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Comparison of two *Bauhinia* species Based on the morphological, antimicrobial and protein markers

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Abstract
The present paper deals with the analysis of inter specific relationship between *Bauhinia tomentosa and Bauhinia* species that is *Bauhinia tomentosa. The morphological and antimicrobial analysis of these species had led to the finding that there was an immense variation of these species.* For SDS-PAGE has been effectively used for the comparative study of protein analysis of leaf and seeds. This results was the most important evidence of these two species.

Keywords: *B. tomentosa*, *B. purpurea*, Fabaceae, SDS-PAGE

Introduction
The genus *Bauhinia* belongs to the family Fabaceae. Members of this family are mostly herbs, shrubs and trees with about 730 genera and 19,400 species [1]. *Bauhinia tomentosa* and *Bauhinia purpurea* is considered to the medicinal plants. There are about two hundred species present in this genus and they are distributed into the world. In India, only three species of this genus are distributed, such as *B.tomentosa*, *B.purpurea* and *B.variegata* and it grows well in the drier parts of tropical and subtropical regions of Maharashtra, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab, Karnataka Jammu and Kashmir ascending up to 1,650 meter in the Himalayas [2] and Tamil Nadu. *B. tomentosa* are regularly erroneous identified it has been estimated about 60% of people worldwide mainly used herbal medicine and it is used to cure of dysentery, diarrhoea, anthelmintic, snakebite and skin disease and yellow bell orchid tree, Kanjana in Tamil [3]. In *B. purpurea* are distributed in South China, Cambodia, Myanmar, Thailand, Vietnam, Malaysia, Brunei, Indonesia, Philippines, Singapore and India. In India, it is known as Rajasthan, Gujarat, Madhya Pradesh, Karnataka and Tamil Nadu. It is commonly called purple orchid tree or butterfly tree, nilattiruvatti in Tamil, It is used as a fat burner and it's possess antibacterial, antidiabetic, analgesic, anti-inflammatory, anti-diarrheal, anticancerous, nephroprotective, anti-ulcer [4]. Therefore, this work was intend to differentiated into the two *Bauhinia* species namely *B. tomentosa* and *B. purpurea* based on the morphology, antimicrobial activity and protein analysis between these two morphologically similar species of the same genus.

Materials and methods
Plant Collection
Plant species were collected from the Vathal malai hills, Dharmapuri district in Tamil Nadu, India. The taxonomic indentified of these species were confirmed by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu

Morphology characters of *Bauhinia* species
In order to analyse the morphological variation between the plants of *B. tomentosa* and *B. purpurea* the following characters were studied habit, stem, leaf arrangement, leaf shape, venation pattern, type of fruits and flower colour.

Plant materials
The collected plant samples were shade-dried at ambient temperature (30˚C) and the dried materials were crushed in fine powder using an electric blender.

Solvent extract
Hundred grams of dried powdered materials (Leaves) were soaked separately in 200 ml each of the solvent i.e Methanol and Chloroform in a soxhlet apparatus for 72hrs at 31°C until

complete exhaustion of the material. Each mixture was stirred at every 24hrs using a sterile glass rod. At the end of 72 hrs, each extract was passed through whatman No 1 filter paper and the filtrates were concentrated in vacuum rotary evaporator at 60 °C in order to reduce the volume. The paste like extracts were stored in labeled screw capped bottles and kept in refrigerator at 4 °C. Each of the extract was individually reconstituted using minimal amounts of the extracting solvent prior to use.

Medium for microbial growth

The nutrient agar and rose bengal agar medium were prepared and sterilized for bacteria and fungi respectively. Three bacterial strains consisted of *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* and fungal strains (*Aspergillus niger*, and *Trychoderma viride*). Were obtained from Government hospital Dharmpuri, Tamil Nadu, both strains were used in agar well diffusion [5] methods and the various temperature likewise 35˚C for 24 to 48 hrs for mother culture. The leaf extracts were taken separately at different concentrations of 25 to 100µl. They were kept under incubation. After incubation the plates were observed for the zone formation and the length (mm) of the zone was measured and tabulated.

SDS-PAGE analysis [6]

The plant parts such as of *B.tomentosa* and *B.purpurea* used for SDS-PAGE electrophoretic analysis (leaf and seed) for the separation and characterization of protein, analysis and comparison of proteins in a large number of samples is easily made on SDS-PAGE. Polyacrylamide gels are formed by polymerizing acrylamide with a cross-linker, bisacrylamide in the presence of detergent SDS, free radical APS and TEMED. The polymerization is initiated when APS undergoes homolytic cleavage in the presence of water, giving rise to persulphate free radicals (unpaird electrons). These persulphate free radicals are short- lived and are transferred to TEMED, which are subsequently transferred to acrylamide monomers. These monomers get activated and polymerize in the presence of bisacrylamide in the form of long chains. In SDS-PAGE, works on the basis that proteins are separated based on their charge and mass. Depending upon the number and kind of aminoacids in proteins, the charges of the proteins vary and hence mobility in an electric field. SDS is an anionic detergent which binds strongly to, and denatures proteins. Thereby, all the proteins get a net, uniform negative charge and hence more towards the anode.

Results and Discussion

Characterization of the morphological variation was an essential first step towards executing any organized plant conservation program. The *B.tomentosa* and *B.purpurea* can be distinguished from each other on the basis of their morphological characters. There are some morphological differences among the populations of *B.tomentosa*. For example, in Malaysia *B.tomentosa*, calyx was small and triangular whereas the India *B.tomentosa* was having longer[7]. It was a small tree; leaves are obtuse and green in colour, the flowers are bell shaped and yellow in colour. The fruit are small, containing 3 to 5 seeds, slender and velvety. They are light green, in mature it becomes pale brown in colour but in the case of *B.purpurea* small deciduous tree, the leaf are small and obtuse, the flower are pink, the fruits is long, brownish black in colour and contained 5 to 7 seeds. Leaves are alternate in both *Bauhinia* species (Fig.1 and 2). Therefore the morphological characters are slightly different in both species. The similarly work reported by Pannerselvam [8] with *Withania species*.

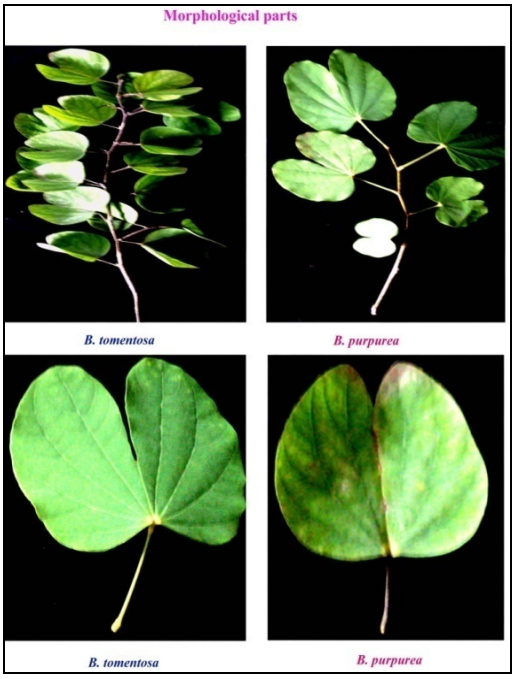

Fig 1

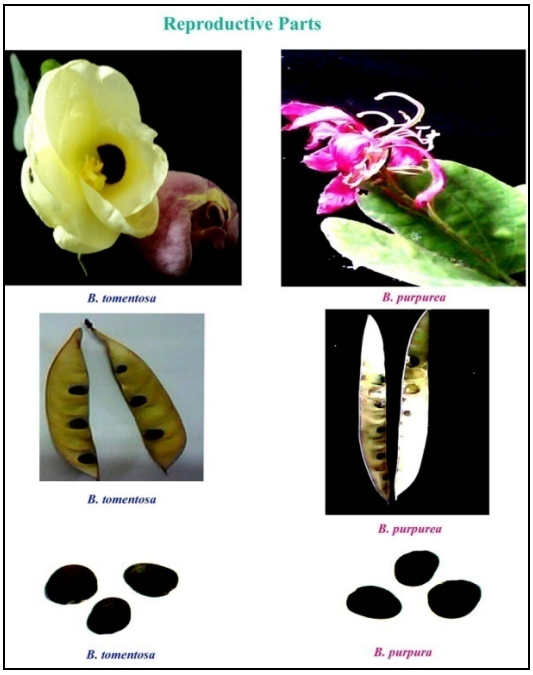

Fig 2

In antimicrobial activity of methanol and Chloroform extract of *Bauhinia* species from leaf samples against microorganisms using agar well diffusion method presented in Table 1-4. Methanol and Chloroform extract was active against bacteria (*Eschrichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae*) and fungi (*Aspergillus niger* and *Trychoderma viride*) strains. As a general rule plant was considered highly active against both bacteria and fungi when the zone of inhibition is greater than 6mm [9].

Effect of *B. tomentosa* methanol extract against bacterial and fungal

The Methanol leaf extract of *B. tomentosa*, a maximum inhibition zone was found to be 25.2.3±3.4mm in the concentration of 100µl, against in *K. pneumoniae* and the minimum inhibition zone 10.5±0.81mm was found in concentration of 25µl *E. coli* bacteria. In fungal pathogen a minimum inhibition zone in concentration of 25µl was observed in *T. viride* with 11.3±0.89mm and the maximum inhibition zone from100µl was observed in *A. niger* with 24.5±4.8mm. (Table.1)

Effect of *B. tomentosa* chloroform extract against bacterial and fungal

In Chloroform, a maximum inhibition zone was found to be 26±5.2mm in the concentration of 100µl against in *K. pneumoniae* and the minimum inhibition zone 10.4±0.91mm was found in concentration of 25µl *S. typhi* bacteria. In fungal pathogen, a maximum inhibition zone was recorded 17.5±2.5mm in the concentration of 100µl against in *T. viride* and a minimum inhibition was found to be 10.4±0.8mm in the concentration of 25µl against in *A. niger*.

Effect of *B. Purpurea* methanol extract against bacterial and fungal

B. purpurea in methanol leaf extract a maximum inhibition zone was found to be 22.3±4.2mm in the concentration of 100µl, against in *K. pneumoniae* and the minimum inhibition zone 10.5±0.81mm was found in concentration of 25µl *S. typhi* bacteria. In fungal pathogen a minimum inhibition zone was found in the concentration of 25µl was observed in *T. viride* with 11.3±0.89mm and maximum inhibition zone from100µl was observed in *A. niger* with 24.5±4.8mm.

Effect of *B. purpurea* Chloroform extract against bacterial and fungal

Chloroform, a maximum inhibition zone was found to be 20.6±5.2mm in the concentration of 100µl against in *E. coli* and the minimum inhibition zone 10.4±0.9mm was found in concentration of 25µl *S. typhi* bacteria. In fungal pathogen, a maximum inhibition zone was recorded 17.5±2.5mm in the concentration of 100µl against an isolated human pathogen *T. viride* and a minimum inhibition was found to be 10.4±0.8mm in the concentration of 25µl against an isolated human pathogen such as *A. niger*. The present study showed enhanced activity in the methanol leaf extract when compared to chloroform in *B. tomentosa*. But in the case of *B. purpurea* a maximum microbial activity was found in the methanol leaf extract when compared to chloroform therefore the application of antimicrobial study has proven to be immense assistance in interpreting problems related to identification of differences between the two species.

Table 1: Leaf Methanol extracts against bacterial and fungal isolates in *B.tomentosa*

Organism	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E. coli	10.5±0.8	11.5±1.3	16.5±1.8	18.5±1.8	10.4±0.9
Salmonella typhi	12.5±1.4	13.3±1.7	18.5±2.4	21.6±2.4	11.3±1.1
Klebsiella pneumoniae	15.4±1.9	18.4±2.4	21.8±2.8	25.2±3.4	11.3±0.9
A. niger	13.4±1.2	13.7±1.8	18.6±2.1	24.5±4.8	10.8±0.8
Trychoderma viride	11.3±0.8	12.2±1.7	16.7±1.8	18±1.5	10.4±0.6

Table 2: Leaf Chloroform extracts against bacterial and fungal isolates in *B. tomentosa*

Organism	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E. coli	10.6±0.9	11.8±1.5	15.3±2.1	18.9±25	10.1±0.7
Salmonella typhi	10.4±0.9	12.3±1.2	16.5±2.2	22.4±2.3	10.3±0.9
Klebsiella pneumoniae	13.7±1.8	18.5±1.8	22.3±4.2	26.5±5.2	10.3±0.7
A. niger	10.2±0.8	14.7±1.3	15.4±1.8	20.3±2.5	11.3±0.9
Trychoderma viride	13.3±1.6	16.7±1.8	18.4±1.5	17.5±2.5	10.4±0.6

Table 3: Leaf Methanol extracts against bacterial and fungal isolates in *B. purpurea*

Organism	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E. coli	10.5±0.8	10.4±0.6	14.8±2.2	18.8±5.5	10.3±0.7
Salmonella typhi	11.3±0.6	12.2±1.7	16.4±1.8	17.5±2.5	10.4±0.6
Klebsiella pneumoniae	12.6±1.5	18.4±2.4	21.6±2.4	22.3±4.2	11.3±0.9
A. niger	10.6±0.9	12.3±1.2	18.8±2.8	24.5±4.8	10.8±0.8
Trychoderma viride	11.3±0.8	11.5±0.9	13.7±1.8	16.7±1.8	10.1±0.4

Table 4: Leaf Chloroform extracts against bacterial and fungal isolates *B. purpurea*

Organism	Zone of inhibition (mm) at volume of leaf sample loaded (µl)				
	25 µl	50 µl	75 µl	100 µl	Control
E.coli	10.5±0.8	13.4±1.4	15.7±1.9	20.6±5.2	10.8±0.8
Salmonella typhi	10.4±0.9	12.7±1.1	14.4±1.6	19.3±2.1	11.3±0.9
Klebsiella pneumonia	12.1±1.5	14.2±1.8	16.7±1.8	18.4±1.5	10.8±0.8
A.niger	10.4±0.8	15.4±2.1	16.6±1.4	18.5±2.4	10.5±0.7
Trychoderma viride	11.3±0.8	11.5±0.9	12.6±1.9	17.5±2.5	10.1±0.5

SDS-PAGE has been effectively used for the comparative study of proteins between the seed, leaf of *B. tomentosa* and *B. purpurea.* In second lane, seed, showed five bands in the range between 20 and 10kda, exactly one band was observed which was prominent and equal in size in both seed (lane 2) and leaf (lane 5) sample (Fig 3). In the results analogous to

the standard 20 band of protein marker, second and third bands were observed in the range between 15 and 10Kda, fourth and fifth bands were seen in 10 and 5Kda. In the lane six of the leaf four bands were observed between 25 and 10Kda, first band is observed in the range between 25 and 20Kda, second band showed and a prominent band in 20, whereas third band was not clear but observed in the range between 15 and 10Kda, fourth band is observed in the range between 10 and 5Kda in *B. tomentosa*.

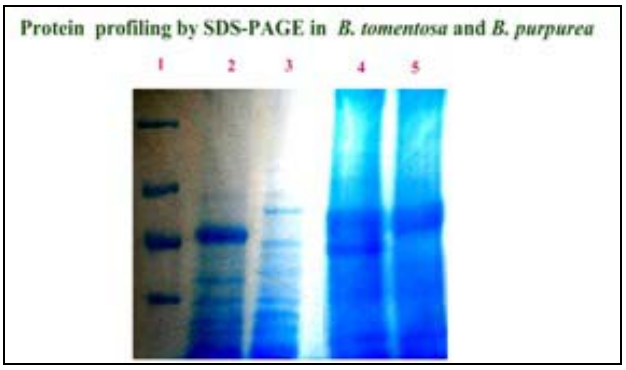

Fig 3: Lane 1: Protein Marker Lane 2: *B. tomentosa* seed Lane 3: *B. purpurea* Lane 4: *B. tomentosa* leaf Lane 5: *B purpurea* leaf

Whereas in *B.purpurea*, seed sample observed in lane three, five bands were seen in the range between 25 and 10Kda, first and second band were observed in between 25and 19Kda whereas third and fourth band were observed in the range between 15and 10 Kda, fifth band was observed in between 10 and 5Kda, the leaf sample of lane seven, among the two bands situated between 25 and 5Kda one band was observed in 25 was prominent and another band was observed in the range between 10 and 5Kda. From the results obtained in *B. purpurea* and *B. tomentosa*, it can be differentiated only based on the protein profiling of leaf and seed. Also there was a presence of unique marker in the plant *B. tomentosa* with a molecular weight of 20Kda present in the lane second and sixth. While comparing both samples, it can be referred that some of the proteins are common for both and some proteins are restricted only to certain parts like seeds and leaf. The outcome of the study is essential for eliminating the problem related to the identification of the species. A similar study was done by Zubaida [10]. in different *Withania* species and exhibited differences in their protein profile.

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