

Determination of Antibacterial Activity and MIC of Crude Extract of *Abrus precatorius* L.

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Abstract

Abrus precatorius L. is the common plant found in the South-Gujarat region. Antibacterial activity from crude Methanolic and Petroleum ether extracts of the legume plant *Abrus precatorius* (commonly known as Crab's Eye) was carried out. Different parts such as leaves, seeds and roots were taken and extract was prepared to study antibacterial activity on pathogenic as well as opportunistic pathogenic bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Escherichia coli* and *Klebsiella pneumoniae*. All the selected organisms were naturally isolated. Antimicrobial activity was determined by the filter paper disc method. Zone of growth inhibition was observed in case of root extract prepared in both Petroleum ether as well as Methanol against *Staphylococcus aureus*. Further the Minimum Inhibitory Concentration (MIC) was also determined for both the extracts against *Staphylococcus aureus* and it was found to be 0.44 mg/ml (440 µg/ml) and 0.40 mg/ml (400 µg/ml) for both petroleum ether extract and methanolic extract respectively.

Key words: Antibacterial activity; Filter paper disc method; Zone of inhibition; MIC

Introduction

Various medicinal plants have been used for years in daily life to treat disease all over the world. Moreover, there occurs an enormous diversity in the phytochemicals derived from plants. Different extracts from traditional medicinal plants have been tested to identify the sources of the therapeutics effect (Parekh and Chanda, 2007). As a result some natural products have been approved as new antibacterial drugs. The common name of *Abrus precatorius* Linn. is Jequerity, crab's eye, Rosary pea. It is medically important plant from *Fabaceae* family. The plant is native to Indonesia and grows in tropical and subtropical areas of the world where it has been introduced. This plant is found throughout India. It is seen on hedges and bushes in exposed areas. A deciduous, wiry climber with tough branches leaves abruptly pinnate with many pairs of leaflets, the rachis ending in a spine the leaflets oblong rounded at both ends. Fruits are short pods containing hard shiny scarlet and black seeds. Their seeds are often used as beads and in percussion instruments. The seed contains the toxic poison abrin analogous to ricin. The seeds paste is applied locally against skin diseases. Leaves are used as substitutes for licorice (mulethi) considered useful in biliousness and in leucoderma, itching and other skin diseases. Roots used as diuretics. Also used in preparations prescribed for gonorrhea, jaundice and haemoglobinuria.

Antimicrobial susceptibility testing (AST) is performed by laboratories every day on clinical isolates often with new antimicrobial agents. The techniques employed are often taken for granted and are frequently abused. The use of absorbent paper as a means of carrying antibacterial solutions was first suggested by Pope in 1940 (Heatley, 1944). The Bauer-Kirby method provided important advances in methodological

standardization and zone diameter interpretation against a quantitative Minimum Inhibitory Concentration (MIC) value.

Materials and Methods

The plant materials used in this study were leaves, seeds and roots of *Abrus precatorius* L. Methanolic and petroleum ether extract were prepared by using Soxhlet apparatus. The solvent was evaporated from plant extract by incubating the extracts in hot air oven at 45°C until the concentrated extract was obtained. Further like this way the extract were concentrated. The final concentration of the extract is shown in the Table 1.

Different organisms like *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* were isolated from natural source. The seven bacterial strains isolated from natural source and were confirmed by biochemical tests Results are shown in Table-2 (Patel, 2008). Antibacterial activity was measured using paper disc method (Qadrie *et al.*, 2009) [with modification].

The paper disc for the antimicrobial test was prepared by taking aliquotes of 1 ml each of the different extracts in the separate eppendorf tubes. The sterilized paper disc prepared from the Whatman paper (diameter = 5.42 mm) were dipped in the extracts for 1 hr. After incubation, the paper discs were incubated in the oven at 45°C overnight to evaporate the solvent from the paper disc. For preparing the paper disc for the determination of the minimum inhibitory concentration (MIC), the sterilized paper discs were dipped in each of the different dilutions prepared from the extract from which the antimicrobial activity was observed. Other working steps were same as above.

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Approximately 10 ml of nutrient agar was poured in Petri-plate for base agar. Further the 24 hr activated culture of respective organisms were inoculated (about 400 µl) in previously melted and then cooled soft agar (5 ml) (at about 45°–50°C). After well mixing, the soft agar was poured over the base agar plate. Composition of Base nutrient agar and Soft nutrient agar are shown in Table-3 and Table-4 respectively. After the solidification of soft agar, the paper disc previously dipped in extract and the solvent from extract being evaporated overnight in the oven & its control is placed over the solidified agar plate. Then for better diffusion of the extract the plates were incubated in refrigerator for 30 min. After that the plates were incubated in the incubator at 37°C for 24 hr. The result was interpreted after 24 hrs.

The determination of minimum inhibitory concentration (MIC) was carried out by placing the paper discs in increasing or decreasing concentration of the extract over the Petri plate containing the soft agar layered over base agar plate. Further procedures are same as above only.

Result and Discussion

We have isolated 7 bacterial strains from the soil named *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Klebsiela pneumonia* and *Staphylococcus aureus*. Biochemical results are shown in the Table-2. Antimicrobial activity of different parts of *Abrus precatorius* like roots, seeds and leaves were studied against all above mentioned bacterial strains. Root extract of *Abrus precatorius* was found to be active against the Gram positive organism *Staphylococcus aureus*.

Root extracts possess good antibacterial potential particularly against *Staphylococcus aureus*. The zone of growth inhibition against *Staphylococcus aureus* is recorded in Table 5. The Minimum Inhibitory Concentration (MIC) of the Petroleum ether extract against *Staphylococcus aureus* was found to be 0.44 mg/ml (440 µg/ml) and of Methanolic extract was found to be 0.40 mg/ml (400 µg/ml) against same. It was considered that if the extracts displayed an MIC less than 100 µg/ml, the antimicrobial activity was good; from 100 to 500 µg/ml the antimicrobial activity was moderate; from 500 to 1000 µg/ml the antimicrobial activity was weak; over 1000 µg/ml the extract was considered inactive (Holetz et al., 2002). Thus for our result to be interpreted, the antimicrobial activity of the root extract is moderate. Although, it has been considered that the extract is crude extract and further identification and the isolation of the active component could be possible.

The activity of tested plant depends on their kind, mode of extraction and used species tested (Mbata, 2006). *Staphylococcus* is gram positive about cell wall of various polypeptide polymers and this could be the more reason for the many of the drugs to have had reduced effect



Figure-1: Zone of inhibition found in MR region of *S. aureus* plate.

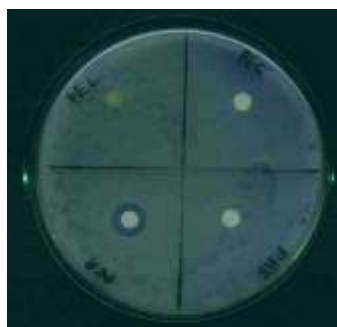


Figure-2: Zone of inhibition found in PER region of *S. aureus* plate.

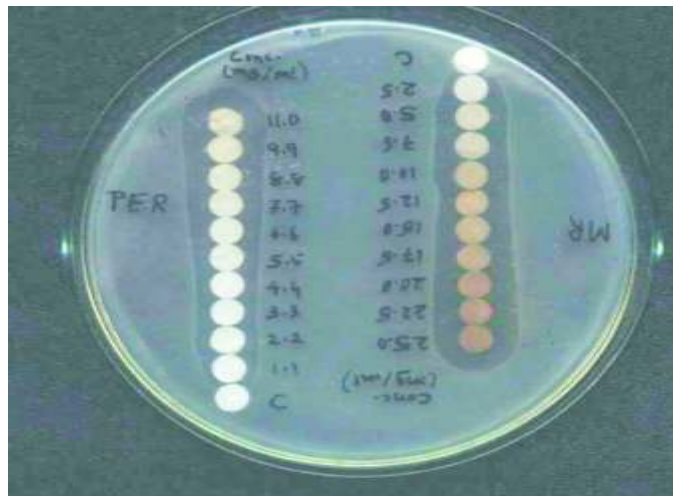


Figure- 3: MIC plate.
Range:- 1.1- 11.0mg/ml (for PER)
2.5- 25.0mg/ml (for MR)
Zone upto:- 1.1mg/ml (for PER)



Figure- 4: MIC plate.
Range:- 0.11- 1.10mg/ml (for PER)
0.25- 2.5mg/ml (for MR)
Zone upto:- 0.44mg/ml (for PER)
0.50mg/ml (for MR)

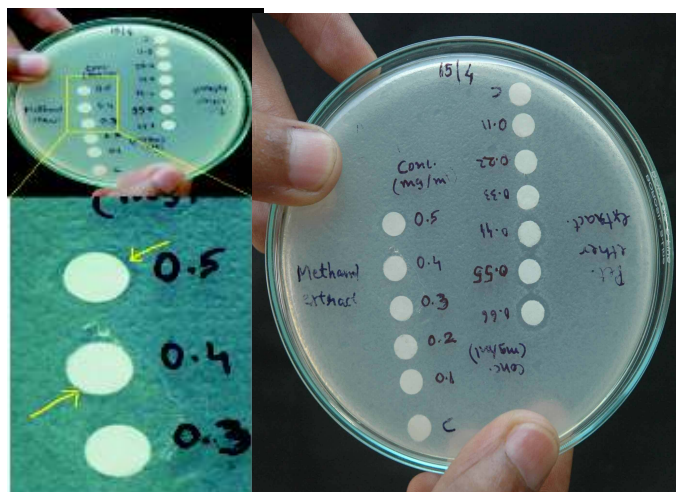


Figure- 5: MIC plate Range:- 0.11- 0.66mg/ml (for PER)
0.10- 0.50mg/ml (for MR)
Zone upto:- 0.44mg/ml (for PER)
0.40mg/ml (for MR)

Plant Part	Extraction Solvent	Concentration of Extract (in mg/ml)
Roots	Methanol (MR)	25.0
	Petroleum ether (PER)	11.0
Seeds	Methanol (MS)	45.0
	Petroleum ether (PES)	5.0
Leaves	Methanol (ML)	104.6
	Petroleum ether (PEL)	33.5

Table-1: Summary of the concentration of the extract obtained from different plant parts in mg/ml.

No.	Test	S.A	P.A	K.P	E.C	S.T	S.P.A	S.P.B
1	Indole test	-	-	-	+	-	-	-
2	Methyl Red Test	-	-	+	+	+	+	+
3	Voges Proskauer test	-	-	-	-	-	-	-
4	Citrate utilization test	-	+	+	-	-	-	+
5	H ₂ S production test	-	-	-	-	+	-	+
6	Urea hydrolysis test	-	-	-	-	-	-	-
7	Nitrate reduction test	+	+	+	+	+	+	-
8	Gelatin liquification test	-	-	-	-	-	-	-
9	Sugar fermentation test							
	Glucose	A	A	AG	AG	A	AG	AG
	Sucrose	-	-	AG	AG	-	-	-
	Lactose	-	-	AG	AG	-	-	-
	Maltose	-	-	AG	AG	A	AG	AG
	Mannitol	A	-	AG	AG	A	AG	AG
	Xylose	-	-	AG	AG	A	-	AG

Table-2: Summary of the Biochemical Tests carried out for the identification of microorganisms isolated from natural source.

Key: + = positive
 - = negative
 A = acid production
 G = gas production
 S.A= S. aureus
 P.A= P. aeruginosa
 K.P= K. pneumonia
 E.C = E. coli
 S.T = S. typhi
 S.P.A= S. paratyphiA
 S.P.B= S. paratyphiB

Components	Gram Percentage
Peptone	0.5
Beef extract	0.3
Sodium chloride	0.5
Agar	3.0
pH	7.4

Table-3: Media composition of the base nutrient agar used in the Antimicrobial Susceptibility Testing.

Components	Gram Percentage
Peptone	0.5
Beef extract	0.3
Sodium chloride	0.5
Agar	1.5
pH	7.4

Table-4: Media composition of the soft nutrient agar used in the Antimicrobial Susceptibility Testing

Plant Part	Extraction Solvent	Zone of inhibition in mm
Roots	Methanol (MR)	15
	Petroleum ether (PER)	12
Seeds	Methanol (MS)	-
	Petroleum ether (PES)	-
Leaves	Methanol (ML)	-
	Petroleum ether (PEL)	-

Table-5: Comparative results of the Zone of Inhibition formed by the respective extracts prepared from different plant parts.

(Ajaiyeoba, 2000). Finally, we concluded that antibacterial activity of this plant provide hope that they can form the basis for an alternative therapeutic agent for control of *Staphylococcus aureus* infection. These results provide promising baseline information for the potential use of this plant extract in the treatment of skin diseases and diarrhea.

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