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Evaluation and comparison of antibacterial properties of three species of *Bauhinia* (*Caesalpiniaceae*)

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ABSTRACT: Background: Plants possess a diverse range of antimicrobial activity against pathogenic micro organisms. Aim: The present study was aimed to explore the comparative analysis of antimicrobial activity of selected *Bauhinia* species against selected strains of bacteria. **Methods:** The three species of Bauhinia selected for the study, were *Bauhinia purpurea*, *Bauhinia tomentosa* and *Bauhinia accuminata*. Fresh plant leaves of three species of *Bauhinia* were collected and washed with tap water, air dried, homogenized to a fine powder and stored in air-tight containers. The powder was dissolved in sterile water and a stock solution was prepared with a final concentration of 5 mg/ml. From the stock solution, the sample was diluted and three concentrations were prepared as 2.5, 1.25 and 0.625 mg/ml. **Result:** Different species of *Bauhinia* responded differently to the selected human pathogenic micro-organisms. Powdered water extract of *Bauhinia tomentosa* showed comparatively good inhibitory activity and *Bauhinia accuminata* in its least concentration showed less activity. **Conclusion:** The crude aqueous extracts of *Bauhinia* may be used for various human ailments especially for various infectious diseases as they showed maximum activity against the pathogens.

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INTRODUCTIONS:

The herbal based traditional medicine system continues to play a vital role in health care, with about 80 % of the Worlds inhabitants relying mainly on traditional medicines for their primary health care ^[1]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs ^[2]. Therefore, such plants should be investigated to better understand their properties, safety and efficacy. Plant based antimicrobials represent a vast untapped source of

medicines even after their enormous therapeutic potential and effectiveness in the treatment of infectious disease; hence, further exploration of plant antimicrobials needs to occur [3]. Many plants have been used because of their antimicrobial traits, which are due to the secondary metabolites synthesized by the plants. These products are known by their active substances like, phenolic compounds which are part of the essential oils, as well as in tanning. The screening of plant products for antimicrobial activity have shown that the higher plants represent a potential source of novel antibiotic prototypes [4]. Even though hundreds of plants species have been tested for antimicrobial properties, the vast majority of them have not yet been evaluated. Considering this, an attempt has been made to investigate the antibacterial activity of aqueous extracts of leaf of three species of Bauhinia.

The plants which are selected for the present study were identified by using available Floras and Literature ^[5,6]. It includes three species from the genus *Bauhinia*. It is a genus of more than 100 species of flowering plants in the family *Caesalpiniaceae* with a pantropical distribution. Bauhinia trees typically reach a height of 6 to 12 m and their branches spread 3 to 6 m outwards. The lobed leaves usually are 10 to 15 cm across. The five-petalled flowers are 7.5 to 12.5 cm diameter, generally in shades of red, pink, purple, orange, or yellow, and are often fragrant. The tree begins flowering in late winter and often continues to flower into early summer. Depending on the species, *Bauhinia* flowers are usually in magenta, mauve, pink or white hues with crimson highlights.

The plant Bauhinia purpurea is a medium sized tree with ashy to dark brown bark, young parts pubescent. Leaves are petiolate, petiole are 2.5 to 5 cm long, lamina 7.0 to 18 cm long, rather longer than broad, 9 to 11 nerved, cleft about halfway down into 2 acute or rounded lobes, minutely pubescent below when young. Inflorescence few flowered panicles at the ends of the branches. Flowers are pedicellate, pedicel is 5 to 13 mm long; tomentose, bract is 3 mm long, and bracteole is 2 mm long. Hypanthium is 7 to 10 mm long. Calyx is 2.5-3.0 cm long, usually splitting into two reflexed segments, one emarginate the other 3 toothed. Petals are 3.7 to 5 cm long, oblanceolate, long clawed, spreading, veined. Stamens usually 3 fertile, others reduced to antherless filaments. Ovary downy, long stalked; style long, stigma oblique. Pod is 15 to 25 cm long, with 1.5 to 2 cm broad;

stalk is 2 cm long. Seeds are 12 to 15, almost round, is 1.2 to 1.3 cm in diameter, brown, smooth (Fig 1).



Fig 1. The flowering plant, Bauhinia purpurea L.

This plant *Bauhinia tomentosa* is a shrub or a small tree. Leaves are petiolate with petiole is 2.5 cm long, stipule is 3 to 5 mm long, lamina is 2.5 to 5 cm long, 4.5 to 6.5 cm broad, bilobed, lobes rounded at apex, 7 to 9 nerved at the base, glabrous above, hairy below. Flowers axillary in pairs, sometimes 1 to 3, each on a pedicel bearing a pair of subulate persistent bracteoles. Bracteole is 3 mm long. Calyx is 1.3 to 1.4 cm long, with 2 teeth at the apex in bud, splitting down on one side when the flower opens. Petals are 5 in number and are 1.8 to 5 cm long, obovate, not clawed, yellow, conspicuously veined, one with a red blotch on the inner surface. Stamens are 10 in number. Ovary stipitate; style is 1.8 to 1.9 cm long. Pods dehiscent, 6 to 10 seeded; stipe is 10 to 12.5 cm long, tomentose or glabrous (Fig 2).



Fig 2. The flowering plant, Bauhinia tomentosa L.

The plant Bauhinia acuminata is a shrub, to about 3 m tall; young stems puberulent; stipules linear, puberulent, 1 to 2 cm long, caducous; leaves subcoriaceous, ovate to subrotund, bilobed for about 1/3 the length, lobes acute to acuminate or rarely, rounded, the base rounded to subcordate, 7 to 11 nerved, glabrous above, puberulent, glabrescent, somewhat glaucous beneath; bracts and bracteoles inflorescences lateral, few-flowered. linear; racemose or cymose; flowers about 3.5 to 6 cm long; buds narrowly ellipsoid with 5 apical teeth about 3 mm long; calyx spathaceous, puberulent, about 3 cm long; petals white, elliptic, obtuse, stamens 10, perfect, the filaments connate at the base, otherwise free, 1.5 to 2.5 cm long, pubescent at the base; anthers 4 to 7 mm long with puberulent connective; ovary stipitate, subglabrous or lightly puberulent; style glabrous; stigma peltate; fruit linear-oblong, somewhat septate within, 3 to 11 seeded, 7 to 15 cm long, 1 to 2 cm wide with sharprimmed, raised margins; seeds about 8 to 10 mm long, 6 mm wide, compressed, brown (Fig 3).



Fig 3. The flowering plant, Bauhinia acuminata L.

MATERIALS AND METHODS:

The solvents ethanol, methanol, n-hexane and ethyl acetate were procured from S.D. Fine Chemical, Mumbai. All other chemicals and reagents used in this study were of analytical grade and procured from a authorized dealer.

The processing of plants Bauhinia species:

The different Fresh plant leaves of three species of *Bauhinia* were collected and washed with tap water, air

dried (avoiding direct sunlight), homogenized to a fine powder and stored in air-tight containers. The powder was dissolved in sterile water and a stock solution was prepared with a final concentration of 5 mg/ml. From the stock solution the sample was diluted and three concentrations were prepared as 2.5 mg/ml (A_1 , A_2 , A_3) 1.25 mg/ml (B_1 , B_2 , B_3) and 0.625 mg/ml (C_1 , C_2 , C_3) for the present study.

Antibacterial activity of the extracts:

Antagonistic activity of the plant extracts against different pathogens was checked by Kirby- Bauer disc diffusion method ^[7]. Bacterial pathogens maintained at the National Centre for Aquatic Animal Health (NCAAH) were used for the study. The different human pathogens checked were *V. cholerae*, *E. coli* and *Staphylococcus aureus*.

Nutrient agar (Peptone 0.5 g; beef extract 0.3 g; sea water (50 %) 100 ml; pH 7.2) plates were prepared and swab inoculations of the pathogens were made on the surface to produce a lawn culture. Sterile filter paper discs impregnated with the extracts were placed immediately on the agar surface. The plates were incubated at 37°C for 24 h and observed for halo zone formation. Presence of halo zone around the discs was recorded as positive for antibacterial property.

RESULTS AND DISCUSSION:

The crude extracts from *Bauhinia* were found to have activity against almost all the human pathogens tested (Fig 4 to 6). Activities of extracts are given in Table 1. Inhibitory activity was comparatively high for *Bauhinia tomentosa* against all the pathogens. B1 which is the highest concentration showed the highest activity. *Bauhinia accuminata* in its lowest concentration showed no activity against the pathogens. A concentration of 2.5 mg/ml showed relatively good activity against all the pathogens. As the concentration decreased the overall activity also decreased.

Similar studies were conducted in other species of *Bauhinia* like *B. variegata* ^[8]. They evaluate the antimicrobial efficacy of the ethanol extract of *Bauhinia variegata* leaves. This was tested against both gram positive and gram negative bacteria (*V. cholera, K. Pneumonia, E. coli* and *S. aureus*). Their results showed varying degree of inhibitory potential against all the tested bacteria. They also concluded that, the antimicrobial activities of selected plant species may be

due to its phytochemical content. They also suggested that, further studies are carried out to isolate and characterize the bioactive compounds responsible for this antimicrobial activity.

Table 1. Antibacterial activity of plant extracts

against pathogens.

Pathogens Pathogens	V. cholerae	E. coli	S. aureus
A1	+	+	+
A2	+	+	+
A3	+	+	+
B1	+	+	+
B2	+	+	+
В3	+	+	+
C1	+	+	+
C2	+	+	+
C3	-	-	-

(+ presence; - Absence)

Similarly the hexane, ethyl acetate and methanol extracts of Bauhinia vahlii roots were tested for their antimicrobial activity against both gram-positive and negative bacteria [9]. Their studies also highlights that, the antibacterial activity showed by this selected plant extract may be due to its rich flavonoid and tannin contents. Because Flavonoids and tannins, these plants are known to exhibit antimicrobial activity through formation of a complex with the bacterial cell wall. The present study provides an important basis for the use of extracts from these plants for the treatment of infections associated with the studied microorganisms. Isolation and characterization of bioactive compounds from the selected species of Bauhinia is also suggested further analysis [10].

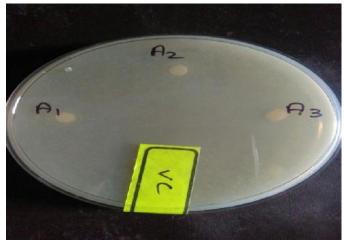


Fig 4. The antimicrobial activity of B. purpurea.



Fig 5. The antimicrobial activity of *B. tomentosa*.



Fig 6. The antimicrobial activity of *B. accuminata*. CONCLUSION:

Plant based antimicrobials have enormous therapeutic potential as they can solve the purpose without any side effects that are often associated with synthetic antimicrobials, continued further research exploration of plant derived antimicrobials is needed today. Medicinal plants were important source for the development of potential, new chemotherapeutic drugs and the in vitro antibacterial test form the basis. The present study was concluded that the antibacterial activity of the crude extracts of Bauhinia species may be used for various human ailments especially for various infectious diseases. Thus these plant species could be utilized as an alternative source of useful antimicrobial drugs. Further studies are also needed to isolate, characterize and elucidate the structure of the bioactive compounds of selected plant species for antimicrobial drug formulation.

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