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Preliminary Phytochemical and Antibacterial activity Studies of *Colvillea racemosa* Bojer

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Abstract

This study investigates the antibacterial properties of an alcoholic extract from *Colvillea racemosa* Bojer against Bacillus subtilis and Escherichia coli. Using the Agar well diffusion method, we assessed the antibacterial activity of the parts of extract on both gram-positive B. subtilis and gram-negative E. coli. The results demonstrated that the alcoholic extract at doses of 200 µg/ml and 400 µg/ml significantly increased the zone of inhibition compared to the control, with a notably larger zone observed at the 400 µg/ml dosage. Furthermore, the difference in mean values between the 200 and 400 µg/ml doses was promising, indicating that the extract has considerable antibacterial efficacy. *Colvillea racemose* Bojer Alcoholic extract, Antibacterial activity, Evaluation, Agar diffusion, Gram positive.

Key Words:

Colvillea racemosa Bojer, Gram-negative E.Coli.

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INTRODUCTION:[1,2]

As natural medicine gains popularity, more people may investigate the potential of employing natural remedies in addition to conventional therapy, as is already the case in some minority cultures. *Colvillea racemosa* Bojer often referred to as the flamboyant tree or yellow flame tree, plant classified as follows

- **Kingdom**: Plantae
- **Clade**: Angiosperms
- **Clade**: Eudicots
- **Clade**: Rosids
- **Order**: Fabales
- **Family**: Casalpiniacea
- **Genus**: *Colvillea*
- **Species**: *C. racemosa*

This species is known for its vibrant yellow flowers and is commonly found in tropical and subtropical landscapes Additionally, natural compounds continue to be a significant source of new drug discoveries. According to a study, many species continue to be used in traditional medicine. There are many plant species for treating skin conditions, for renal and urinary tract problems, for treating diabetes, for digestive system problems, for liver problems, and for treating respiratory conditions and aliments

A Twig of *Colvillea racemosa* Bojer

MATERIALS AND METHODS:[3]

The material parts of plant of *Colvillea racemosa* Bojer were collected from Andhra University area in Visakhapatnam, Andhra Pradesh, India. The collected specimens were botanically identified and confirmed using local flora references, including "Flora of Andhra Pradesh" and "Excursion of Flora of Andhra Pradesh." Fresh materials were gathered, and their morphological features were studied in the field, with photographs taken for documentation. The materials of the plant samples were placed in separate polythene bags and then dried in the laboratory under shade for 3-4 days. The dried materials were stored in dry polythene bags for subsequent phytochemical and biological investigations.

Preparation of alcoholic extract:[4]

Fresh materials of the plant were collected, dried in the shade, and then ground into a powder. To prepare the alcoholic extract, 100 g of the powdered material was mixed with alcohol (methanol) and allowed to extract for 24 hours. Filtration was performed twice at room temperature for 4 hours using a mechanical shaker and Whatman filter paper. The crude extract was subsequently dried at 40°C under reduced pressure and vacuum. The resulting extract was then used for further pharmacological analysis.

Preliminary phytochemical screening:[5,7,8,9]

The extract underwent preliminary phytochemical screening prior to being subjected to the established procedures.

Antimicrobial activity:[6]

The alcoholic extract of the selected plant material was prepared at various concentrations, specifically 200 µg/ml and 400 µg/ml. These concentrations were then used to assess antimicrobial potential in comparison to the respective standard antibiotic at 200 µg/ml.

Test microorganism:

The following bacterial strains were used to evaluate the effectiveness of various antibiotics. All human pathogenic microbial strains were obtained. The strains included Escherichia coli, a Gram-negative bacterium, and Bacillus subtilis a Gram-positive bacterium.

Determination of antimicrobial activity: [10,11,13,14,15,16,17]

The antibacterial activity of the alcoholic extract of *Colvillea racemosa* Bojer was assessed against the Gram-positive bacterium Bacillus subtilis and the Gram-negative bacterium Escherichia coli using the Well Agar Diffusion method. Agar, peptone, and beef extract were accurately weighed, diluted in distilled water, and autoclaved at 121°C for 15 minutes to ensure sterility. After cooling the assay medium to 50°C, plates were prepared with wells. The test organisms were inoculated onto these plates, and the agar wells were filled with 0.1 ml of the test compounds, along with standard and control samples. The plates were then incubated at 37°C for 18 to 24 hours. After incubation, the zones of inhibition produced by the alcoholic extract at concentrations of 200 µg/ml and 400 µg/ml were measured using a scale.

RESULTS:

Preliminary phytochemical screening:

The preliminary phytochemical screening (**Table 1**) indicates the presence of carbohydrates, phenols, flavonoids, and tannins. In contrast, proteins, glycosides, steroids, and terpenes were not detected.

Phytoconstituents	Result
Carbohydrates	+
Proteins	-
Glycosides	-
Phenols	+
Flavonoids	+
Steroids	-
Terpenes	-
Tannins	+

Table 1: Preliminary phytochemical screening of alcoholic extract of *Colvillea Racemosa.*Bojer

Tests for Carbohydrates

1. Benedict's Test

Objective: To identify reducing sugars, such as glucose and fructose.

Method:

- Combine 1 mL of the sample with 2 mL of Benedict's reagent in a test tube.
- Heat the mixture in a boiling water bath for 5-10 minutes.
- Observe any color changes:
  - Blue: No reducing sugars
  - Green: Trace amounts
  - Yellow: Moderate levels
  - Orange/Red: High levels

2. Fehling's Test

Objective: To detect reducing sugars.

Method:

- Mix equal volumes of Fehling’s A (copper sulfate solution) and Fehling’s B (alkaline tartrate solution).
- Add 1 mL of the sample to this mixture.
- Heat in a boiling water bath.
- Look for a color change from blue to brick red, indicating the presence of reducing sugars.

3. Iodine Test

Objective: To identify starch.

Method:

- Add a few drops of iodine solution (iodine in potassium iodide) to the sample.
- A blue-black coloration signifies the presence of starch.

4. Molisch's Test

Objective: To detect carbohydrates in general.

Method:

- Add 2-3 drops of Molisch's reagent to the sample.
- Carefully layer concentrated sulfuric acid down the side of the test tube.
- A purple or violet ring at the interface indicates the presence of carbohydrates.

5. Barfoed's Test

Objective: To differentiate between monosaccharides and disaccharides.

Method:

- Add the sample to Barfoed’s reagent and heat.
- Monosaccharides will produce a red precipitate within 2-3 minutes, whereas disaccharides require a longer time to react.

Tests for Phenols

1. Ferric Chloride Test

Objective: To identify phenolic compounds.

Method:

- Dissolve a small amount of the sample in either water or alcohol.
- Add a few drops of ferric chloride ($FeCl_3$) solution.
- Look for a color change:
  - A purple, blue, green, or red color indicates the presence of phenols.

2. Bromine Water Test

Objective: To confirm the presence of phenolic compounds.

Method:

- Add bromine water to the sample.
- If phenolic compounds are present, the reddish-brown color of the bromine will fade, signaling a reaction.

3. Sodium Metal Test

Objective: To detect phenols by their reaction with sodium.

Method:

- Introduce a small piece of sodium metal into the sample.
- If phenols are present, effervescence (bubbling) will occur due to the release of hydrogen gas.

4. Acidified Dichromate Test

Objective: To identify phenolic compounds through oxidation.

Method:

- Combine the sample with acidified potassium dichromate solution.

- A color change from orange to green indicates the presence of phenols, as they can reduce dichromate.

5. Thymol Test
Objective: To test for specific phenolic compounds.
Method:
- Mix the sample with concentrated sulfuric acid and add thymol.
- A blue color indicates the presence of certain phenolic compounds.

Tests for Tannins
1. Ferric Chloride Test
Objective: To identify the presence of tannins.
Method:
- Dissolve a small amount of the sample in water.
- Add a few drops of ferric chloride ($FeCl_3$) solution.
- A blue, green, or black color change indicates the presence of tannins.

2. Gelatin Test
Objective: To confirm the presence of tannins.
Method:
- Prepare a 1% gelatin solution.
- Add a few drops of the sample to the gelatin solution.
- The formation of a precipitation suggests the presence of tannins.

3. Lead Acetate Test
Objective: To detect tannins through precipitate formation.
Method:
- Mix the sample with lead acetate solution.
- The appearance of a white precipitate indicates the presence of tannins.

4. Modified Stiasny Test
Objective: To identify condensed tannins.
Method:
- Combine a small amount of the sample with a mixture of hydrochloric acid and ethanol.
- Gently heat the mixture, then allow it to cool.
- A red color indicates the presence of condensed tannins.

5. Protein Precipitation Test
Objective: To assess the ability of tannins to precipitate proteins.
Method:
- Combine the sample with a protein solution (e.g., egg albumin).
- The formation of a precipitate indicates the presence of tannins.

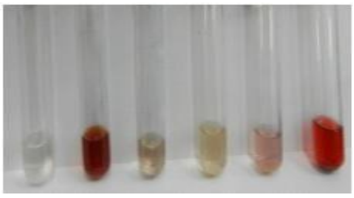

Fig 1. Test for Flavanoids

Fig 2 Test for Carbohydrates

Fig 3 Test for Tannins

Microorganisms	Zone of inhibition in mm			
	Colivllea racemosa **alcoholic extract 200µg/ml**	***Colivillea racemosa*** **alcoholic extract 400µg/ml**	**Standard 200µg/1ml**	**Control**
Escherichia coli	16	18	25	12
Bacillus subtilis	14	21	23	13

Table 2: Antimicrobial activity of alcoholic extract of *Colivillea racemosa* Bojer

Antimicrobial activity:
The analysis of the antibacterial activity of the alcoholic extract of *Colvillea racemosa* Bojer demonstrates that treatment at two dose levels 200 µg/ml and 400 µg/ml—significantly inhibited microbial growth compared to the control group. Notably, the 400 µg/ml group exhibited a substantial zone of inhibition. The significant difference in mean results between the 200 µg/ml and 400 µg/ml doses suggests that the extract's activity is dose-dependent. The outcomes are presented in the following results.

DISCUSSION:
Phytochemical studies of the alcoholic extract of *Colvillea racemosa* Bojer *revealed* the presence of phenols, flavonoids, tannins, and reducing sugars. It has been suggested that phenols and flavonoids may contribute to the antimicrobial activity observed. These findings indicate that the plant contains a diverse array of chemical compounds, which could be responsible for its wide-ranging pharmacological effects. While the specific roles of these compounds were not explored in this study, it is known that many active plant constituents include flavonoids, steroids,

glycosides, and alkaloids. Despite the availability of numerous antibiotics, the increasing ability of microorganisms to develop multidrug resistance has prompted researchers to search for new, effective bioactive compounds from herbal sources. The findings of this study indicate that further tests were conducted on the alcoholic extract of *Colvillea racemosa.*Bojer

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