

Antibacterial Efficacy of Methanolic Leaf Extracts of Some Trees Against some Common Pathogenic Bacteria.

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Abstract - Majority of the world population cannot afford the allopathic drugs and have to depend upon the use of traditional medicines. Plants have variety and huge source of phytochemicals with proven potential of treating communicable infection with lesser side effects compared to the chemotherapeutic agents. The main aim of the present study was to evaluate and determine an *in-vitro* antibacterial efficacy of methanolic leaf extracts of some trees: *Albizia lebbbeck*, *Dillenia pentagyna*, *Kigelia pinnata*, *Parkia biglandulosa*, *Peltophorum pterocarpum* and *Pterospermum acerifolium* against some common pathogenic bacteria by agar-well diffusion method. Antibacterial potential of leaf extract was determined by measuring the zone of inhibition. It was concluded from the results that methanolic leaf extracts of trees were significant antibacterial efficacy. Therefore, the leaf extracts of this plant can be selected for further investigation to determine their therapeutic potential.

Keywords - antibacterial efficacy, leaf extract, agar well diffusion, zone of inhibition.

I. INTRODUCTION

There are so many evidence from the different countries around the world indicates an overall decrease in the total stock of antibiotic effectiveness: resistance to all the first line of drugs and last resort antibiotics is increasing. The patterns of which pathogenic bacteria are resistant to specific antibiotics differ region to region and country to country [1]. It is estimated that 58,000 neonatal sepsis deaths are attribute to drug resistant infections in India [2]. According to World Health Organization (WHO, 2014) *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* are most recent worldwide estimated global antibiotic resistance, which are of the greatest concern, associated with both hospital and community acquired infections.

There is a continuous increase in antibiotic resistance & emerging threats globally [3]. This situation forced scientists to search for new alternative antimicrobial substances. Majority of the world population cannot afford the allopathic drugs and have to depend upon the use of traditional medicines. More than 30% of the entire plant species were used for medicinal purposes [4]. Plants have variety and huge source of phytochemicals with proven potential of treating communicable infection with lesser side effects compared to the chemotherapeutic agents. Recently much more emphasis has been given to biologically active compounds from plants used in the alternative medicine [5]. The main aim of the present study was to evaluate and determine the antibacterial efficacy of leaf extracts of some trees: *Albizia lebbbeck*, *Dillenia pentagyna*, *Kigelia pinnata*, *Parkia biglandulosa*, *Peltophorum pterocarpum* and *Pterospermum acerifolium* against some common pathogenic bacteria.

II. MATERIALS AND METHODS

A. *Preparation of leaf extracts:*

Fresh and diseased-free leaves of *Albizia lebbbeck*, *Dillenia pentagyna*, *Kigelia pinnata*, *Parkia biglandulosa*, *Peltophorum pterocarpum* and *Pterospermum acerifolium* were collected from local area in and around Vyara, Gujarat. The leaves of these plants were washed thoroughly under running tap water and then dry at in an oven at 55 ± 2 °C for 24 hours. The dried plant material was pulverized to fine powder, stored in air tight bottle. A 5 gm dried powder was soaked in 50 ml of methanol in flask. The flasks were covered with aluminium foil and allowed to stand in a dark for 72 hrs for extraction. These extracts were filtered through Whatmann filter paper no. 1 and filtrate was evaporated at 55 ± 2 °C in an oven to get dark greenish residue (crude extract), which was stored at 4°C prior to use [6], [7]. These crude extract was further dissolved in DMSO to prepare the stock solution of 100 mg/ml.

B. *Sources of pathogenic bacteria*

The antibacterial activity of crude methanolic extracts of some trees was tested against common pathogenic bacteria i.e. *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These pathogenic bacteria were isolated from urine & pus sample of a patient suffering from urinary tract infection & wound infection by standard microbiological procedure and identified biochemically [8], [9].

C. *Evaluation of antibacterial efficacy*

Evaluation of antibacterial efficacy of crude methanolic extracts of tree plants were tested by agar well diffusion method [10]. A nutrient agar plate was seeded with 100 µl suspension of pathogenic bacteria (standard inoculums), spread uniformly over a plate by spread plate technique. After 10-15 minute a sterile stainless steel borer 8 mm in diameter was used to make a well in each plate. These wells were filled with 20 µl of respective methanolic crude extract. After prediffusion, plates were incubated at 37 ± 1 °C for 24 hours. After overnight incubation results were recorded by measuring the diameter of zone of inhibition in mm.

MIC of the crude methanolic extracts of tree plants was also determined against three most important common pathogenic bacteria i.e. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* by two fold dilution followed by well diffusion method [11]. The stock solution of 100 mg/ml of methanolic crude extract of tested trees which showed antibacterial potential were diluted two fold and 20 µl of respective diluents were transferred into well in a previously seeded N. agar plates with standard inoculums of three most important common pathogenic bacteria. Then plates were kept for prediffusion & incubated at 37 ± 1 °C for 24 hours. After overnight incubation diameter of zone of inhibition were measured in mm.

III. RESULTS AND DISCUSSION

All the methanolic crude extract of tree showed good antibacterial activity against tested pathogenic bacteria isolated from clinical specimens, which are shown in the Table 1 and Figure 2. Methanolic leaf extract of *Peltophorum pterocarpum* showed significant antibacterial activity against all the pathogenic bacteria except *E. coli* and *Enterobacter aerogenes*. Methanolic leaf extract of *Dillenia pentagyna*, *Peltophorum pterocarpum* and *Pterospermum acerifolium* showed significant antibacterial activity against *Pseudomonas aeruginosa*.

Figure 1. N. agar plates showed the antibacterial activity of methanolic leaf extract of trees against *Pseudomonas aeruginosa* - PA and *Klebseilla pneumonia* - KP.

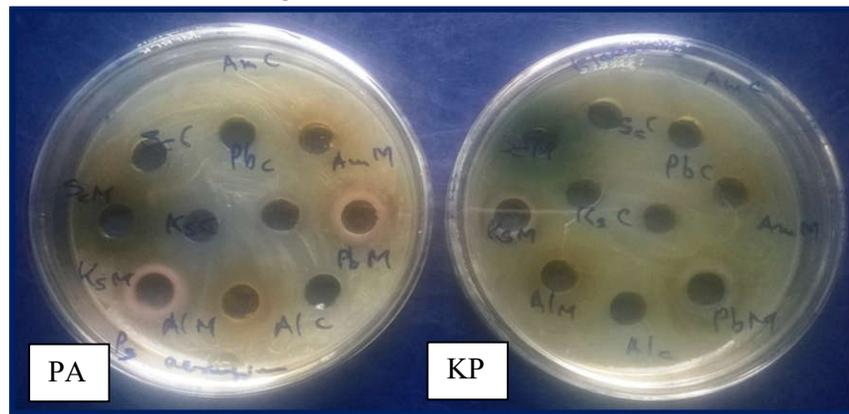


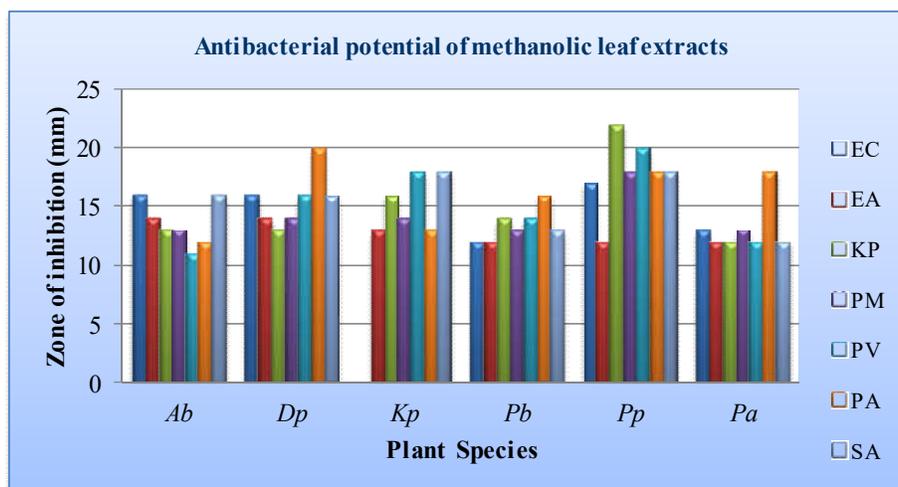
Table 1. Antimicrobial activity of methanolic leaf extracts of trees.

Sr. No	Trees	Zone of inhibition (mm)						
		E C	EA	KP	PM	PV	PA	SA
1	<i>Albizia lebbek</i>	16	14	13	13	11	12	16
2	<i>Dillenia pentagyna</i>	16	14	13	14	16	20	16
3	<i>Kigelia pinnata</i>	--	13	16	14	18	13	18
4	<i>Parkia biglandulosa</i>	12	12	14	13	14	16	13
5	<i>Peltophorum pterocarpum</i>	17	12	22	18	20	18	18
6	<i>Pterospermum acerifolium</i>	13	12	12	13	12	18	12
7	DMSO - Negative control	--	--	--	--	--	--	--

Note: EC : *E. coli*, EA : *Enterobacter aerogenes*, KP : *Klebseilla pneumoniae*, PM : *Proteus mirabilis*, PV : *Proteus vulgaris*, PA : *Pseudomonas aeruginosa*, SA : *Staphylococcus aureus*.

Note: 8 mm well was loaded with 20 µl of crude extract (100mg/ml).

Figure 2. Antibacterial activity of methanolic leaf extract of trees against common pathogenic bacteria.



Note: Al : *Albizia lebbeck*, Dp : *Dillenia pentagyna*, Kp : *Kigelia pinnata*, Pb : *Parkia biglandulosa*, Pp : *Peltophorum pterocarpum* and Pa : *Pterospermum acerifolium*

MIC of methanolic leaf extracts of plants against three most important common pathogenic bacteria i.e. *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* are given in **Table 2**. MIC of methanolic leaf extract of *Dillenia pentagyna*, *Peltophorum pterocarpum* and *Pterospermum acerifolium* showed significant antibacterial activity that is 6.25 and 12.5 mg/ml against all three common pathogenic bacteria i.e. *E. coli*, *Staph. aureus* and *P. aeruginosa*.

Figure 3. MIC of the methanolic leaf extract of DP (Khalio Saag – Ks) and PP with significant result against *Pseudomonas aeruginosa*.

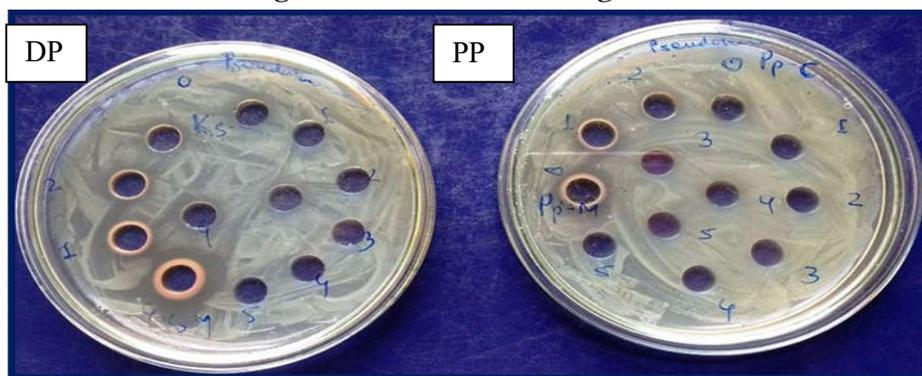


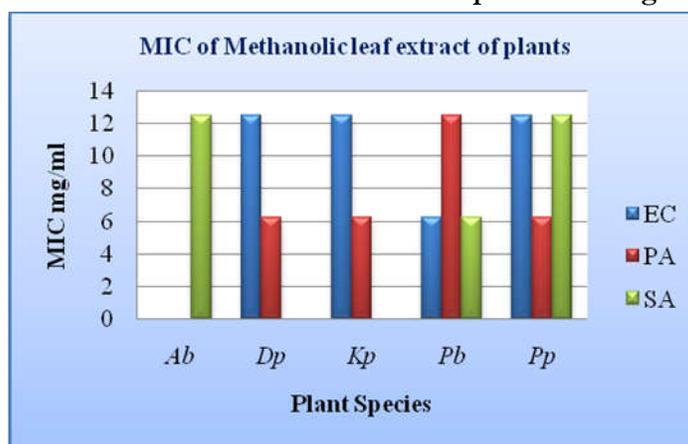
Table 2. MIC of methanolic leaf extracts of plants.

Sr. No.	Plants	MIC mg/ml		
		EC	PA	SA
1	<i>Albizia lebbeck</i>	50	25	12.5
2	<i>Dillenia pentagyna</i>	12.5	6.25	6.25
3	<i>Kigelia pinnata</i>	---	25	100
4	<i>Parkia biglandulosa</i>	6.25	12.5	6.25
5	<i>P. pterocarpum</i>	12.5	6.25	12.5
6	<i>P. acerifolium</i>	25	25	100

Note: EC : *Escherichia coli*, PA : *Pseudomonas aeruginosa*, SA : *Staph. aureus*

Note: 8 mm well was loaded with 20 µl of respective dilution of crude extracts.

Figure 3. MIC of the methanolic leaf extract of plants with significant result.



Note: Al : *Albizia lebbbeck*, Dp : *Dillenia pentagyna*, Kp : *Kigelia pinnata*, Pb : *Parkia biglandulosa* and Pp : *Peltophorum pterocarpum*

Note: EC : *Escherichia coli*, PA : *Pseudomonas aeruginosa*, SA : *Staph. aureus*

IV. CONCLUSIONS

It is concluded from the results that all the methanolic leaf extract of tree showed very good antibacterial activity against common pathogenic bacteria. It is clear evidence that these methanolic extracts of tree have potential as an antibacterial compound against pathogenic bacteria and they can be further evaluated for bioactive natural product. This investigation would help to formulate a new, cheaper and alternative antibacterial drug.

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