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# Sensitivity of Two Acacia Species on Some Resistant Isolates

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## ABSTRACT:

Almighty God already planned to create the plants before human being in this world. Human has been depending totally on plants till their ends. In India about 17000 plants have good medicinal value. 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 aim of the present investigation was to evaluate and determine the sensitivity of crude extracts of two acacia species - *Acacia auriculiformis* and *Acacia mangium* against some clinical isolates by agar-well diffusion method. Antibacterial potential of crude extract of leaves was determined by measuring the zone of inhibition. It was concluded from the results that methanolic leaf extracts of both the acacia species were significant antibacterial activity. Therefore, the leaf extracts of these plant has very good sensitivity against clinical isolates and can be selected for further investigation to determine their pharmacological and therapeutic potential.

**KEYWORDS:** traditional medicines, crude extracts, agar well diffusion, zone of inhibition.

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## INTRODUCTION

Almighty God already planned to create the plants before human being in this world. Human has been depending totally on plants till their ends. In India about 17000 plants have good medicinal value. However, approximate 8000 species are used for their medicinal values in traditional systems of medicines. Natural products have been an integral part of the ancient traditional medicine systems, e.g. Chinese, Ayurveda and Egyptian.[1] About 3.4 billion people in the developing world depend on plant based traditional medicines. This represents about 88 % of the world's inhabitants, who rely mainly on traditional medicine for their primary health care. Majority of the world population cannot afford the allopathic drugs and have to depend upon the use of traditional medicines. Antimicrobial properties of many plants have been investigated extensively.[2] More than 30% of the entire plant species were used for medicinal purposes.[3]

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.[4] *Escherichia coli, Klebseilla 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.[5]

Plants have enormous and huge variety of chemical substances with proven potential of treating infectious diseases with lesser side effects compared to the allopathic drugs. Recently much more emphasis has been given to biologically active compounds from plants used in the alternative medicine.[6] Increasing in antibiotic resistance & emerging the new threats throughout the world forced the scientists to search for new alternative cheap, safe and green drugs. The aim of the present investigation was to evaluate and determine the sensitivity of crude extracts of two acacia species - *Acacia auriculiformis* and *Acacia mangium* against some clinical isolates by agar-well diffusion method.

## MATERIALS AND METHODS

### *Preparation of leaf extracts*

Fresh and diseased-free leaves (Phyllodes) of *Acacia auriculiformis* and *Acacia mangium* 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 $\pm$ 2 $^{o}$C for 24 hours. The dried plant material was pulverized to fine powder in a grinder, and then stored in an air tight bottle. A 5 gm of each dried powder was soaked in 50 ml of chloroform and methanol in flask. The flasks were covered with aluminum foil and allowed to stand in a dark for 72 hrs for extraction. These extracts were filtered through Whatmann filter paper no. 1 in a preweighted test tubes and filtrate was evaporated at 55 $\pm$ 2 $^{o}$C in an oven to get dark greenish residue ( crude extract ), which was stored at

4°C prior to use.[7, 8] These crude extract was further dissolved in DMSO to prepare the stock solution of 100 mg/ml.

### *Sources of clinical isolates*

Sensitivity of crude extract of leaves of two Acacia species were tested against some clinical isolates i.e. *Escherichia coli, Enterobacter aerogenes, Klebseilla pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa and Staphylococcus aureus.* These common pathogens were isolated from urine and pus sample of a patient suffering from urinary tract infection & wound infection by standard microbiological procedure & identified biochemically.[9, 10] Antibiotic sensitivity testing of clinical isolates was carried out by WHO recommended Kirby-Bauer, NCCLS modified disc diffusion technique. More than thirteen antibiotics (1st line, 2nd line & 3rd line) were tested. Antibiogram were prepared.

### *Sensitivity of leaf extracts*

Sensitivity of leaf extract (chloroform and methanol) of *Acacia auriculiformis* and *Acacia mangium* were evaluated by agar well diffusion method.[11] A nutrient agar plate was inoculated with 100 µl of standard inoculums of clinical isolate, spread uniformly over a plate by spread plate technique. After 10 - 15 minute, wells were prepared in each plate by a sterile stainless steel borer (8 mm in diameter). These wells were inoculated with 20 µl of respective chloroform and methanol extract. N. agar plates kept at 2 - 8˚ C for 15 - 30 minutes. After prediffusion plates were incubated at 37 $\pm$ 1˚ C for 24 hours. After overnight incubation results were recorded by measuring the diameter of zone of inhibition in mm.

Minimum inhibitory concentration of the crude extracts of *Acacia auriculiformis* and *Acacia mangium* were also determined against three most important resistant isolates i.e. *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* by two fold dilution of crude extract followed by well diffusion method.[12] The stock solution of 100 mg/ml of crude extract 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 these resistant isolates. Then plates were kept for prediffusion & incubated at 37 $\pm$ 1˚ C for 24 hours. After overnight incubation diameter of zone of inhibition was measured in mm. MIC is defined as – the lowest concentration of crude extracts that will prevent the growth of tested clinical isolates.

Total activity (TA) was also determined. TA is the volume or amount at which the tested crude extract can be diluted without losing their antimicrobial activity. The quantity of material extracted from one gram of dried plant material is divided by the minimal inhibitory concentration value to give the total activity of the plant. It is calculated by dividing the amount of extract from 1 gm of plant material by the MIC of the same extract and is expressed in ml/gm.[13]

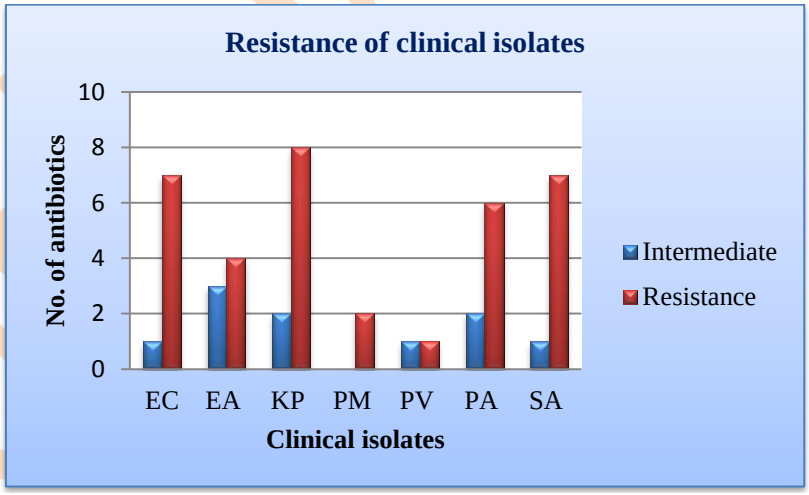
## RESULTS AND DISCUSSION

There were 21 urine specimens and 3 pus samples were examined microbiologically for clinical isolates. On the basis of Gram staining, colony morphology on selective media, hemolytic pattern on Blood agar and result of various biochemical tests, seven clinical isolates were identified & confirmed. Antimicrobial susceptibility test were performed with more than twelve antibiotics were studied against seven clinical isolates. Antibiogram of all the isolates were tabulated with the reference range of antibiotics given in Performance standards of Antimicrobial Disc Susceptibility Tests.[14] Clinical isolates & their resistance to the total no. of antibiotic is showed in the **Table 1 and Figure 1.**

**Table 1. Clinical isolates & their resistance to antibiotics.**

| Sr. No. | Clinical isolates | Resistant to |
|---|---|---|
| 1 | *Escherichia coli* | Ampicillin, Cefixime, Chloramphenicol, Ciprofloxacin, Doxycycline, Norfloxacin & Tetracycline. |
| 2 | *Enterobacter aerogenes* | Ampicillin, Cefixime, Doxycycline & Tetracycline. |
| 3 | *Klebseilla pneumoniae* | Amoxyclave, Ampicillin, Cefixime, Ciprofloxacin, Cotrimexazol, Doripenem, Nalidixic Acid, Norfloxacin. |
| 4 | *Proteus mirabilis* | Doxycycline & Tetracycline. |
| 5 | *Proteus vulgaris* | Doxycycline. |
| 6 | *Pseudomonas aeruginosa* | Amoxyclave, Ampicillin, Cefixime, Doxycycline, Nalidixic Acid & Tetracycline. |
| 7 | *Staphylococcus aureus* | Amoxyclave, Ampicillin, Ciprofloxacin, Nalidixic Acid, Norfloxacin, Azithromycin, Erythromycin & Penicillin. |

**Figure 1. Resistance of Clinical isolates.**




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

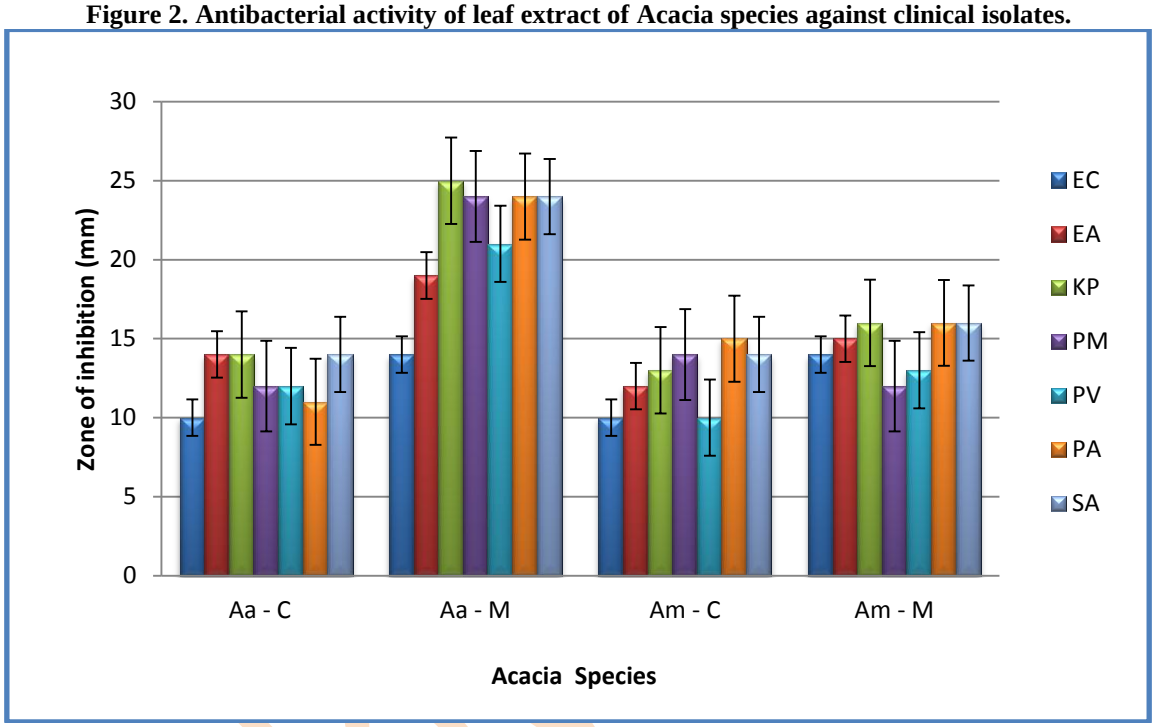
Chloroform and methanol extract of both the Acacia species were effective against all the clinical isolates. Methanol extract of *Acacia auriculiformis* showed very good sensitivity against all the clinical isolates compare to *Acacia mangium.* Antimicrobial activity of leaf extracts of two Acacia species are shown in the **Table 2 and Figure 2.**

**Table 2. Antimicrobial activity of leaf extracts of Acacia species.**

| Sr. No. | Acacia species | Extract | Zone of inhibition (mm) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | EC | EA | KP | PM | PV | PA | SA |
| 1 | *Acacia auriculiformis* | Chloroform | 10 | 14 | 14 | 12 | 12 | 11 | 14 |
| | | Methanol | 14 | 19 | 25 | 24 | 21 | 24 | 24 |
| 2 | *Acacia mangium* | Chloroform | 10 | 12 | 13 | 14 | 10 | 15 | 14 |
| | | Methanol | 14 | 15 | 16 | 12 | 13 | 16 | 16 |

**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).

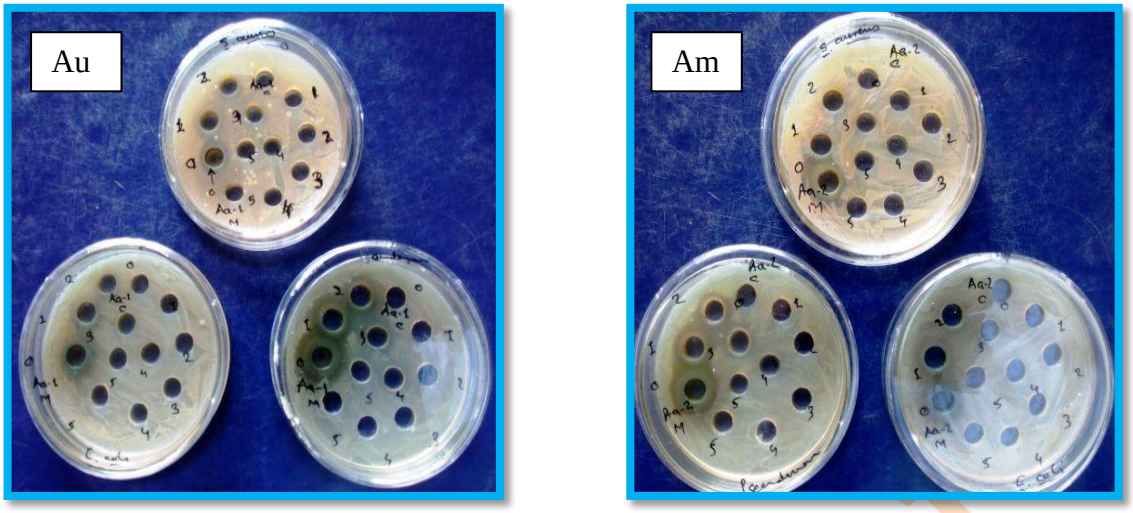
**Figure 2. Antibacterial activity of leaf extract of Acacia species against clinical isolates.**




**Note:** Aa : *Acacia auriculiformis*, Am : *Acacia mangium*, C : Chloroform extract, M : Methanol extract
**Note:** EC : *E. coli*, EA : *Enterobacter aerogenes*, KP : *Klebseilla pneumoniae*, PM : *Proteus mirabilis*, PV : *Proteus vulgaris*, PA : *Pseudomonas aeruginosa*, SA : *Staphylococcus aureus.*

MIC of chloroform and methanol extracts of both the Acacia species against three most important common pathogenic bacteria & resistant isolates i.e. *Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa* are given in the **Figure 3 and Table 3.**

**Figure 3. MIC of the leaf extract of Acacia spp. against resistant isolates.**




**Note:** In all Plates for MIC determination, two fold dilution of crude extract; **0** – 100 mg/ml, **1** – 50 mg/ml, **2** – 25 mg/ml, **3** – 12.5 mg/ml, **4** – 6.25 mg/ml and **5** – DMSO. **Au :** *Acacia auriculiformis* **Am :** *Acacia mangium*
**Note:** 8 mm well was loaded with 20 µl of respective dilution of crude extracts.

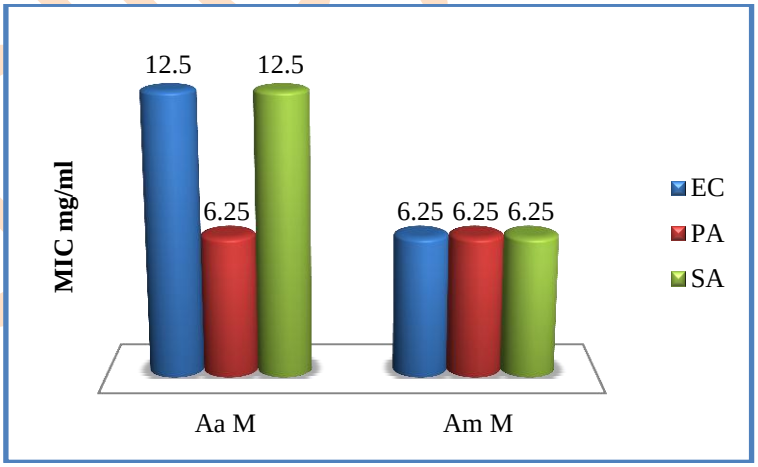
**Table 3. MIC of leaf extracts of Acacia species.**

| Sr. No. | Plants | Extract | MIC mg/ml | | |
|---|---|---|---|---|---|
| | | | EC | PA | SA |
| 1 | *Acacia auriculiformis* | Chloroform | 100 | 100 | 100 |
| | | Methanol | 12.5 | 6.25 | 12.5 |
| 2 | *Acacia mangium* | Chloroform | 100 | 50 | 100 |
| | | Methanol | 6.25 | 6.25 | 6.25 |

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

MIC of methanolic extract of both the Acacia species showed significant antibacterial activity against all three resistant isolates, which is shown in the **Figure 4.**

**Figure 4. MIC of the methanolic leaf extract of Acacia species against resistant isolates.**




**Note:** Aa : *Acacia auriculiformis*, Am : *Acacia mangium*, M : Methanol extract
**Note:** EC : *Escherichia coli*, PA : *Pseudomonas aeruginosa*, SA : *Staph. aureus*

Total activity indicates the largest volume to which the biologically active compounds in one gram can be diluted and still inhibit the growth of bacteria. Result revealed that methanol extract of

both the Acacia showed high total activity. Total activity of leaf extracts of Acacia spp. shown in **Table 4.**

**Table 4. Total activity of leaf extracts of Acacia spp.**

| Sr. No. | Plants | Extract | Yields of crude extract | | MIC mg/ml | | | Total activity ml/gm | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Per 5 gram | Per 1 gram | EC | PA | SA | EC | PA | SA |
| 1 | *Acacia auriculiformis* | Chloroform | 39 mg | 7.8 mg | 100 | 100 | 100 | 0.078 | 0.078 | 0.078 |
| | | Methanol | 275 mg | 55 mg | 12.5 | 6.25 | 12.5 | 4.4 | 8.8 | 4.4 |
| 2 | *Acacia mangium* | Chloroform | 110 mg | 22 mg | 100 | 50 | 100 | 0.22 | 0.44 | 0.22 |
| | | Methanol | 419 mg | 83.8 mg | 6.25 | 6.25 | 6.25 | 13.41 | 13.41 | 13.41 |

## CONCLUSIONS

It is concluded from the results of antibiogram of clinical isolates that *Escherichia coli*, *Staphylococcus aureus and Pseudomonas aeruginosa* are most common multidrug resistant prevalent in community acquired and hospital acquired infection. It is concluded that both the Acacia species showed very good sensitivity activity against common clinical isolates. On the basis of total activity, it is also concluded that *Acacia mangium* possessed good sensitivity against all the three multidrug resistant clinical isolates than *Acacia auriculiformis.* It is clear evidence that methanolic extracts of both the Acacia species have potential as an antibacterial compound against resistant isolates and they can be further evaluated for bioactive natural product. This investigation would help to formulate a new, cheaper and alternative antibacterial drug.

## REFERENCES


1. Sarker, S. D., Nahar, L. and Kumarasamy, Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth and its application in the in vitro antibacterial screening of phytochemicals, *Methods*, 2007; 42: 321-324
2. Adamu, H. M., Abayeh, O. J., Agbo, M. O., Abdullahi, A. L., Uba, A., & Dukku, H. U. *Journal Ethan Pharmacology* , 2005; *99*(1): 1-4.
3. P. P. Joy, J. Thomas, Samuel Mathew and Baby P. Skaria. Medicinal plants. *Aromatic and medicinal plants research station*, Kerala agricultural university1998; 3-9.
4. O'Neill, J. Antimicrobial resistance: Tackling a crisis for the health and wealth of Nations. *Review on Antimicrobial Resistance*, London, 2014.
5. WHO. Antibiotic resistance - A report on infectious diseases, 2014.
6. Nair, R. and Chanda, S. In vitro antimicrobial activity of *Psidium guajava* L. leaf extracts against clinically important pathogenic microbial strains. Brazilian Journal of Microbiology, 2007; 3 (8): 452-458.

7. Harborne J B. Phytochemical methods. A Guide to Modern Techniques of plant analysis, Chapman and Hall, International edition, 1974.
8. Prashant Tiwari & et. al. Phytochemical screening and extraction: A Review, *Internationale Pharmaceutica Sciencia*, 2011; 1(1): 98-106
9. WHO. Basic procedure in clinical bacteriology, 2nd edition, 1991
10. American Society for Microbiology, Washington D. C. Manual of Clinical Microbiology, 7th Edition, 1999; 1526-1543.
11. Perez, C., Pauli, M. and Bazerque, P. An Antibiotic Assay by Agar Well Diffusion Method. *Acta Biologiae et Medicinae Experimentalis*, 1990; 15: 113-115.
12. Esimone, C.O., Adiuku, M.U. and Okonta, J.M. Preliminary Antimicrobial Screening of the Ethanolic Extract from the Lichen Usnea subfloridans (L). *J. Pharmaceutic Res. Dev*. 1998; 3(2): 99-102
13. Eloff J. N. A proposal on expressing the antibacterial activity of plant extracts - a small first step in applying scientific knowledge to rural primary health care in South Africa. *South African Journal of Science*. 1998; 96, 116-118
14. NCCLS. Performance Standards for Antimicrobial disc susceptibility tests; 8th Edition, 2003;23 (1).