

## Antimicrobial Activity of *Bauhinia Purpurea* (L) by Minimum Inhibitory Concentration (MIC) Method

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**Abstract:** MIC methods are widely used in the comparative testing of new agents, or when a more accurate result is required for clinical management. As there are no CLSI (formerly NCCLS) recommendations for the determination of MICs of various bio-compounds against gram positive and gram negative organisms. The minimum inhibitory concentration is the lowest concentration (in µg/ml) of an antibiotic that inhibits the growth of a given strain of bacteria. In Diagnostic laboratories these MIC'S are used to confirm resistance and also most often used to determine antimicrobial invitro activity. In the present MIC method, one can get the information about Antibiotic agent, preparation of stock solutions, media and inoculation, conditions of incubation, ultimately reading and interpretation of results. The present study is focused to evaluate antibacterial activities of ethanolic extract and purified fractions of *Bauhinia purpurea* using a gram-positive and a gram-negative organisms *Staphylococcus aureus*, *Klebsiella*, *E.coli* and *enterococcus*.

**Keywords:** Antimicrobial activity MIC, Cefixim, *Bauhinia purpurea* (L), column-chromatography

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### I. Introduction

It is a well known fact that the interactions between microorganisms, plants and animals are natural and constant. The ecological role of microorganisms and their importance in the bio-geochemical cycles in nature is well documented. The human food supply consists basically of plants and animals or products derived from them. Microorganisms are ubiquitous and are so small that they can only be visualized with the aid of high-resolution microscopes. Microbes are a heterogeneous group of several classes of living things. These were originally classified under the plant and animal kingdoms. Antimicrobial activity and are a source of powerful drugs [1]. The present research work on these local medicinal plants like *Bauhinia purpurea* (BP) is expected to reveal the Antimicrobial potency against the test organisms [2]. The phytoconstituents like flavonoids, *Isoflavonoids* found in the plant. They may be the responsible active moieties exhibiting the anti microbial activities [3, 4].

### II. Materials and Methods

#### A] Preparation of Agar media and Agar dilution plates [5,6,7] :

Nutrient agar weighing 28g is diluted in 1000ml of distilled water and is subjected to autoclave process for 15min at 121<sup>0</sup>C. Allow it to cool. Add 19ml of cooled molten agar to each container, including the antibiotic free control. It is mixed well before pouring into 90mm petridishes. After pouring the agar along with extract, fraction and cefixim with 0.1, 0.2, 0.3 and 0.4µg each into petridish. Later incubation is done for 10min.

#### B] Preparation of Inoculum:

The procedure describes a method for preparing the desired inoculums by comparison with a 0.5Mc Farland standard. [0.5ml of 0.048 BaCl<sub>2</sub> in 99.5ml of 0.18M H<sub>2</sub>SO<sub>4</sub> with constant stirring]. The density of organism suspension was adjusted to equal that of the 0.5Mc Farland standard. Broth is incubated at 35-37<sup>0</sup>C for 3-4 hr until the visible turbidity is equal to 0.5Mc farland. Comparison of test and standard against white background with a contrasting blackline, in which suspensions was equal to 1.5x10<sup>8</sup> CFU/ml. Further 1-2µg of broth is inoculated in agar media.

#### C] Test organisms used:

The strains used for the present test were: *E.coli*, *S.aureus*, *Klebsiella* and *Enterococcus*



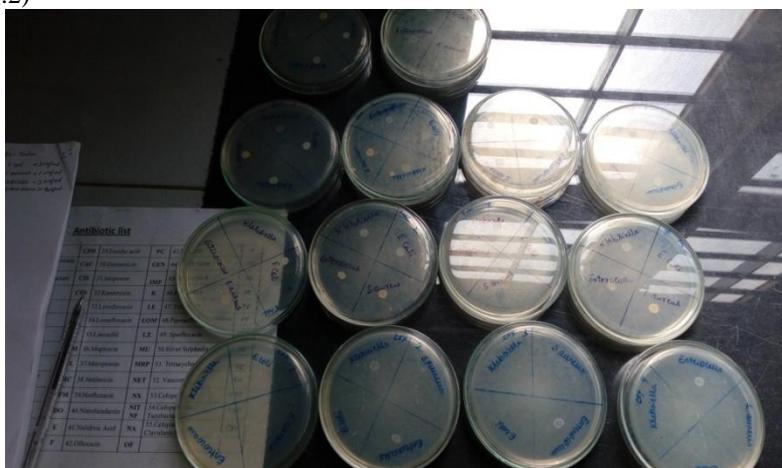
**Fig.1:**

**D] Determination of Minimum Inhibitory Concentration:**

The MIC was observed after overnight incubating the petridishes containing test organisms in agar media along with extract and fraction (F1) of *Bauhinia purpurea* (L) plant for 16-18h at 35-37°C. The lowest concentration of the sample required to inhibit the growth of test organism was noted for each organism as the minimum inhibitory concentration (MIC). The extract and fraction (F1) were dissolved in dimethylsulphoxide (DMSO).

**III. Results and Discussions**

The preliminary phytochemical test of stem bark extract and fraction of *Bauhinia purpurea* (L) plant has been revealed that the presence of secondary metabolites like Flavonoids, isoflavonoids has the potency against many microorganisms.[8][9] Results of the antimicrobial activity of the plant extract and fraction1 are shown in table1(fig.2)



**Fig.2**

**Table1:** Results of Minimum Inhibitory Concentration ethanolic extract and isolated fraction of *Bauhinia purpurea*

SL.NO	Organisms	Drug Concentration In µg	Antibacterial activity by MIC method		
			BP- Ethanolic extract	BP-fraction 1	Cefixime
1	E.coli.	10	-	-	+
		20	-	+	+
		30	+	+	+
		40	+	+	+
2	S.aureus	10	-	-	+
		20	-	+	+
		30	+	+	+
		40	+	+	+
3	Enterococcus	10	-	-	+
		20	-	+	+
		30	+	+	+
		40	+	+	+
4	Klebsiella	10	-	-	+
		20	-	-	+
		30	-	+	+
		40	+	+	+

**Indications: ‘+’ → Inhibition of growth**

**‘-’ → No inhibition**

The ethanolic extract and isolated fraction (30mg and 40mg) of Bauhinia purpurea exhibits an excellent antimicrobial activity by MIC method against S.aureus and Klebsiella. Whereas the same extract fraction (20mg and 30mg) showed least antimicrobial activity against E.coli and Enterococcus.

#### IV. Conclusion

The MIC test is relatively straight forward, which naturally can be done on a very small scale without using much antimicrobial agent. In this current investigation, the results revealed that both extract and isolated fraction are exhibited antimicrobial activity. Considerable minimum inhibitory concentration of extract and isolated fraction was observed showed antimicrobial activity against *E.coli and Enterococcus* when compared to the standard drug.

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