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# Quantitative, qualitative phytochemical analysis and *in vitro* antibacterial activity of *Bauhinia tomentosa* L.

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**ABSTRACT**

*The flower Bauhinia tomentosa L. where extracted and the extracts were subjected to quantitative, qualitative and antibacterial analysis. Quantitative analysis of some phytochemical was also done on the sample. The various extract revelaed the presence of constituents such as flavonoids, alkaloids and saponins. A preliminary phytochemical screening was conducted on the selected medicinal plant extract using standard qualitative procedures that revealed the presence of several secondary metabolites. The antibacterial activity of the crude n-butanol, chloroform and distilled water extract of Bauhinia tomentosa L. using by agar well diffusion assay against five human pathogenic strains of bacterial species, viz., Bacillus subtilis, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhi, and Staphylococcus aureus. n-Butanol extract showed highest activity against Salmonella typhi and Enterobacter aerogenes compared to the other extract. The generated data has provided the basis for its wide use as the therapeutic both in traditional and folk medicine.*

**Key words:** Phytocompounds, Antibacterial activity, Bacterial cultures, *Bauhinia tomentosa* L.

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## INTRODUCTION

The value of medicinal plants to the mankind is very well proven. India harbors about 15 percent (3000 – 3500) medicinal plants, out of 20,000 medicinal plants of the world. About 90 percent of these are found growing wild in different climatic regions of the country. It is estimated that 70 to 80% of the people worldwide rely chiefly on traditional health care system and largely on herbal medicines (Shanley and Luz, 2003). Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations (Shanmugam *et al.*, 2009).

Phytochemistry deals with the analysis of plant chemicals called natural products, and with changes occurring in such chemicals due to alterations in environmental conditions. These compounds are involved as well in allelopathy, dealing with the interactions between two plants, which process can change depending upon variations in the phytochemicals produced under particular environmental conditions (Zobel *et al.*, 1999). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such

efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly due to synthesized during secondary metabolism of the plant (Prusti, 2008).

The development of antimicrobial agents has been undeniably one of the greatest accomplishments of modern medicine. Multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The limited life span of antibiotics has rendered a neccessity to search for new antimicrobial substances from various sources such as medicinal plants. Plants used in traditional medicine are one of the most promising areas in the search for new biologically active compounds. Medicinal plants are well known natural sources for the treatment of various diseases since antiquity. Furthermore, natural products, either pure compounds, or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity (Cos *et al*., 2006). This has urged microbiologists all over the world for formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents (Pandian *et al*., 2006).

Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem. One of the methods to reduce the resistance to antibiotics is by using antibiotic resistance inhibitors from plants (Kim *et al.*, 1995; Alagesaboopathi, 2011). Plants are known to produce a variety of compounds to protect themselves against a variety of pathogens. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens (Ahmad and Beg, 2001). Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the World. Hence, researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (Pavithra *et al.*, 2010).

In recent years, multiple drug resistance has developed due to indiscriminate use of existing antimicrobial drugs in the treatment of infectious diseases. Antimicrobial resistance is a threat to mankind because most of the infection causing bacteria has become multidrug resistant. Antibiotic resistant bacteria may keep people sick longer, and sometimes people are unable to recover at all. Because of the concern about the side effects of conventional medicine, the use of natural products as an alternate to conventional treatment in healing and treatment of various diseases has been on the rise in the last few decades (Kumari *et al.*, 2011).

## MATERIALS AND METHODS

### *Plant Collection*

The flowers of *Bauhinia tomentosa* L. was collected from Thanjavur (Dt.) brought into the laboratory for further processes.

### *Sterilization of Plant Materials*

The disease free and fresh flowers were selected for this investigation. About 2gm of fresh and healthy flowers were taken for each solvent extract including distilled water. Then surface sterilized with 0.1% mercuric chloride or alcohol for few seconds. Again, the plant materials were washed thoroughly with distilled water (Three times).

### *Preparation of Plant Extract*

Two grams of sterilized plant flowers were kept in the 10ml organic solvents such as n-butanol, chloroform and distilled water. Then there are ground with the help of mortar and pestle. The ground plant materials were subjected to centrifugation for further antibacterial screening purpose.

### *Selection of Bacterial Cultures*

Totally five pathogenic bacterial cultures (*Bacillus subtilis, Enterobacter aerogenes, Klebsiella pneumoniae, Salmonella typhi,* and *Staphylococcus aureus*) were selected for the present investigation. The bacterial cultures were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur and used for present investigation.

***Preparation of Microbial Inoculums***
The young microbial cultures were prepared and used during the research period. The Nutrient Broth (NB) was prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures were collected from the institute and inoculated in the tubes by using inoculation needles or loops. After these tubes were incubated ($37^oC$ for 24-28 hrs for bacteria). After incubation the cultures were used for the experiments.

***Preparation of Nutrient Agar Medium***
1000ml of Nutrient agar medium are prepared pH was adjusted to 6.8, using a pH meter by the addition of either acid or alkali. The medium are sterilized by using autoclave of $121^oC$ for 15lbs pressure for 15 minutes and allowed to cool.

***Screening for Antibacterial Activity Assay (Agar well diffusion method)***
The antibacterial activities of the flowers were tested against the selected bacterial cultures. The 20ml of sterilized Nutrient agar medium was poured into each sterile petriplates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. Then a well of 0.5mm are made in the medium by using a sterile cork borer, 150µl of each chloroform, n-butanol, distilled water extracts (flowers) were transferred into separate wells. After these plates were incubated at $37^oC$ for 24-48 hours. After incubation period, the results were observed and measure the diameter of inhibition zone around the each well.

***Antibiotic sensitivity test on bacteria (Positive control)***
The antibiotic sensitivity test using standard antibiotics (kanamycin, methicillin and ampicillin) were analysed by the method of Bauer *et al*., (1996). The sterilized nutrient agar medium was poured into each sterile petriplates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic discs namely kanamycin, methicillin and ampicillin were placed on the bacterial plates. Then, the plates were incubated for 24 hours at $37^oC$. After the incubation period, the results were observed and the diameter of the inhibition zone was measured around the isolates.

***Quantitative analysis on phytochemical constituents of Bauhinia tomentosa L.***
Total alkaloids, flavonoids and saponins were determined using the method described by Krishnaiah *et al*, 2009.

***Determination of alkaloids***
Five grams of the plant sample was placed in a 250ml beaker and 200ml of 10% $CH_3CO_2H$ in $C_2H_5OH$ was added. The mixture was covered and allowed to stand for 4 hours. It was then filtered and the filtrate was concentrated on a water bath until it reaches a quarter of its original volume. Concentrated $NH_4OH$ was added until precipitation was complete. The mixture was allowed to settle and the precipitate collected on a weighed filter paper and washed with dilute $NH_4OH$. The precipitate, alkaloid, was dried and weighed. The percentage alkaloid was calculated by difference.

***Determination of flavonoids***
Ten grams of plant sample was repeatedly extracted with 100ml of 80% aqueous methanol at room temperature. The mixture was then filtered through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and allowed to evaporate to dryness and weighed. The percentage flavonoid was calculated by difference.

***Determination of saponins***
Twenty grams of plant sample was weighed into a 250ml conical flask. 100 ml of 20% $C_2H_5OH$ was added. The mixture was heated over a hot water bath for 4 hours with continuous stirring at about 55°C. It was then filtered with a Whatman No.42 paper. The residue was re-extracted with another 200ml of 20% $C_2H_5OH$. The combined extract was reduced to 40ml over a water bath at about 90°C. The concentrated extract was then transferred into a 250ml separator funnel and 20ml of $(CH_3CH_2)_2O$ was added to the extract and shaken vigorously. The aqueous layer was recovered while the $(CH_3CH_2)_2O$ layer was discarded. This purification process was repeated.

60ml of n-butanol was added and the combined n-butanol extract was washed twice with 10ml of 5% NaCl. The remaining solution was then heated on a water-bath in a pre-weighed 250ml beaker. After evaporation the residue was dried in a Gallenkamp moisture extraction oven (Size 1) to a constant weight. The % saponin was calculated by difference.

***Preliminary Phytochemical Screening***
All the extracts such as chloroform, n-butanol, distilled water of *Bauhinia tomentosa* L. was subjected to routine qualitative chemical analysis to identify the nature of phytochemical constituents present in them (Brindha *et al*., 1982; Harborne, 1998).

***Alkaloids***
A 2 ml of test solution are taken with 2N HCl. Aqueous layer formed was decanted and then added with one or a few drops of Mayer's reagent. Formation of white precipitate or turbidity formed indicates the presence of alkaloids.

***Sterols***
A 2 ml of test solution and minimum quantity of chloroform are added with 3-4 drops of acetic anhydride and one drop of concentrated $H_2SO_4$. Formation of purple color changes into green color that indicates the presence of steroids.

***Phenols***
A 2 ml of test solution in alcohol are added with one drop of neutral ferric chloride (5%) solution. Formation of intense blue color indicates the presence of phenols.

***Saponins***
A 2 ml of test solution are added with $H_2O$ and shaked. Formation of foamy lather indicates the presence of saponins.

***Lignins***
Phloroglucinol with HCl are added with the test solution. Formation of pink color indicates the presence of lignins.

## RESULTS AND DISCUSSION

WHO, (1978) reported that the Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care.

***Antibacterial activity of flower in Bauhinia tomentosa L.***
Silva Peixoto Sobrinho *et al.*, (2012) investigated that the extracts were inactive against *P. aeruginosa*, *K. pneumoniae* and *E. coli*. The samples of *C. infestus*, *C. urens* and the leaves of *C. pubescens* did not show antibacterial activity. The two extracts of *C. quercifolius* were active against strains of *Staphylococcus* and were less active against *E. faecalis*. In our study the n-butanol extract are exhibited zone of inhibition against in *Salmonella typhi* (25mm), *Enterobacter aerogenes* (22mm) and *Klebsiella penumoniae* (20mm). In chloroform extract are showed significant antibacterial activity against salmonella typhi (20mm) and *Enterobacter aerogenes* (20mm). There is no antibacterial activity was observed in distilled water (Table 1). Salihu *et al.*, (2012) studied that the sensitivity test showed that the extracts were active against *S. typhi* and *S. dysenteriae*. Only methanol and chloroform extracts showed activity against *S. aureus* and *E. coli*. While only methanol extract was active against *P. aeruginosa*. By comparison, methanol extract showed the highest activity against the test organisms.

***Antibiotic sensitivity test on bacteria (Positive Control)***
Vaghasiya *et al.*, (2011) suggested that was comparable with some of the standard antibiotics studied. Amongst the Gram negative bacteria studied. The antibacterial activity was comparable with that of standard antibiotics. Amongst the Gram positive bacteria *Enterococci* species showed maximum antibacterial activity. In the present study the antibiotic sensitivity test using standard antibiotics such as methicillin, kanamycin and ampicillin were tested against bacteria studied. The result of antibiotic sensitivity tested presented in table 2. Rosy *et al*., (2010) investigated that the antibiotic disc ampicillin showed antibacterial activity against *Stapphylococcus aureus*, *Staphylococcus epidermidis*, *E. coli*, *Klebsiella* sp. and *Enterobacter* sp. It doesn't show any activity against *Proteus vulgaris* and *Proteus mirabilis.* It had the maximum inhibitory action against almost all the bacteria.

***Effect of solvents on bacteria (Negative Control)***
The results of negative control experiments (solvents) showed no antibacterial activity (Table 3).

***Quantitative phytochemical analysis of flower extract in Bauhinia tomentosa L.***

Salihu *et al.,* (2012) suggested that the phytochemical of the crude extract of *C. alata* revealed the presence of carbohydrate flavonoids and saponins and all extracts tannins, steriols, alkaloids and anthraquinones were not detected in the hexane extract. The test also revealed that the methanol extract have higher contents of the phytochemical. Results for the quantitative analysis carried out on the sample of *Bauhinia tomentosa* L. flower as shown in table 4 revealed that *Bauhinia tomentosa* L. has flavonoid percentage content of 15.80%, alkaloids percentage content of 5.61% and saponins percentage content of 2.1%.

***Qualitative phytochemical analysis of in Bauhinia tomentosa L.***

Krishnaiah *et al.,* (2009) reported a range of 0.24-0.52% alkaloids, 1.1-2.3% saponins and 0.32 - 0.62% flavonoids in various Malaysian herbs. This showed that plants have different concentration of constituents and *Bauhinia blakeana* compares favorably. Phytochemical analysis for methanol extract of *Balanites aogytiaca* fruit revealed the presence of saponin, terpenoids, phenolic compounds and alkaloids with considerable quantities of total phenolic and total flavonoids. In the present study qualitative phytochemical analysis of the different solvent extracts such as chloroform, n-butanol and distilled water of the flower in *Bauhinia tomentosa* L. indicated the presence of lignins, saponins, sterols, alkaloids and phenols (Table 5).

**Table: 1 Antibacterial activity of flower extract in *Bauhinia tomentosa* L.**

| Bacterial cultures | Solvent Extracts (Zone of inhibition in mm) | | |
|---|---|---|---|
| | Chloroform | n-Butanol | Distilled water |
| *Bacillus subtilis* | - | 5 | - |
| *Enterobacter aerogenes* | 20 | 22 | - |
| *Klebsiella penumoniae* | 12 | 20 | - |
| *Salmonella typhi* | 20 | 25 | - |
| *Staphylococcus aureus* | - | 10 | - |

**Table: 2 Antibiotic sensitivity test on bacteria (Positive control)**

| Bacterial culture | Antibiotics (Zone of inhibition in mm) | | |
|---|---|---|---|
| | Methicillin | Kanamycin | Ampicillin |
| *Bacillus subtilis* | 4 | 8 | 3 |
| *Enterobacter aerogenes* | 5 | 10 | 4 |
| *Klebsiella penumoniae* | 3 | 5 | 4 |
| *Salmonella typhi* | 3 | 6 | 4 |
| *Staphylococcus aureus* | 4 | 8 | 3 |

**Table: 3 Effect of solvents on bacteria (Negative control)**

| Bacterial cultures | Solvent Extracts (Zone of inhibition in mm) | | |
|---|---|---|---|
| | Chloroform | n-Butanol | Distilled water |
| *Bacillus subtilis* | - | - | - |
| *Enterobacter aerogenes* | - | - | - |
| *Klebsiella penumoniae* | - | - | - |
| *Salmonella typhi* | - | - | - |
| *Staphylococcus aureus* | - | - | - |

**Table: 4 Quantitative phytochemical analysis of flower extract in *Bauhinia tomentosa* L.**

| Quantitative phytocompounds | Percentage (%) |
|---|---|
| Flavonoids | 15.80 |
| Alakloids | 5.61 |
| Saponins | 2.1 |

**Table: 5 Qualitative phytochemical analysis of flower extract in *Bauhinia tomentosa* L.**

| Phytocompounds | Chloroform | n-Butanol | Distilled water |
|---|---|---|---|
| Lignins | + | - | - |
| Saponins | + | + | + |
| Sterols | + | + | - |
| Alkaloids | - | + | - |
| Phenols | + | - | + |

*+ Present, - Absent*

## CONCLUSION

In the presence study as a source for new potential drugs is still largely on explored and only a small percentage of them has been subjected to phytochemical investigations and the fractions submitted to pharmacological screening is very low. Search screening of various natural organic compounds and identifying active agents is needed of the hour as due to successful prediction of leed molecules and drug like properties at the onset of drug discovery will pay of later in drug development.

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