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In Vitro Antibacterial Activity and Phytochemical Profiling of Flower Extracts from *Hibiscus rosa-sinensis, Cassia auriculata, Clitoria ternatea,* and *Catharanthus roseus* as Potential Natural Therapeutic Agents Against Pathogenic Bacteria

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KEYWORDS

Antibacterial activity; Medicinal plants; Phytochemical analysis; Natural Antibacterial agents.

ABSTRACT:

Introduction: Medicinal plants are abundant in bioactive compounds with significant therapeutic potential, making them a cornerstone for drug discovery and alternative therapies. *Hibiscus rosa-sinensis, Cassia auriculata, Clitoria ternatea,* and *Catharanthus roseus* have been traditionally used in medicine and are known for their antibacterial properties, offering promise in combating antibiotic-resistant bacteria.

Objectives: This study aims to evaluate the in vitro antibacterial efficacy of flower extracts from these plants and identify their bioactive compounds to support the development of novel therapeutic agents for bacterial infections.

Methods: Standard microbiological techniques were employed to assess the antibacterial activity of the flower extracts against pathogenic bacteria. Phytochemical analyses were conducted to profile the bioactive compounds present in the extracts, integrating traditional knowledge with modern scientific approaches.

Results: The extracts demonstrated notable antibacterial activity against pathogenic bacteria, underscoring their potential as natural remedies. Phytochemical profiling revealed diverse bioactive compounds that could serve as the basis for developing novel antibacterial agents.

Conclusion: The findings validate the efficacy of these medicinal plant extracts in combating bacterial infections, offering a promising avenue to address the global challenge of antibiotic resistance. The study highlights the importance of leveraging traditional knowledge and scientific innovation to explore plant-based solutions for healthcare challenges.

1. Introduction:

Medicinal plants provide primary health care for most people worldwide. Similar to their past accomplishments, plants continue to be crucial for conserving human health because they serve as significant sources for the research and creation of potent antimicrobial drugs [1]. According to the World Health Organization, between 25 and 50 per cent of pharmaceuticals worldwide are derived from plants, and only a small number have been used as antimicrobials [2].

Journal of Chemical Health Risks
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India is renowned for its vast diversity of plant species, many of which have been traditionally used for medicinal purposes over the years. The Herbal industry stands as the top revenue-generating sector [3]. Most of the potential for using plants as a source of pharmaceuticals remains unexplored. The primary medicinal qualities of phytochemicals found in plants are their antipyretic, antibacterial, and antioxidant properties [4]. Alkaloids, glycosides, volatile oils, tannins, saponins, and other phytochemicals are primarily responsible for the medicinal properties of plants [5]. Medicinal plants frequently utilize antimicrobial agents, making it crucial to explore their antibacterial capabilities. Research indicates that essential oils and various plant extracts are used in traditional medicine, sparking interest in natural products as possible alternatives to conventional treatments for a range of infectious diseases [6]. An in-depth exploration of the antibacterial qualities of plants utilized in traditional cultures can pave the way for the creation of invaluable plant-based treatments for severe diseases. This research focused on identifying phytochemicals and examining the antibacterial characteristics of Cassia auriculata flowers. Known as Tanner's Cassia and "Aavaarai" in Tamil, this plant is a common weed found in the dry regions of Rajasthan, Madhya Pradesh, and Tamil Nadu. The plant produces clusters of flowers. The petals and sepals are five in number, separate, with wavy edges, large (almost 5 cm in diameter), bright yellow with orange veins, and smooth, with pedicles measuring 2.5 cm in length. There are about ten distinct anthers, with three upper stamens being sterile; the ovary is superior, single-chambered, and contains marginal ovules [7]. This study examined the antimicrobial performance of unique approaches to extraction with acetone, chloroform, ethanol, petroleum ether, and aqueous extracts from the flowers of *Hibiscus rosa-sinensis, Casia auriculata, Clitoria ternatea, and Catharanthus roseus*) which were examined using the agar well diffusion method against a few pathogenic bacteria and indicated the phytochemicals present in various flower extracts [8].

2. Materials and Methods: (Plate 1)

2.1. Delectation of Plants:

The medicinal flowers used in this study (*H. rosa-sinensis, C. auriculata, C. ternatea, and C. roses*) were collected from homes and streets in Surandai, Tenkasi district, Tamil Nadu, and authenticated by the Department of Botany, Sadakathullah Appa College, Rahmath Nagar, Tirunelveli-627011. Their physical attributes identified them. Table 1 provides the ethnobotanical information for *H. rosa-sinensis, C. auriculata, C. ternatea,* and *C. roses*. The flowers were thoroughly washed and rinsed with sterile distilled water before being allowed to dry at room temperature. They were then crushed into small pieces using a pestle and mortar.

2.2. Preparation of extracts:

The powdered flowers, including *H. rosa-sinensis, C. auriculata, C. ternatea, and C. roses*, were sequentially extracted using 250 mL of Ethanol, Petroleum ether, Chloroform, and Acetone in a Soxhlet apparatus. The resulting extracts were concentrated into a liquid form and stored at 4°C in an airtight container for future use [9].

2.3. Preliminary Phytochemical analysis:

The ethanol, petroleum ether, chloroform, acetone, and aqueous extracts of flowers of *H. rosa-sinensis, C. auriculata, C. ternatea, and C. roses*, were analyzed by the following procedures for the presence of alkaloids, steroids, Fehling's test, anthrone test, reducing sugar, tannins, saponin, flavonoids, terpenoids, glycosides, amino acids, and essential oils [10].

2.3.1. Alkaloids: Each plant extract, in a volume of one mL, was individually mixed with 3 mL of a diluted 2N HCl solution and then filtered. The resulting filtrate was then treated with two drops of Mayer's reagent. The appearance of a cloudy white or light-yellow precipitate signified the presence of alkaloids [11].

2.3.2. Steroids: The plant extract (2 ml) was subjected to treatment with a few drops of methyl chloride, three to four drops of acetic anhydride, and one drop of concentrated sulfuric acid. The transition of the purple color to blue or green serves as an indicator of the presence of steroids [12].

2.3.3. Fehling's test: Plant extract (2 ml) was treated with 2 ml of Fehling's reagent and 3 ml of water. The mixture was boiled for a few seconds. The red-orange color indicates the presence of sugars [13].

2.3.4. Anthrone Test: Each plant extract (2 mL) was combined with an equivalent volume of anthrone, followed by the addition of a few drops of concentrated

Journal of Chemical Health Risks
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JCHR (2026) 16(2), 1000-1009 | ISSN:2251-6727

sulfuric acid, and subsequently heated in a water bath. The emergence of a dark-green coloration serves as an indicator of the presence of carbohydrates [14].

2.3.5. Tannins: The plant extract was diluted with water and subsequently treated with lead acetate powder. The emergence of a white precipitate served as an indicator of the presence of tannins within the extract [15].

2.3.6. Saponins: Each plant extract (2 ml) was diluted with distilled water and thoroughly agitated. The formation of a foamy lather served as an indicator of the presence of saponins [16].

2.3.7. Flavonoids: A volume of 2 ml of each plant extract was combined with a small quantity of magnesium powder and subsequently treated with one or two drops of concentrated hydrochloric acid. The mixture was then subjected to heating. The emergence of a red or orange-red coloration signified the presence of flavonoids [16].

2.3.8. Terpenoids: The extracts were subjected to chloroform treatment, followed by filtration and the addition of a few drops of concentrated sulfuric acid. The mixture was then agitated and allowed to settle. The emergence of a reddish-brown coloration indicated the presence of terpenoids [17].

2.3.9. Glycosides: The extracts were treated with a ferric chloride solution and subsequently hydrolyzed using diluted hydrochloric acid. They were then immersed in boiling water for five minutes. After cooling, an equal volume of benzene was introduced. A modified Borntrager test was employed to separate and treat the benzene layer with an ammonia solution. The formation of a pink color signified the presence of anthranol glycosides [18].

2.3.10. Amino Acids: The extracts were treated with a 0.25% ninhydrin reagent and subjected to boiling for a few minutes before being cooled. The development of a blue color indicated the presence of amino acids [19].

2.3.11. Test for essential oils: One millilitre of the test solution, alcoholic K2Cr2O7, and a few drops of phenolphthalein soap were added. Presence of essential oils [20].

2.4. Antibacterial Activity Procedure:

2.4.1. Test Organism: The test microorganisms, *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Lactobacillus acidophilus,* were purchased from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh. Bacterial strains were maintained on nutrient agar (NA).

2.4.2. Nutrient Broth Preparation: Pure cultures were inoculated onto nutrient agar plates and subcultured at 37 °C for 24 h. Inoculum was prepared by adding fresh culture into 2 ml of sterile 0.145 mol/L saline tube, and cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. This standardized inoculum was used for antimicrobial testing [21].

2.4.3. Antibacterial Test: Antibiotic susceptibility tests used the agar disc diffusion (Kirby-Bauer) method. The medium was prepared by dissolving 38 g Mueller-Hinton Agar Medium (Hi-Media) in 1000ml distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121^0C for 15 min (pH 7.3), cooled and poured into Petri plates (25 ml/plate). The plates were swabbed with bacterial cultures of *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Lactobacillus acidophilus*. The sample-loaded disc was placed on the Mueller-Hinton agar medium. Standard drug discs, including ciprofloxacin (5 mcg), vancomycin (30 mcg), and ampicillin (10 mcg), served as positive controls, with an empty sterile disc as a negative control. The plates were incubated at 37°C for 24 hours. After incubation, inhibition zones around discs were measured in millimeters using a transparent ruler. The absence of zone inhibition indicated no activity [22].

3. Result and Discussion:

3.1. Phytochemical Analysis: This study conducted phytochemical analysis of flower extracts from *Hibiscus rosa-sinensis, Cassia auriculata, Clitoria ternatea,* and *Catharanthus roseus* to screen phytochemical components and evaluate their antibacterial potential, given increasing antibiotic resistance. Initial investigations identified alkaloids, tannins, saponins, flavonoids, terpenoids, and essential oils in the extracts. The ethanol, petroleum ether, and acetone extracts of *Hibiscus rosa-sinensis* showed greater inhibitory effects compared to others (Table 2). Flavonoids, glycosides,

Journal of Chemical Health Risks
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phenolic compounds, tannins, terpenoids, phytosterols, carbohydrates, proteins, amino acids, gum, and mucilages were detected in all morphotypes, while alkaloids, saponins, fixed oils, and fats were absent [23]. The petroleum ether and acetone extracts of *Catharanthus roseus* demonstrated stronger inhibitory effects than other extracts (Table 5). The petroleum ether extracts of *Cassia auriculata* exhibited stronger inhibitory effects, with investigations revealing alkaloids, saponins, terpenoids, phenols, tannins, flavonoids, carbohydrates, proteins, and amino acids [24]. The ethanol and methanol extracts of *Cassia auriculata* showed superior inhibitory effects, suggesting active constituents are better extracted with ethanol and methanol than with acetone, chloroform, and aqueous extracts (Table 3). *Cassia auriculata* flowers contain active principles with therapeutic value against various diseases [25]. The petroleum ether and acetone extracts of *Clitoria ternatea* showed pronounced effects (Table 4). Phytochemical screening tested for tannins, anthraquinones, alkaloids, saponins, phlorotannins, flavonoids, cardiac glycosides, volatile oils, terpenoids, and steroids. *Clitoria ternatea* leaves contain intermediate amounts of tannins, cardiac glycosides, and steroids, and small amounts of alkaloids [26]. The flowers contain moderate amounts of phlorotannins, flavonoids, and terpenoids, while roots contain small amounts of flavonoids, volatile oils, and terpenoids. Anthraquinones and saponins are absent in the plant [27].

3.2. Antibacterial Activity: Four medicinal plant extracts (*Hibiscus rosa-sinensis, Cassia auriculata, Clitoria ternatea, and Catharanthus roseus*) were evaluated for antimicrobial activity against four bacterial strains using the disc diffusion method, revealing solvent-dependent efficacy and differential bacterial susceptibility. *Hibiscus rosa-sinensis* exhibited the highest inhibition with lipophilic solvents, notably petroleum ether against *K. pneumoniae* (14 mm) and acetone against *L. acidophilus* (17 mm), whereas aqueous extracts demonstrated reduced activity (Table 6, Graph 1 & Plate 2). *Cassia auriculata* displayed selective antimicrobial effects, with ethanolic extracts most effective against *S. aureus* (15 mm) and aqueous extracts against *E. coli* (11 mm), but showed no activity against *K. pneumoniae* with certain solvents (Table 7, Graph 2 & Plate 3). *Clitoria ternatea* presented a balanced antimicrobial profile across solvents, particularly aqueous extract against *L. acidophilus* (15 mm) and ethanolic extract against *E. coli* (12 mm) (Table 8, Graph 3 & Plate 4). *Catharanthus roseus* exhibited a narrower spectrum, with ethanolic and petroleum ether extracts active primarily against *E. coli* and *L. acidophilus*, while aqueous and acetone extracts were largely ineffective (Table 9, Graph 4 & Plate 5) [28]. Overall, *Lactobacillus acidophilus* were the most susceptible organism (13–17 mm zones), followed by *S. aureus, E. coli*, and *K. pneumoniae*, which demonstrated the greatest resistance. These variations correspond to differences in phytochemical solubility and bacterial structural defences, including the accessibility of gram-positive cell walls versus the protective outer membranes and efflux systems of gram-negative bacteria [29]. The antimicrobial activity is attributed to secondary metabolites that disrupt bacterial membranes and inhibit enzymatic functions, with solvent polarity influencing the extraction of bioactive compounds [30]. These findings underscore the therapeutic potential of *Hibiscus rosa-sinensis* and *Cassia auriculata* against Gram-positive infections, and *Clitoria ternatea* for broad-spectrum applications, while highlighting the necessity to consider the effects on beneficial microbiota, such as *L. acidophilus* [31].

4. Conclusion:

This study's outcomes reveal that the flower extracts examined possess varying levels of antimicrobial effectiveness. The antibacterial potential found in extracts from *Hibiscus rosa-sinensis, Cassia auriculata, Clitoria ternatea,* and *Catharanthus roseus* suggests their promise as potent herbal antibacterial agents. These early findings point to the notable antimicrobial properties of these plants. However, extensive pharmacological research is essential to confirm and support the therapeutic use of these herbal extracts.

Table 1: Ethnobotanical data for medicinal plants

S No	**The Botanical Identity**	**Family**	**English Name**	**Local Mame**	**A Section of The Plant**	**Area of Collection**

					Using	
1	*Hibiscus rosa-sinensis*	Malvaceae	Chinese hibiscus	Sembaruthi	Flowers	Surandai
2	*Casia auriculata*	Caesalpiniaceae	Tanners' cassia	Aavarai	Flowers	Surandai
3	*Clitoria ternatea*	Fabaceae	Butterfly pea	Sangupoo	Flowers	Surandai
4	*Catharanthus roseus*	Apocynaceae	Periwinkle	Nithiyakalyani	Flowers	Surandai

Table 2: phytochemical screening results for *Hibiscus rosa-sinensis* using various solvent extracts

Test name	Petroleum ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	+	-
Steroids	-	-	-	+	-
Reducing sugar	+	+	-	+	-
Tannins	+	+	+	+	-
Saponins	-	-	+	+	-
Flavonoids	+	-	+	+	-
Terpenoids	+	-	+	+	+
Glycosides	-	-	+	+	-
Amino acid	-	-	-	+	-
Essential oils	+	-	+	+	+

Table 3: phytochemical screening results for *Cassia auriculata* using various solvent extracts

Test name	Petroleum ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-
Steroids	+	-	-	-	-
Reducing sugar	+	+	+	+	-
Tannins	-	-	-	-	-
Saponins	-	-	+	-	-
Flavonoids	+	-	-	-	+
Terpenoids	+	+	-	+	+
Glycosides	+	-	-	-	-
Amino acid	-	-	-	-	-

Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 1000-1009 | ISSN:2251-6727

Essential oils	+	+	+	+	+

Table 4: phytochemical screening results for *Clitoria ternatea* using various solvent extracts

Test name	Petroleum ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-
Steroids	-	-	-	-	-
Reducing sugar	+	+	+	+	-
Tannins	+	-	-	-	-
Saponins	-	-	+	-	-
Flavonoids	+	-	-	-	-
Terpenoids	+	+	+	+	+
Glycosides	-	-	-	-	-
Amino acid	-	-	-	-	-

Essential oils	+	+	+	+	+

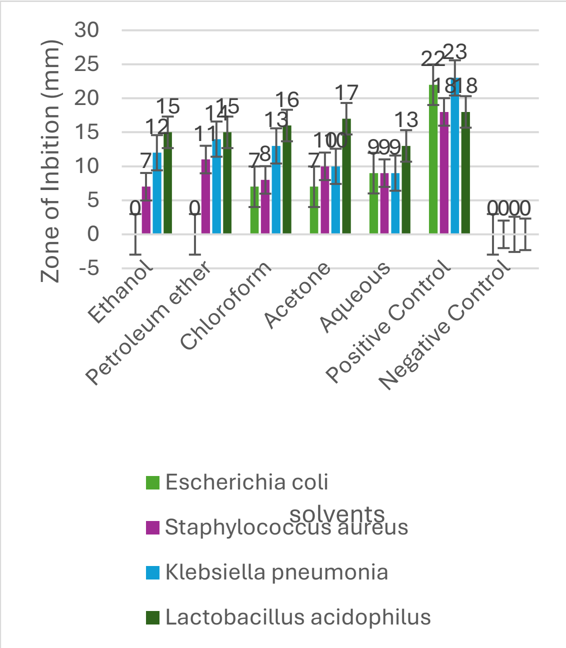
Table 5: phytochemical screening results for *Catharanthus roseus* using various solvent extracts

Test name	Petroleum ether	Chloroform	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-
Steroids	+	+	+	+	-
Reducing sugar	+	+	+	+	+
Tannins	+	+	-	-	-
Saponins	+	-	+	-	-
Flavonoids	+	-	-	-	-
Terpenoids	+	+	+	+	+
Glycosides	-	-	+	+	-
Amino acid	-	-	-	-	-

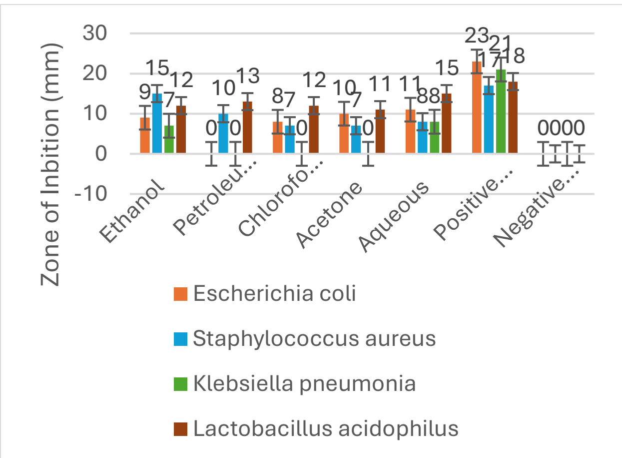
Journal of Chemical Health Risks
www.jchr.org
JCHR (2026) 16(2), 1000-1009 | ISSN:2251-6727

Essential oils	+	+	+	+	+

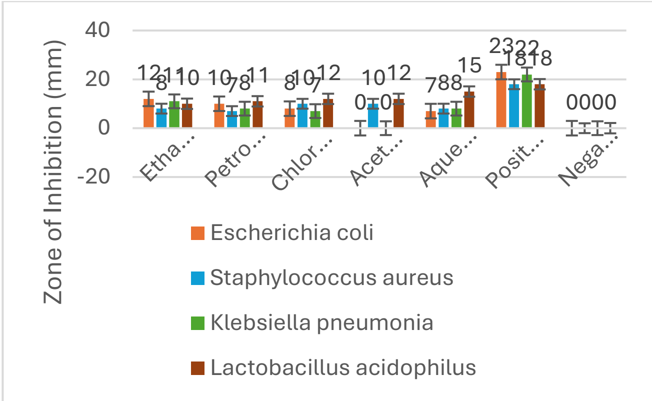
Graph 1: The antimicrobial activity of various solvents of *Hibiscus rosa-sinensis* against different microorganisms



Graph 2: The antimicrobial activity of various solvents of *Cassia auriculata* against different microorganisms



Graph 3: The antimicrobial activity of various solvents of *Clitoria ternatea* against different microorganisms



Graph 4: The antimicrobial activity of various solvents of *Catharanthus roseus* against different microorganisms

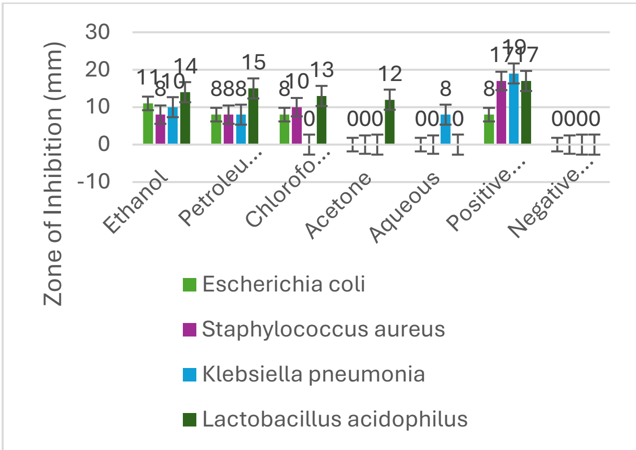


Plate 1: Graphical Abstract

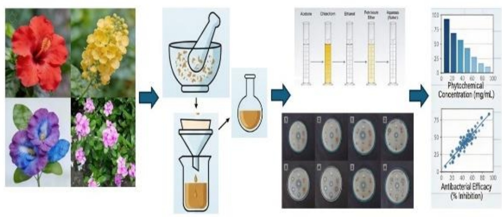


Plate 2: The antimicrobial activity of various solvents of *Hibiscus rosa-sinensis* against different microorganisms

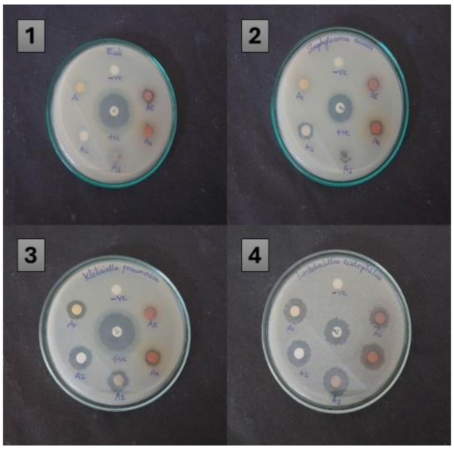


Plate 3: The antimicrobial activity of various solvents of *Cassia auriculata* against different microorganisms

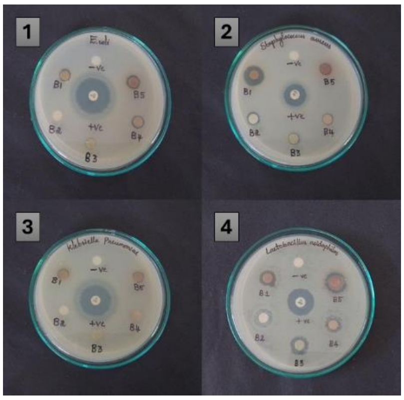


Plate 4: The antimicrobial activity of various solvents of *Clitoria ternatea* against different microorganisms

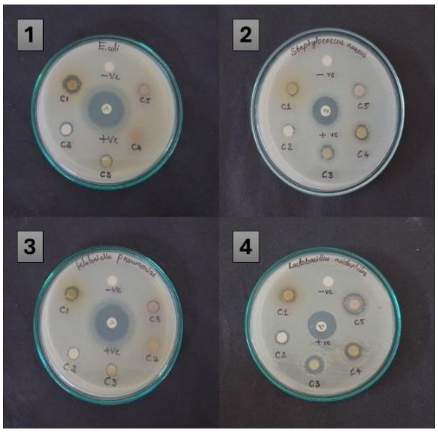
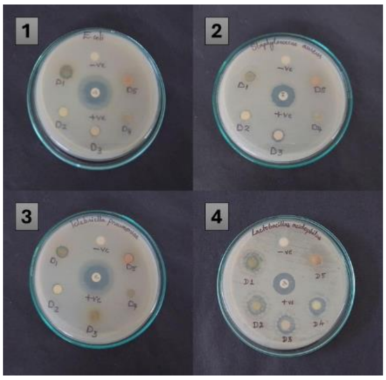


Plate 5: The antimicrobial activity of various solvents of *Catharanthus roseus* against different microorganisms



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conflict of interest:

Nil

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