<8 mm	<8 mm

Table 4. MIC and MBC of ethanolic and methanolic extracts of studied leaves

Test organism	Ethanolic extract (mg/ml)		Methanolic extract (mg/ml)	
	MIC	MBC	MIC	MBC
Escherichia coli	200	200	200	200
Staphylococcus aureus	-	-	-	-
Pseudomonas aeruginosa	-	-	-	-

MIC=Minimum Inhibitory Concentration
MBC= Minimum Bactericidal Concentration

Determination of MIC and MBC of Justicia adhatoda L. leaf extracts

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of *Justicia adhatoda* L. leaf extracts were determined by broth dilution method. MIC and MBC of ethanolic extract and methanolic extracts of *Justicia adhatoda* L. leaves were 200 mg/ml for *Escheriachia coli.* These results of ethanolic and methanolic extracts are shown in Table 4.

DISCUSSION

From phytochemical investigations, it was observed that alkaloids, glycosides, steroids, cardiac glycosides, amino acids, starch, flavonoids, proteins, phenols, tannin and carbohydrates were significantly present and cyanogenetic substant was absent in the leaf. The phenolic compounds were among the most active components against gram positive and gram negative bacteria.[13] Regarding the medicinal value of *Justicia adhatoda* L. leaves, antimicrobial properties may be due to the presence of phenolic compounds.

Antimicrobial activity of *Justicia adhatoda* L. leaf extracts was determined by agar disc diffusion method. The inhibition zones ranged between 7 mm to 10 mm on

Escherichia coli, Pseudomonas aeruginosa and *Staphylococcus aureus.* MIC and MBC values of methanolic and ethanolic extracts were each of 200 mg/ml on *Escherichia coli*. The previous study showed that MIC and MBC values of methanolic extract *Justicia adhatoda* L. was 3.125 µg/ml and 6.25 µg/ml on *Escherichia coli* and did not detected on *Pseudomonas aeruginosa.*[14] This difference may be due to the disparity of extraction methods, species of strains and resistance of organisms.

Conclusion

In this study, the plant *Justicia adhatoda* L. was identified. The phytochemical analysis revealed the presence of various phytochemical constituents such as alkaloids, glycosides, steroids, phenols, amino acids, starch, flavonoids, proteins, tannin and carbohydrates. Both methanolic and ethanolic extracts of *Justicia adhatoda* L. had antibacterial activity at the dosage of 200 mg/ml on *Escherichia coli*.

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