

## Screening for Antimicrobial Activity of the Stem Bark of *Bauhinia purpurea* Linn

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**Abstract:** The aim of the present study was, to carry out the phytochemical and antimicrobial screening of the n-hexane extract of the stem bark of *Bauhinia purpurea*. The preliminary phytochemical studies revealed the presence of fatty acids, triterpenoids, steroids, alkaloids and phytol esters. The antimicrobial screening of the n-hexane extract of the stem bark of *Bauhinia purpurea* by Well Diffusion and Tube Dilution Method was carried out against 6 bacterial strains; *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella species* and 2 fungal strains; *Aspergillus niger* and *Claviceps purpurea*. The present evaluation revealed that the n-hexane extract exhibited activity towards all the tested microbial strains showing a broad spectrum of activity against gram positive as well as gram negative microorganisms.

**Key words:** *Bauhinia purpurea* • Leguminosae • Tube Dilution • Well Diffusion

### INTRODUCTION

Natural products obtained from the plant resources have been the major supplements to combat many serious diseases in the developing countries [1]. With the advancement of modern medicinal systems, herbal medicines have always maintained their popularity in treatment of diseases due to their easy availability and safe effectiveness. The capacity of herbs to produce innumerable bioactive secondary metabolites has increased their usage in healing systems.

Natural products, as a basis of new drugs, have a great promise and it is gratifying to note that the World Health Organization is showing abiding interest in plant derived medicines, described in the folk medicine of different countries. According to the WHO (2001), 80% of the world population uses natural remedies and traditional medicines [2].

During the last few decades, the number of upcoming infectious diseases is increasing in the developing countries [2]. Plant based products may be a new boon to the development of antibiotics effective against antibiotic

resistant strains. Despite a great step towards development of new antibiotic drug therapies there has been a decline in advancement of new drugs.

The rapid emergence of multiple drug resistance strains of pathogens to current antimicrobial agents has generated an urgent intensive for new antibiotics from medicinal plants. Many medicinal plants have been screened extensively for their antimicrobial potential worldwide. Free radicals which have one or more unpaired electrons (superoxide, hydroxyl, peroxy) are produced in normal or pathological cell metabolism and the compounds that can scavenge free radicals have great potential in ameliorating the diseases and pathological cells [3,4].

The genus *Bauhinia* L is often called as 'Orchid Tree' of ornamental value [5]. *Bauhinia purpurea* (Linn.) is a medium sized deciduous flowering tree, bark ashy to dark brown belonging to the family Leguminosae and subfamily Caesalpinioideae [6, 7]. It is an erect tree with a round, symmetrical, moderate dense crown of 25 feet growing to a height of 20-35 feet tall [8]. The plant commonly known as Purple Orchid Tree or Butterfly Tree

is a native from the foot of the West Himalayas, Khasia Mountains and also found distributed in other parts of India and China [9].

The plant is well known for its traditional uses to treat roundworm infections, conjunctivitis, anthrax, ulcerations, dysentery, blood-poisoning, leprosy, lung and skin diseases. The use of different parts of the plant like roots as carminative and flowers as laxative are folk claimed. The bark is commonly used for treatment of diarrhoea and other gastrointestinal complaints [10,11].



Leaf and Stem Bark of *Bauhinia purpurea* linn.

## MATERIAL AND METHODS

### Authentication and Collection of the Plant Material:

The stem bark of *B. purpurea* was collected from Altinho, Panaji - Goa during November, 2011. It was identified and authenticated by Prof. G. I. Hukkeri, Dept. of Botany, Dhempe College of Arts and Science, Miramar - Goa, India.

**Preparation of n-hexane Extract:** The stem bark of *B. purpurea* was collected from Altinho, Panaji - Goa during the month of November, 2011. It was dried under shade. The dried stem bark was powdered (500g) and exhaustively extracted by maceration with n-hexane for three days. After three days, the n-hexane layer was decanted off. The process was repeated thrice. The solvent from the total extract was distilled off and the concentrate was evaporated to a syrupy consistency and then evaporated to dryness (5g).

**Preliminary Phytochemical Analysis:** Qualitative analysis was carried out of the crude n-hexane extract which revealed the presence of fatty acids, triterpenoids, steroids, alkaloids and phytol esters.

**Isolation of Phytoconstituents from N-hexane Soluble Fraction:** Chromatographic elution's led to the isolation of 9 plant constituents namely Myristic Acid, Octadecanoic Acid, 9, 12- Octadecadienoic Acid, isopropyl-24-methyl-pentacosanoate, Stigmasterol,  $\beta$ -Amyrin,  $\beta$ -Sitosterol, Lupeol and ethyl 9, 12-Hexadecadienoate [12].

**Microbial Strains:** The microbial strains used for the antimicrobial screening techniques were acquired from National Chemical Laboratories, Pune. The bacterial strains used for the study are *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 19429,

*Salmonella typhimurium* ATCC 23564, *Klebsiella species* and 3 fungal strains like *Aspergillus niger* ATCC 10864, *Claviceps purpurea* NCIM No. 1046 and *Candida albicans*.

**Determination of Antimicrobial Activity:** The n-hexane extract of stem bark of *B. purpurea* was subjected to antibacterial as well as antifungal screening by Well Diffusion Method (Cup Plate Method) [13-15] and Broth Dilution method (Tube Dilution Method). Mueller Hinton Agar/Broth and Sabouraud's Dextrose Agar/Broth were used as the seed medium for the antibacterial and antifungal screening respectively. The Minimum Inhibitory Concentration was performed by two-fold dilution of the test extract in the respective medium under sterile conditions [14, 16]. The stock inoculums of the bacteria and fungi were prepared in sterile normal saline (0.9%) previously autoclaved at 120°C with 15lbs bar pressure. The fungal and bacterial cultures were maintained on potato dextrose agar and nutrient agar slants respectively and refrigerated at 5°C. The inoculum was verified by streaking on specific medium for colony identification and purification. Appropriate controls were maintained.

The plates were observed visually and the diameter of zones was measured using an mm scale. The activity was indicated by the presence of clear zones around the well size. The bioassay was repeated thrice and the mean was recorded to check the effectiveness of the procedure. The MIC was determined by turbidometric method by measuring the Optical Density using Elico colorimeter (Filter No. 60). The MIC results were further reinforced by determining viable counts using pour plate method.

## RESULTS

The phytochemical screening led to the isolation of many bioactive phytoconstituents. The n-hexane extract was found to be significantly effective towards bacterial as well as fungal strains showing a broad spectrum of

Table 1: Effect of n-hexane extract of *Bauhinia purpurea* against various bacterial strains by Well Diffusion Method.

Sample	Diameter of Zone of Inhibition in mm					
	SA	BS	PA	ST	EC	KS
Extract	17	16	39	21	18	12
Standard (Streptomycin)	40	39	50	28	15	34

Concentration of the stock solution was 25mg/ml.

SA (*Staphylococcus aureus* ATCC 6538), BS (*Bacillus subtilis* ATCC 6633), PA (*Pseudomonas aeruginosa* ATCC 19429), ST (*Salmonella typhimurium* ATCC 23564), EC (*Escherichia coli* ATCC 35218), KP (*Klebsiella species*) and 2 fungal strains; AN (*Aspergillus niger* ATCC 10864), CP (*Claviceps purpurea* NCIM No. 1046).

Table 2: Effect of concentration of n-hexane extract on Bacterial cultures by Tube Dilution Method

Concentration (µg/ml)	SA	BS	PA	ST	EC	KS
+ve	0.76	0.56	0.63	0.71	0.70	0.53
31.25	0.56	0.42	0.66	0.74	0.71	0.59
62.5	0.55	0.38	0.53	0.54	0.65	0.47
125	0.52	0.26	0.44	0.34	0.62	0.41
250	0.23	0.22	0.39	0.30	0.51	0.30
500	0.22	0.19	0.35	0.25	0.41	0.25
1000	-	-	-	-	-	-

Concentration of the stock solution was 2mg/ml.

Extract control O.D.-0.10 (turbidity of the extract)

-Indicates no growth

Table 3: Effect of concentration of n-hexane extract on fungal cultures by Broth Dilution Method

Sample	AN						CP					
	1000	500	250	125	62.5	31.25	1000	500	250	125	62.5	31.25
Extract	-	-	-	+	+	+	-	-	-	-	-	+

Concentration of the stock solution was 2mg/ml.

+ positive indicating presence of growth -negative indicating absence of growth

activity. The extract shows significant antibacterial activity towards *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Klebsiella species* and antifungal activity towards the fungal strains; *Aspergillus niger* and *Claviceps purpurea* as cited below (Tables 1-3).

## DISCUSSION

*Bauhinia* traditionally is well known to have multiple uses in medicine. Since it is used in treatment of several infectious diseases such as diarrhoea, fever, dysentery, skin diseases, cancer etc [17]; it was decided to analyse the extract against various bacterial and fungal strains. *S. aureus*, *P. aeruginosa* and *E.coli*, well known to be a causative agent for boils, skin infections, abscesses, dysentery and diarrhoea [18, 19] appears to be strongly inhibited by n-hexane extract by both well

diffusion and MIC methods (Tables 1 & 2). The large zone of inhibition obtained with *P. aeruginosa* is particularly significant as the incidence of drug resistance in this culture is well known and many of its strains are implicated in nasoonial infections. Off late there are many bioactive results regenerated mainly focussing on antimicrobial activity of aqueous and methanolic extracts of *B. purpurea* [2, 20- 22]. This paper reports the antimicrobial activity of n-hexane extract of the stem bark of *B. purpurea* Linn and further accelerates our study on bioactive compounds.

It was seen from the present study that there was negligible growth seen at 500µg/ml for most bacterial cultures and no growth at 1000µg/ml. (Table 2). This further indicates that the MIC value lies between 500µg/ml to 1000µg/ml. From the above results it is evident that as the concentration of the n-hexane extract increases the viable count decreases indicating the bioactivity of the plant extract (Fig 1).

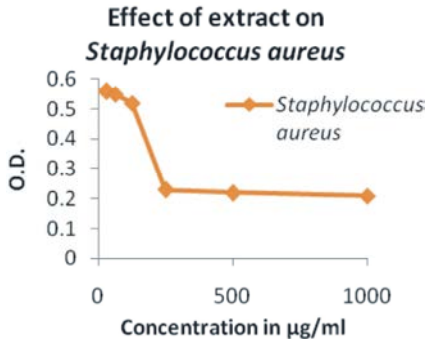


Fig. 1:

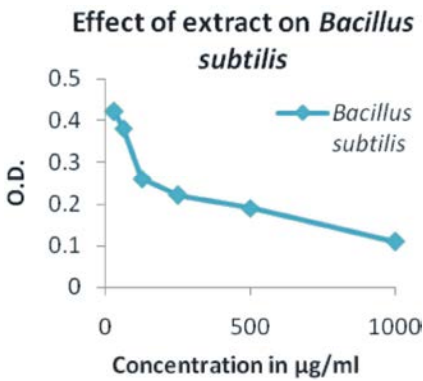


Fig. 2:

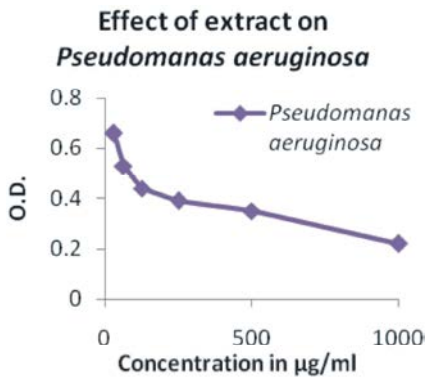


Fig. 3:

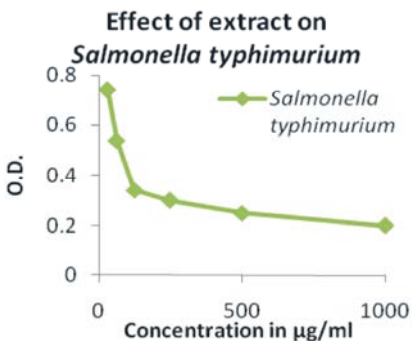


Fig. 4:

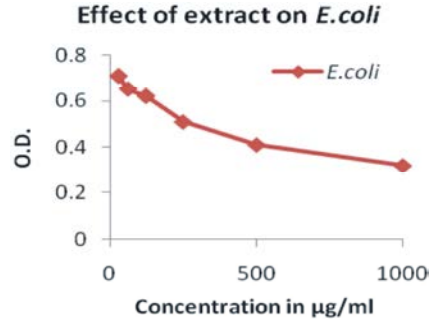


Fig. 5:

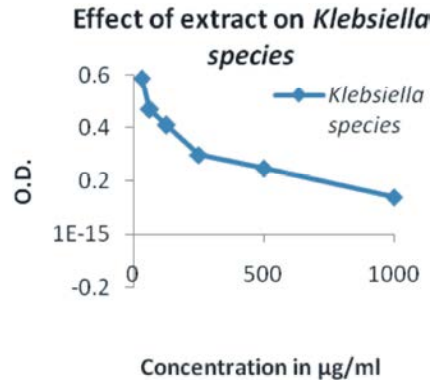


Fig. 6:

In our study the chromatographic elution led to the isolation of Myristic acid, Octadecanoic acid (Stearic acid), 9,12-Octadecadienoic acid, Stigmasterol,  $\beta$ -Amyrin,  $\beta$ -Sitosterol and Lupeol. It is well documented that the above constituents possess antimicrobial activity and hence this confirms and reinforces our study on antimicrobial activity of n-hexane extract of the stem bark of *B. purpurea* [23-28].

## CONCLUSION

The present study establishes that the n-hexane extract of stem bark of *B. purpurea* shows antibacterial as well as antifungal activity. The phytochemical investigation led to the isolation of steroids, triterpenoids, alkaloids, fatty acids and phytol esters. The above antimicrobial activity may be attributed to the presence of these bioactive constituents in the plant.

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