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Research Article

IN VITRO ANTIBACTERIAL ACTIVITIES STUDY OF *ALBIZZIA LEBBECK* (L) BENTH LEAF EXTRACTS

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ABSTRACT
To know the *in vitro* antibacterial activities of benzene, chloroform, acetone, ethyl acetate, ethanol and methanol extracts of the leaves of *Albizzia lebbek*(L) Benth. (Shirish), Family: *Mimosaceae*, present study was conducted. All the extracts were used at 3mg/ml and 6mg/ml concentrations. Against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli*, antibacterial activities of the different extracts were studied. Considering the overall antibacterial activity pattern, it can be mentioned that both the chloroform and ethanol extracts possessed antibacterial activities against all the strains used. Out of these two extracts, chloroform extract was more potent than the ethanol extract as far as their antibacterial activities were concerned. The extracts, which displayed antibacterial activity, produced such activity in a dose dependent manner. For the study Tetracycline (25µg/ml) was used as positive control, while DMSO was the negative control.

KEYWORDS: Leaf extracts, Antibacterial activity, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, Tetracycline, DMSO.

INTRODUCTION

The use of plants as medicines is as old as human civilization itself. Many of the existing medicinal systems such as Ayurveda, Unani, Homeopathy, Naturopathy, Siddha and other alternative medicinal systems have been utilizing plants as effective medicines to cure many harmful diseases. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world[1].

Albizzia lebbek(L) Benth. (Shirish), Family: *Mimosaceae* is a deciduous tree with compound leaves, flat oblong fruits, round cream coloured seeds, which grows wild. The plant is found throughout India, Bangladesh, tropical and subtropical Asia and Africa. Barks are used in toothache and diseases of the gum. Decoction of the leaves and barks are protective against bronchial asthma and other allergic disorders. Barks and seeds are astringent and are given in piles and diarrhea.[2]

Till now reports on the antibacterial activity study using leaves of *Albizzia lebbek* are scanty. However, Chulet et al. (2010a), Chulet et al. (2010b) and Maji et al. (2010) mentioned the antibacterial activities of *Albizzia lebbek* leaves[2,3,4]**.** In addition, Anthonamma et al. (2010) found such activities of their seeds[1]. Considering this information, we tried to explore the antibacterial activities of the leaves of *Albizzia lebbek*.

MATERIALS AND METHODS

Pant Material –The leaves of the plant were collected from, Ring road, Rourkela, during May 2011. Authenticated sample was used for the study. The shade dried leaves were powdered and stored in a desiccator until evaporation.

Preparation of extract- The powdered leaves (40.2 gm) of *Albizzia lebbek* were successively extracted using solvents in order of increasing polarity, viz. benzene, chloroform, acetone, ethyl acetate, ethanol and methanol. After extraction, each time the marc was dried and later extracted with the next solvent. All the extracts were dried by distilling the solvents in a rotary vacuum evaporator[4]. The yield of different extracts was as follows: benzene extract 5.7gm, chloroform extract 3.47gm, acetone extract 2.0gm, ethyl acetate extract 1.7gm, ethanolic extract 2gm and methanolic extract 1.9gm. The extractive values of the extracts have been mentioned in the Table-1. All the extracts were dissolved in Dimethylsulfoxide (DMSO)[5].

Antibacterial activity study - In order to determine the *in vitro* antibacterial activity of some extracts of the leaves of *Albizzia lebbek*, the nutrient agar well diffusion method as described by Schillenger and Luke (1989) was performed. Sterile nutrient agar medium was inoculated with 0.1ml of fresh overnight nutrient broth culture of each bacterium (approx.10^7CFU/ml) and poured into sterile petriplates[7]. For our study we used bacterial suspensions of *Staphyloccus aureus, Bacillus subtilis* and *Escherichia coli*. In each plate, wells of 6mm in diameter were punched using a sterile borer and the plates were allowed to dry for 5min[7,8]. For the present study benzene, chloroform, acetone ethyl acetate, ethanol and methanol extracts of the plant were used at 3mg/ml and 6mg/ml concentrations[1,4]. Each concentration of the of benzene, chloroform, acetone ethyl acetate, ethanol and methanol extracts of *Albizzia lebbek* was, at first, dispensed separately into different wells using sterile micropipettes. In addition, DMSO (negative control) and tetracycline at a concentration of 25µg/ml (positive control) were also dispensed separately into different wells[9]. The volume of different solutions used in each well was 50 µl. After holding the plates at room temperature for 2 hours to allow diffusion of the extracts and controls into the nutrient agar medium, the plates were incubated at 37 ^{0}C for 24 hrs. After the incubation period, the plates were examined for inhibition of the bacterial growth around the wells. The diameters of the zones of inhibition in each case were measured[1,4].

RESULTS

Against, *Staphylococcus aureus,* benzene extract did not produce any zone of inhibition. Amongst other extracts used in our study, minimum zone of inhibition was found in case of ethyl acetate extract, while chloroform extract was more or less as potent as positive control. (Table-2) Against *Bacillus subtilis*, ethyl acetate and acetone extracts did not produce any zone of inhibition whereas other extracts were to some extent effective. (Table-3) Ethyl acetate, acetone and methanol extracts were totally ineffective against *Escherichia coli*. On the other hand, chloroform extract was the most

potent amongst all the extracts used against it. (Table-4) Considering the overall antibacterial activity pattern, it can be mentioned that both chloroform and ethanol extracts possessed such activities against all the strains used. Out of these two extracts, chloroform extract was more potent than the ethanol extract as far as their antibacterial activities were concerned. (Table-2, 3 and 4)

The negative control (DMSO) did not produce any zone of inhibition against all the strains used in the study. The extracts, which displayed antibacterial activity, produced such activity in a dose dependent manner. (Table-2, 3, 4)

DISCUSSION

Like Anthomma et al. (2010), we found that chloroform extract was effective against *Bacillus subtilis* and *Escherichia coli*. On the other hand, they reported that the methanol extract was also active against those strains, but we did not observe any zone of inhibition against *Escherichia coli*[1]. Like Chulet et al. (2010b), our study also indicates that ethyl acetate extract was effective against *Staphylococcus aureus*[3]. On the other hand, Maji et al. (2010) reported antibacterial activity of both acetone and benzene extracts against *Staphylococcus aureus* as well as *Escherichia coli*[4]. From our investigation, it may be mentioned that out of those two extracts, acetone extract was effective against *Staphylococcus aureus* while benzene extract was active against *Escherichia coli*. Considering our overall result, it may be concluded that chloroform extract was the most potent against all the strains used in the study.

Further work using various extracts from the different parts of the same plant is necessary to establish its exact antibacterial activities. Then only the plant could be considered as a potential source of antibacterial agent. Moreover, further studies are required to identify the actual chemical constituents that are present in the crude drug extracts of this plant which are responsible for antibacterial activity. It is, however, suggested to conduct further research on pure chemical constituents to critically evaluate their antibacterial activities.

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Table -1 Extractive values of different extracts of *Albizzia lebbek* leaves.

Extracts	Extractive values (%w/w)
Benzene extract	5.72
Chloroform extract	3.47
Acetone extract	4.98
Ethyl acetate extract	4.21
Ethanolic extract	4.98
Methanolic extract	4.71

Table -2 Antibacterial activities of the different extracts of *Albizzia lebbeck* leaves against *Staphylococcus aureus*

Sample	Concentration	Zone of Inhibition(mm)
Benzene extract	6 mg/ml	0
	3 mg/ml	0
Chloroform extract	6 mg/ml	36
	3 mg/ml	34
Acetone extract	6 mg/ml	27
	3 mg/ml	26
Ethyl acetate extract	6 mg/ml	15
	3 mg/ml	14
Ethanolic extract	6 mg/ml	25
	3 mg/ml	22
Methanolic extract	6 mg/ml	25
	3 mg/ml	18
Tetracycline	25 µg/ml	40
DMSO	-	0

Table-3 Antibacterial activities of the different extracts of *Albizzia lebbek* leaves against *Bacillus subtilis*

Sample	Concentration	Zone of Inhibition(mm)
Benzene extract	6 mg/ml	13
	3 mg/ml	15
Chloroform extract	6 mg/ml	30
	3 mg/ml	27
Acetone extract	6 mg/ml	0
	3 mg/ml	0
Ethyl acetate extract	6 mg/ml	0
	3 mg/ml	0
Ethanolic extract	6 mg/ml	24
	3 mg/ml	22
Methanolic extract	6 mg/ml	22
	3 mg/ml	18
Tetracycline	25 µg/ml	36
DMSO	-	0

Table -4 Antibacterial activities of the different extracts of *Albizzia lebbek* leaves against *Escherichia coli*

Sample	Concentration	Zone of Inhibition(mm)
Benzene extract	6 mg/ml	16
	3 mg/ml	15
Chloroform extract	6 mg/ml	34
	3 mg/ml	28
Acetone extract	6 mg/ml	0
	3 mg/ml	0
Ethyl acetate extract	6 mg/ml	0
	3 mg/ml	0
Ethanolic extract	6 mg/ml	21
	3 mg/ml	22
Methanolic extract	6 mg/ml	0
	3 mg/ml	0
Tetracycline	25 µg/ml	30
DMSO	-	0

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