

Original Research Article

Comparative Anti bacterial study in the leaves of four *Bauhinia* species

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ABSTRACT

Keywords

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Disc diffusion
method;
MIC technique;
inhibition zones;
TLC test.

The aim of the present study is to investigate the anti-bacterial effect by using the leaf extract of *Bauhinia* species. Four species of *Bauhinia* viz..*B.purpurea*, *B.galpini*, *B.roxburghii*, *B.vahli* among which anti bacterial effect of *B.galpini*, *B.roxburghii* were not reported, were collected and washed thoroughly & dried. The leaf samples were extracted by the methanol, acetone, distil water as solvents. Those extracts were tested on 5 species of pathogenic bacteria viz.. *Micrococcus luteus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia* by sterile filterpaper discs with methanol as solvent and MIC technique with acetone & Distil water as solvents. Different concentration of methanolic extract of *Bauhinia* leaf samples viz.. 50µg/ml, 100µg/ml, 200µg/ml, 400µg/ml were tested. Different zones of inhibition appears on agar plates and measured in millimetres. Comparatively, *B. purpurea* & *B.galpini* showed inhibition zones against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *B.galpini* & *B.roxburghii* showed inhibition zones against *Bacillus subtilis* and *Klebsiella pneumonia*. Successfully, anti bacterial effect was determined in *B.galpini* & *B. roxburghii*, which its antibacterial property was not reported. The anti-bacterial activity of all the leaf extracts of 4 *Bauhinia* species showed positive results even at least concentration of 50µg/ml. The effects of acetone & distil water extracts were Nil. The average results were obtained by Combination test. The methanolic extract of *Bauhinia* species were more effective than the standard anti biotic on most resistant bacteria viz.. *Micrococcus luteus* and *Pseudomonas aeruginosa*.

Introduction

There was many trees in my school. Trees were attacked by pests and diseases frequently. Among them I observed one tree which was healthy and not affected by any pests or diseases. My curiosity about this species increased. By detailed

survey the species was found to be *Bauhinia* species.

Plants of the genus *Bauhinia* are widely distributed in the tropics and important for animal's nutrition because of their high

protein content (Anonymous, The Wealth of India, 526-555(2004)). Some plants species also have been used in folk medicine for the treatment of diabetics, diuretics and also as Antimicrobial(Sahu *et al.*, (2012), The plant show great variation in their habitat ,they may be trees or Shrubs and may be woody climber also .

Bauhinia purpurea, Linn. Is found mainly in Deccan and carnic, in deciduous forest chiefly along water courses; W.Coast, in dry forest; often cultivated in gardens.A moderate – sized evergreen tree, often bushy, with rater large leaves, bark grey to brown; wood dark brown. Moderately hard, used for implements. The bark is used for tanning, Adey and fibers are obtained from its bark. *Bauhinia vahli*, Linn. is mainly found in Assam Madhya Pradesh, Bihar and Punjab.

It is a gigantic climber with very thick stem and long branches bearing cream coloured flowers with shortly-clawed petal. It is destructive to forest trees and has to be cut .The leaves are used as plates and for many purposes; the seed are roasted and eaten. The bark yields a fiber which is used for making ropes. The bark is used for tanning.

Bauhinia roxburghii, Cor. is mainly found in Malabar and Travancore and near the cost. It is not very abundant and apparently scarce. A Large very long climber with curious stem alternately twisted one away and the other between the straight margins. Also they are used for folk medicine .

Bauhinia galpini , N.E.Br. is a straggling and prostrate shrub. This is native of south and Tropical Africa. This is grown in the garden as on ornamental for its bright

scarlet flowers. They are used for traditional medicine purpose in tribal areas.

Among which anti bacterial effect of *Bauhinia galpini*, *Bauhinia roxburghii* is unknown still today . So, I planned to study its anti bacterial effect ,by getting above Information .

Materials and Methods

Three sets of Petri plates were prepared to test for control, standard and experiment for each sample of *Bauhinia* species.

Standard plates are also tested by taking different concentrations viz..

1 µg/ml, 2.5 µg/ml, 5 µg/ml, 10 µg/ml, 25 µg/ml, 50 µg/ml and 100 µg/ml of Tetracycline hydrochloride Batch No.: CMS 219, Lot No: 0000114334, Exp /Date: Jan 2014

Preparation of plant extracts

The healthy and mature leaves of 4 *Bauhinia* species viz...*B.purpurea*, *B.vahli*, *B.galpini*, *B.roxburghii* were collected & also Identified by H.O.D of Botany Department ,Government Science College ,Bangalore . The leaves were washed with water and air dried. This samples were converted to powered form . The samples (1g) were taken in pestle & mortar and extracted (1g+10ml methanol × 3 times) with methanol as solvent. This extracts were squeezed through three layer cheese cloth and filtrate was collected and centrifuge at 5000RPM for 10 min. The liquid supernatant was collected and dried. It was dissolved in 2ml distil water.

The leaf samples were also extracted with acetone and distil water. 25g of leaf samples was taken in water bath; acetone and distil water were added separately, the

setup was Reflexed for 1hr, filtrates were collected and concentrated. The filtrates were dissolved in 10ml distilled water.

Determination of anti bacterial activity (Disc diffusion technique)

Freshly grown 5 bacteria viz., *Micrococcus luteus* (ATCC 10240), *Bacillus subtilis* (MTCC 441), *Pseudomonas aeruginosa* (MTCC 1688), *Staphylococcus aureus* (MTCC 737) and *Klebsiella pneumonia* (MTCC 109) in NA (18 hr, 37°C) were taken. The agar media was prepared and poured into Petri plates and 0.1ml of bacteria was spread entirely on agar media with sterile cotton swab at aseptic condition. The sterile filter paper of 6mm diameter dipped in plant extract was placed on Petri plates. The plates were incubated for 24 to 48 hr at 37°C.

Minimum Inhibitory concentration (MIC) Determination

In addition MIC protocol using agar dilution technique method was adopted to test leaf extracts with acetone and distil water as solvents on bacteria. Water extracts: 25% samples is made using sterile DM water. Acetone extracts : 10% stock of test samples is made using Methanol. Tetracycline hydrochloride: 0.5% stock of the test samples is made using methanol. Varying concentrations of the test agents are incorporated into labeled plates and mixed along with agar at temperature not exceeding 50-55°C. Plates were allowed to set at RT for an hour. Duplicate analysis is done for all concentration. Plates were dried before streaking. The aliduo(1µg) of the bacteria was streaked on surface of agar using calibrated loop and incubated for 24 to 48 at 37°C under aerobic conditions.

Thin layer chromatography (TLC) test

5 grams of leaf samples was extracted with methanol and reflexed for 45 min. Then concentrated to 100ml, this sample was used for TLC. Toluene:Ethyle acetate:Acetic acid (5.5:4.5:0.5) were used as Mobile phase and Anisaldehyde sulfuric acid reagent (ANS) was spray reagent. Detection: 254,366 nm and after spray

Results and Discussion

The four species of *Bauhinia* viz.. *B. purpurea*, *B. galpini*, *B. roxburghii* and *B. vahli* were tested for their anti bacterial activity. Methanol, acetone & distil water was used as solvents for the extraction of anti microbial compounds. There are four concentrations viz...50µg/ml, 100µg/ml, 200 µg/ml, 400 µg/ml were tried against the pathogens like *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Staphylococcus aureus* and *Klebsiella pneumonia* with methanol as solvent. And Concentrations viz..100 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml were tested on bacteria with acetone and distil water as solvents, but results were Nill, even at 1000 µg/ml.

The combination trail of *Bauhinia* species was found to exhibit average results, when compared to effect of individual species. Similarly it was found that all the four sample of *Bauhinia* species exhibited positive effect on bacteria with methanol as solvent. Successfully anti bacterial effect was found in *B. galpini* & *B. roxburghii*, which its anti bacterial property was not reported.

Comparatively of all the species *Bauhinia purpurea* and *B. galpini* showed positive inhibition zones of +7mm+7mm against *Pseudomonas aeruginosa* (table.4) and

Table.1 Control test on bacteria. (N/I = No inhibition)

S. No.	Bacteria	Stain no. (or) code no.	Control			
			Methanol	Media	Acetone	DM water
1	<i>Micrococcus luteus</i>	ATCC 10240	N/I	N/I	N/I	N/I
2	<i>Staphylococcus aureus</i>	MTCC 737	N/I	N/I	N/I	N/I
3	<i>Pseudomonas aeruginosa</i>	MTCC 1688	N/I	N/I	N/I	N/I
4	<i>Bacillus subtilis</i>	MTCC 441	N/I	N/I	N/I	N/I
5	<i>Klebsiella pneumoniae</i>	MTCC 109	N/I	N/I	N/I	N/I

Table.2 Anti bacterial activity of 4 *Bauhinia* species on *Micrococcus luteus*

S. No	Sample Used	Methanol extract				Acetone extract Minimum inhibitory concentration (MIC)	Distil water extract Minimum inhibitory concentration (MIC)
		Concentration					
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml		
1	<i>B.purpurea</i>	N/I	N/I	(8mm)	(9mm)	>1,000 µg/ml	>1,000 µg/ml
2	<i>B.vahli</i>	N/I	N/I	(10mm)	(12mm)		
3	<i>B.galpini</i>	(6mm)	(8mm)	(12mm)	(13mm)		
4	<i>B.roxburghii</i>	N/I	(7mm)	(9.5mm)	(12.5mm)		
5	Combination B.p+,B.v.,+B.g,+B.r	N/I	N/I	(10mm)	(12mm)		

The MIC of Standard antibiotic i.e. Tetracycline hydrochloride against *Micrococcus luteus* is >100 µg/ml.

(mm =inhibitory zones & >1,000 µg/ml means no,effect still 1,000 µg/ml.)

Table.3 Anti bacterial activity of 4 *Bauhinia* species on *Staphylococcus aureus*

S.No	Sample Used	Methanol extract				Acetone extract Minimum inhibitory concentration (MIC)	Distil water extract Minimum inhibitory concentration (MIC)
		Concentration					
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml		
1	<i>B.purpurea</i>	(7mm)	(10mm)	(13mm)	(16mm)	>1,000 µg/ml	>1,000 µg/ml
2	<i>B.vahli</i>	N/I	N/I	(8mm)	(9mm)		
3	<i>B.galpini</i>	(8mm)	(10mm)	(12mm)	(13mm)		
4	<i>B.roxburghii</i>	N/I	(7mm)	(9mm)	(9mm)		
5	Combination B.p+,B.v.,+B.g,+B.r	N/I	(6.3mm)	(9.5mm)	(11.2mm)		

The MIC of Standard antibiotic i.e. Tetracycline hydrochloride against *Staphylococcus aureus* is 2.5 µg/ml

(mm =inhibitory zones & >1,000 µg/ml means no,effect still 1,000 µg/ml.)

Table.4 Anti bacterial activity of 4 *Bauhinia* species on *Pseudomonas aeruginosa*

Sl. No	Sample Used	Methanol extract					Acetone extract	Distil water extract
		Concentration						
		25 µg/ml	50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml		
1	<i>B.purpurea</i>	N/I	(7mm)	(8mm)	(9mm)	(13mm)	>1,000 µg/ml	>1,000 µg/ml
2	<i>B.vahli</i>	(6mm)	(7mm)	(7mm)	(10mm)	(10mm)		
3	<i>B.galpini</i>	(6mm)	(7mm)	(12mm)	(13mm)	(14mm)		
4	<i>B.roxburghii</i>	N/I	N/I	N/I	(6mm)	(10mm)		
5	Combination B.p+,B.v.,+B.g,+B.r	N/I	N/I	(7mm)	(10.1mm)	(12mm)		

MIC of The Standard antibiotic i.e. Tetracycline hydrochloride against *Pseudomonas aeruginosa* is 50 µg/ml.
(mm =inhibitory zones & >1,000 µg/ml means no,effect still 1,000 µg/ml.)

Table.5 Anti bacterial activity of 4 *Bauhinia* species on *Bacillus subtilis*

Sl. No	Sample Used	Methanol extract				Acetone extract	Distil water extract
		Concentration					
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml		
1	<i>B.purpurea</i>	N/I	N/I	N/I	N/I	>1,000 µg/ml	>1,000 µg/ml
2	<i>B.vahli</i>	N/I	N/I	N/I	N/I		
3	<i>B.galpini</i>	N/I	N/I	(7mm)	(11mm)		
4	<i>B.roxburghii</i>	(6mm)	(8mm)	(9mm)	(12mm)		
5	Combination B.p+,B.v.,+B.g,+B.r	N/I	N/I	N/I	(6mm)		

The MIC of Standard antibiotic i.e. Tetracycline hydrochloride against *Bacillus subtilis* is ≤ 1 µg/ml.

(mm =inhibitory zones & >1,000 µg/ml means no,effect still 1,000 µg/ml.)

Table.6 Anti bacterial activity of 4 *Bauhinia* species on *Klebsiella pneumonia*

S.No	Sample Used	Methanol extract				Acetone extract	Distil water extract
		Concentration				Minimum inhibitory concentration (MIC)	Minimum inhibitory concentration (MIC)
		50 µg/ml	100 µg/ml	200 µg/ml	400 µg/ml		
1	<i>B.purpurea</i>	N/I	N/I	N/I	N/I	>1,000 µg/ml	>1,000 µg/ml
2	<i>B.vahli</i>	N/I	N/I	N/I	(6mm)		
3	<i>B.galpini</i>	N/I	N/I	N/I	(7mm)		
4	<i>B.roxburghii</i>	N/I	N/I	(6mm)	(7mm)		
5	Combination B.p+,B.v,+B.g, +B.r	N/I	N/I	N/I	N/I		

The MIC of Standard antibiotic i.e. Tetracycline hydrochloride against *Klebsiella pneumoniae* is 5 µg/ml µ
(mm =inhibitory zones & >1,000 µg/ml means no,effect still 1,000 µg/ml)

Table.7 Anti bacterial activity of 4 *Bauhinia* species on *Micrococcus luteus* with 100% increase in concentration

S.No	Sample Used	Sample Concentration			
		200 µg/ml	400 µg/ml	800 µg/ml	1,600 µg/ml
1	<i>B.purpurea</i>	(8.1mm)	(9mm)	(11mm)	(12,8mm)
2	<i>B.vahli</i>	(10mm)	(12.1mm)	(13.mm)	(13.1mm)
3	<i>B.galpini</i>	(12mm)	(13mm)	(14mm)	(14.5mm)
4	<i>B.roxburghii</i>	(9.6mm)	(12.6mm)	(13.8mm)	(14mm)

Table.8 Comparison of RF values of four *Bauhinia* species 366nm under UV light

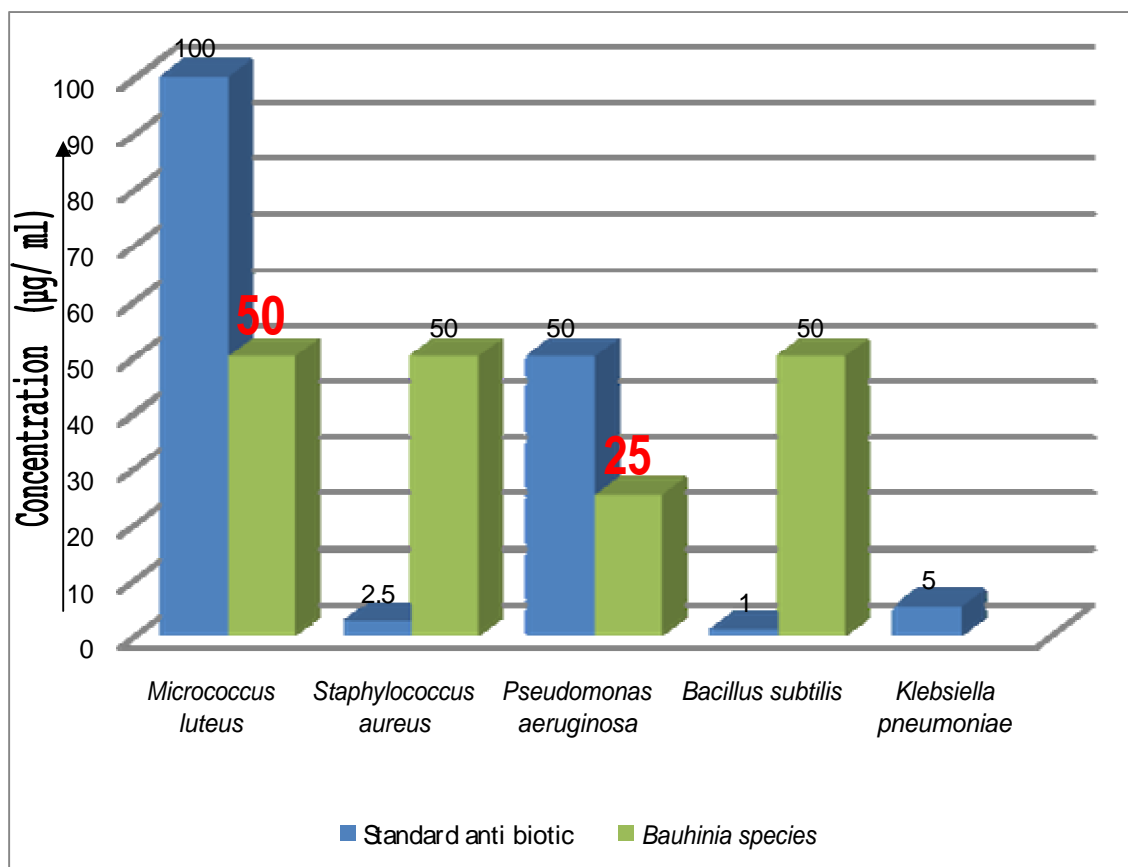
Bands	R.F values			
	B.purpurea	B.vahli	B.galpini	B.roxburghii
1	0.18	0.11	0.31	0.31
2	0.32	0.15	0.34	0.35
3	0.36	0.26	0.44	0.4
4	0.4	0.32	0.51	0.43
5	0.45	0.35	0.6	0.47
6	0.5	0.45	0.66	0.53
7	0.55	0.48	0.74	0.6
8	0.61	0.6	0.8	0.66
9	0.66	0.66	---	0.74
10	0.74	0.7	---	0.8
11	0.77	0.73	---	---
12	---	0.77	---	---

Table.9 After Anisaldehyde sulfuric acid reagent (ANS) spray

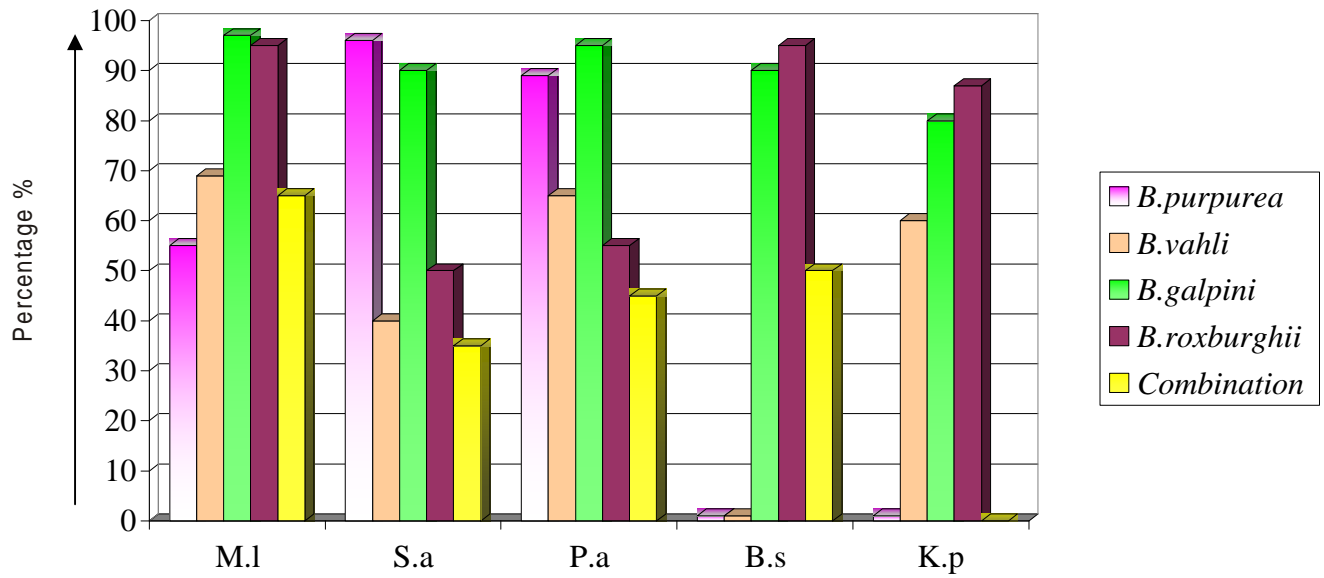
Bands	R.F values			
	B.purpurea	B.vahli	B.galpini	B.roxburghii
1	0.43	0.08	0.35	0.44
2	0.5	0.44	0.44	0.64
3	0.65	0.5	0.65	0.85
4	0.72	0.66	0.72	---
5	0.85	0.72	0.85	---
6	---	0.83	---	---
7	---	0.85	---	---

*This TLC determination will further carry on for purification of potential compounds present in the leaves of 4 *Bauhinia* species*

Graph.1 Comparison between the effect *Bauhinia* species and (MIC) Minimum inhibitory concentration of Standard anti-biotic (Tetracycline hydrochloride) on Bacteri, based on concentrations

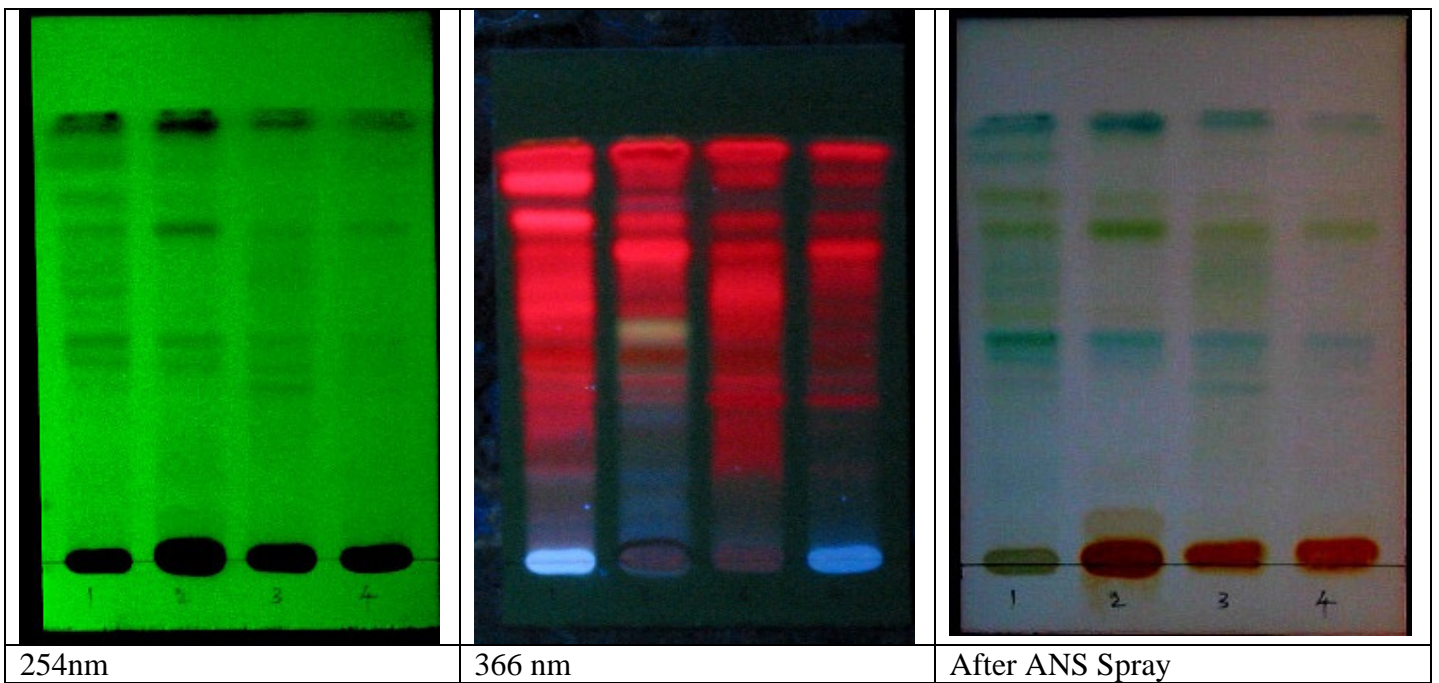


Graph.2 Over all effect of 4 *Bauhinia* species on bacteria



M.l= *Micrococcus luteus*; *S.a* = *Staphylococcus aureus*; *P.a* =*Pseudomonas aeruginosa*; *B.s* =*Bacillus Subtilis*; *K.p* =*Klebsiella pneumoniae*

Fig.1 Chromatograms of 4 *Bauhinia* species under U.V light .



Track 1: *Bauhinia purpurea*; Track 2: *Bauhinia vahlii*;
Track 3: *Bauhinia galpini* ; Track 4: *Bauhinia roxburghii*

+7mm+8mm against *Staphylococcus aureus* (Table.3) even at least concentration of 50 µg/ml. While *Bauhinia roxburghii* showed inhibition zones of +12mm against *Bacillus subtilis* (Table.5) and +12.5mm against *Micrococcus luteus* (Table.2).

The activity of *Bauhinia vahli* was found to be less when compared to other samples. Considerable inhibitory zones this was found only in case of *Pseudomonas aeruginosa* (table.4). *Bauhinia* species also worked better than standard anti-biotic on most resistant bacteria i.e. *Pseudomonas aeruginosa* & especially against *Micrococcus luteus* (Graph.1), which has no clarity on Infection dosage, Immunisation, vector etc.

Wide spectrum of bands (compounds) are obtained in TLC test (Fig.1) , which the RF values are mostly varying with each other species (Table.8,9).

Plates containing only the solublizer, Methonal and sterile D.M water separately, used to solublize the test agents, are tested. A plate containing only the media was tested.

The main aim of increasing concentration on *Micrococcus luteus*, because there is no dosage. No incubation and No immunization found still now. The concentration of *Bauhinia* leaf samples were increased by 100% i.e. 200µg,400µg, 800µg, 1,600µg to observe, whether the result decreases or Increases.

By increasing the sample concentration the, comparatively *Bauhinia galpini*, *Bauhinia roxburghii* showed better effect of +14.5mm and +14mm against *Micrococcus luteus* }
Successfully, anti bacterial effect was determined in *Bauhinia galpini* & *Bauhinia roxburghii* ,which its

antibacterial property was not reported . The methanolic extract of 4*Bauhinia* species exhibits positive results against all the above 5 pathogenic bacteria. Effective results were obtained by *Bauhinia* species, when compared to Standard antibiotic on most resistant bacteria i.e., *Pseudomonas aeruginosa* and especially against *Micrococcus luteus* which has no clarity on infection dosage, Immunizations, vector etc. till today. By the results obtained, I conclude that this research work will, also further lead to development of low cost and affordable antibiotics from 4 *Bauhinia* species against most dreadful pathogenic bacterial diseases, especially caused by *Micrococcus luteus*. The future works will carry on for purification of this potential compounds .

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