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Preliminary Phytochemical Analysis, Metal Scavenging Activity and Anti-Bacterial activity of *Catharanthus roseus* against Human Infections

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Abstract

The biological production of natural compounds from plants is a fascinating technique in the fields of science and biotechnology. *Catharanthus roseus (C. roseus)*, an ethnomedicinal species, has been used for generations to address various health issues like inflammation, diabetes, kidney, liver and cardiovascular diseases in humans. The leaves of *C. roseus* contains secondary metabolites like flavonoids, alkaloids, tannins, saponins, phenols that exhibit antimicrobial and anticancer properties. The flowers of *C. roseus* are characterized by the presence of Anthocyanin as a pigment. In the current era of rapid industrialization, there has been a notable rise in cadmium contamination. Cadmium (Cd) is emitted into the environment through industrial activities and can pollute air, water, and soil. Because of its long half-life, cadmium is associated with numerous health issues such as liver and kidney toxicity, neurological damage, and degenerative bone diseases. This study focuses on the risks associated with Cd, Hg, and Pd, as well as the potential of *C. roseus* extract as an alternative medicinal remedy to alleviate symptoms. The leaf extract of *C. roseus* will undergo analysis for secondary metabolites through preliminary tests and thin-layer chromatography. The antimicrobial properties

assessed against pathogenic microorganisms using the Kirby-Bauer method. The ability to scavenge metals was evaluated using anthocyanin extracts in relation to heavy metals (Cd, Hg, and Pb). Additionally, the anthocyanin pigment extracted from the fresh flowers of *C. roseus* can serve as a pH indicator. The anticipated results include identifying bioactive compounds, assessing antimicrobial activity, and determining the metal scavenging efficiency of the tested plant. Future possibilities involve the use of these plants to lower metal levels in human organs and prevent microbial growth. This research seeks to offer an environmentally friendly approach to address serious human infections.

Keywords: Catharanthus roseus, Phytochemical, Metal scavenging activity, Anti-Bacterial activity, pH indicator

Introduction

Plants are primarily utilized for their potential in treating various human diseases. They generate a range of secondary metabolites, such as alkaloids, flavonoids, saponins, steroids, cyanogenic glycosides, and terpenoids, to defend against naturally occurring pathogens, insect pests, and environmental stresses (Chinna Venkataraman G and Rajendran S., 2012) & (Vosburgh W. C and Cooper GP., 1941). Herbal plants traditionally used in medicine are rich in diverse bioactive compounds that may serve as alternative therapeutic options for the prevention of numerous infectious diseases. Medicinal plants are regarded as effective and safer alternatives to synthetic antibiotics. *Catharanthus roseus* is a significant medicinal plant belonging to the Apocynaceae family, which includes over 70 distinct alkaloids and chemotherapeutic agents effective in combating various types of cancer, including breast cancer, lung cancer, uterine cancer and melanomas as well as Hodgkin's and non-Hodgkin's lymphoma (Monika S and Vandana S 2013) & (Sarabjot K and Poonam M., 2014). Commonly referred to as *Vinca rosea*, *Ammocallis rosea*, and *Lochnera rosea*. *Catharanthus roseus* is an herb native to India that grows wild in the Indian subcontinent across southern Asia. There are two commonly grown varieties of *Catharanthus roseus*, which are named based on the color of their flowers: Pink (Rosea) and White (Alba) (Rischer H *et al.*, 1970). Traditionally, the leaves of *Catharanthus roseus* are utilized medicinally to treat a variety of conditions, including menorrhagia, rheumatism, dyspepsia, indigestion, dysmenorrhea, diabetes, hypertension, cancer, menstrual disorders, skin diseases, bleeding diarrhea and they also possess sedative properties (Chinna Venkataraman G and Rajendran S., 2012) & (Sarabjot K., Poonam M., 2014).

Plant Morphology

Catharanthus roseus is a perennial sub-shrub that can reach heights of up to 1 meter. The leaves are oval to oblong in shape, measuring 2.5 to 9.5 cm in length and 1 to 3.5 cm in width, exhibiting a glossy green color, devoid of hairs, with a pale midrib and a short petiole approximately 1 to 1.8 cm long, arranged in opposite pairs. The flowers range from white to deep pink, featuring a dark red center, with a tubular base measuring about 2.5 to 3 cm in length and a corolla that is 2 to 5 cm in diameter with five petal-like lobes. The fruit consists of two follicles that are about 2 to 4 cm long and 3 mm in width (Rischer H *et al.,* 1970).

Materials and Methods

Collection of Plant Material: *Catharanthus roseus* is a type of flowering plant that is also valued for its ornamental and healing properties. Healthy and fresh leaves from *C. roseus* plants were collected from P. Andipatti, Virudhunagar district. Afterward, these leaves were dried in the sun shade. The dried leaves were then ground into a powder using an electric mixer.

Extraction of Leaves: 20 grams of dried *Catharanthus roseus* powder were placed in filter paper. The packed leaf powder was positioned in the extractor of the Soxhlet apparatus. Subsequently, 300 ml of ethanol solution was added to the extractor. The condenser was then attached to the extractor. After that, the electrical current and heating mantle (set at 60-70ºC) were connected and activated. Following a 72-hours, the extract was settled in the collection flask. The contents of the distillation flask were filtered using Whatman No. 1 filter paper. Finally, the extract was kept alone for the evaporation of excess solvent to converted to the semi-solid form and the extract was used for further processes. This method was carried out repeatedly for the extraction of powdered leaves from *Catharanthus roseus* with petroleum ether.

Phytochemical Analysis

Detection of Alkaloids: Add 1 mL of extract with 1 mL of Mayer's reagent and a few drops of iodine solution. The formation of a yellow precipitate signifies the presence of alkaloids.

Detection of Terpenoids: Mix 1 mL of crude extract with 1 mL of concentrated H_2SO_4 and heat for 2 minutes. A grayish hue indicates the presence of terpenoids.

Detection of Phenol and Tannins: Add1 mL of crude extract with 1 mL of $FeCl_3$. A blue-green or black color suggests the presence of tannins.

Detection of Reducing Sugar: To 1 mL of extract, add 1 mL of Fehling's A solution and 1 mL of Fehling's B solution. The appearance of a red color confirms the presence of sugar.

Detection of Saponins: Add 2 mL of distilled water to 1 mL of extract, shake vigorously, and the formation of a 1 cm foam layer indicates the presence of saponins.

Detection of Flavonoids: To 1 mL of extract, add small pieces of magnesium ribbon and a few drops of concentrated HCl gradually. The development of a pink scarlet color confirms the presence of flavonoids.

Detection of Quinones: Mix 1 mL of extract with 1 mL of 1% NaOH. The appearance of a blue-green or red color indicates the presence of quinones.

Detection of Protein: Add a few drops of mercuric chloride to 1 mL of extract. The formation of a yellow color signifies the presence of protein.

Detection of Steroids: Mix 1 mL of extract with 1 mL of chloroform and add concentrated H_2SO_4 along the side. A red color in the lower chloroform layer indicates the presence of steroids.

Metal Scavenging Activity

Pigment Extraction: A total of 25 grams of fresh *Catharanthus roseus* flowers were placed in a conical flask containing 100 ml of acidified ethanol solution (85 ml of ethanol and 15 ml of Hcl). The flask was then positioned on a mechanical shaker for a duration of three days. After this period, the mixture in the flask was filtered through Whatman No. 1 filter paper. The residue left on the filter paper washed with the same solvent and the filtration process was repeated several times until a clear solution of anthocyanin was obtained (Harborne JB., 1998).

Purification of Anthocyanin: 5 ml of the extract was mixed with 10 ml of petroleum ether (boiling point 40 ºC - 60 ºC), followed by the addition of 10 ml of ethyl acetate to eliminate non-polar impurities and other flavonoids (Kratika K and Sharmita G 2013). The aqueous layer was subsequently concentrated under vacuum at 40°C to obtain pure anthocyanin pigment.

Preparation of pigment: 0.0225 grams of the pigment were dissolved in 20 ml of ethanol in a 100 ml flask, and then the solution was brought up to the mark with ethanol to create a ($0.5x10^{-3}$) M concentration.

Preparation of the Metal solutions: Three different types of heavy metals such as Mercuric II chloride, Silver II nitrate, and Lead II nitrate were chosen for this study.

Mercuric (II) chloride solution: 9 grams of Mercuric II chloride was dissolved in 20 ml of distilled water and then the solution was brought up to the mark with distilled water to yield a ($0.5x10^{-3}$) M concentration.

Lead (II) nitrate solution: 0.0165 grams of Lead II nitrate was dissolved in 20 ml of distilled water and the solution was then brought up to the mark with distilled water to achieve a (0.5x10^-3) M concentration.

Silver (II) nitrate solution: 1.6987 grams of silver II nitrate was dissolved in 20 ml of distilled water, followed by adjusting the solution to the mark with distilled water to provide a ($0.5x10^{-3}$) M concentration.

Absorption Spectra: The UV-Visible spectrum of the pigment solution ($0.5x10^{-3}$) M made from the standard was determined. A total of 2.5 ml and 2 ml of the ($0.5x10^{-3}$) M pigment solutions were mixed with 1 ml of $0.5x10^{-3}$M solutions of each Pb $(NO_3)_2$, Ag $(NO_3)_2$, and $Hgcl_2$, and the resulting volume was brought to 10 ml with distilled water. The UV-Vis spectra for both the mixtures and the pure pigment were measured.

Continuous variation method: Master solutions of equal molar concentrations ($0.5x10^{-3}$ M) of the pigment in ethanol and Pb $(NO_3)_2$ in distilled water were prepared (Vosburgh W. C and Cooper GP., 1941). A range of 10 ml mixtures consisting of complementary proportions of the two solutions (1:9, 2:8, 3:7, up to 9:1) were poured into various test tubes. These mixtures were maintained at a pH of 4.0 to allow color development, after which the absorbance was recorded at 555 nm. The experiment was conducted again with other metal solutions. The metal to ligand mole ratios were obtained from the graphs of absorbance versus mole fractions.

Anthocyanin as pH indicator: Anthocyanin displays a range of colors at varying pH levels, making it a useful natural indicator for pH. An accurate measurement of 1 ml of the anthocyanin extract was taken and placed into several clean test tubes. The pH of the solution in each test tube was adjusted from 1 to 12 using 1 N Hcl and 1 N NaOH and buffer solutions sequentially, with the help of a pH meter for precise adjustments. The contents of each tube were thoroughly mixed, and the development of color was observed and recorded.

Isolation and Identification of microorganisms: The urine sample was obtained from Jana medical Diagnostics Lab in Virudhunagar. To isolate microorganisms from urine sample for antibacterial testing, Eosin Methylene Blue (EMB) agar and Salmonella Shigella (SS) Agar plates were prepared. A loopful of the urine sample was streaked onto the surfaces of both EMB agar and SS agar. After that, the plates were incubated at 37°C for 24 hours. Following incubation, the EMB agar plates displayed two types of colonies: pink mucoid colonies and colonies with a metallic sheen. Similarly, the SS agar plates showed black colonies as well as pink colonies. These colonies were then sub

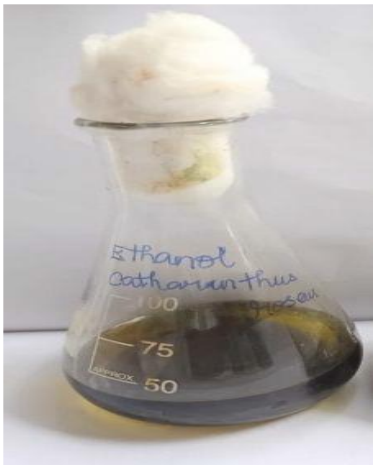
cultured separately onto the same type of medium. Then, these colonies were further confirmed by Gram staining and Biochemical test.

Anti-bacterial activity: The antibacterial assay was conducted using the agar well diffusion technique. Petri dishes were prepared by adding 20 ml of Mueller Hinton Agar medium, which was then allowed to solidify. Once solid, a bacterial culture of test pathogens was evenly spread across the agar surface with a cotton swab. The wells measuring 6 mm in diameter were made using a sterile stainless-steel cork borer. The wells were appropriately labeled, and each well received either 40 µl or 60 µl of the plant extract. The plates were then incubated at 37° C for a duration of 24 hours. The antibacterial activity was assessed by measuring the diameter of the inhibition zones surrounding the tested bacteria.

Results

Fig 1: *Catharanthus roseus* plant

Fig 2: Soxhlet extraction



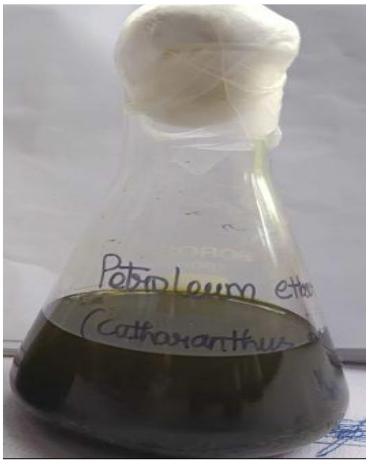


Fig 3: Ethanol and Petroleum ether extract of *Catharanthus roseus*

Table 1: Phytochemical test Result

S. No	Test Name	Leaf extract of *Catharanthus roseus*	
		Ethanol	**Petroleum ether**
1.	Alkaloid	Negative	Negative
2.	Terpenoid	Negative	Positive
3.	Phenols &Tannins	Negative	Positive
4.	Reducing sugars	Positive	Negative
5.	Saponins	Positive	Positive
6.	Flavonoids	Positive	Negative
7.	Quinines	Positive	Negative
8.	Proteins	Positive	Positive
9.	Steroids	Negative	Negative

Metal scavenging activity

Continuous Variation Method (Job's method)

Table 2: Job's method for determination complex molar ratio (ligand: Pb (II))

L:M	XL=VL /(Vm+VL)	Absorbance of Pb (II) complex
0:1	0	0
1:9	0.1	0.250
2:8	0.2	0.306
3:7	0.3	0.377
4:6	0.4	0.391
5:5	0.5	0.409
6:4	0.6	0.366
7:3	0.7	0.273
8:2	0.8	0.169
9:1	0.9	0.087
1:0	1	0

Graph 1: The relationship between Absorbance and pigment mole fraction XL for Pb (II) complex

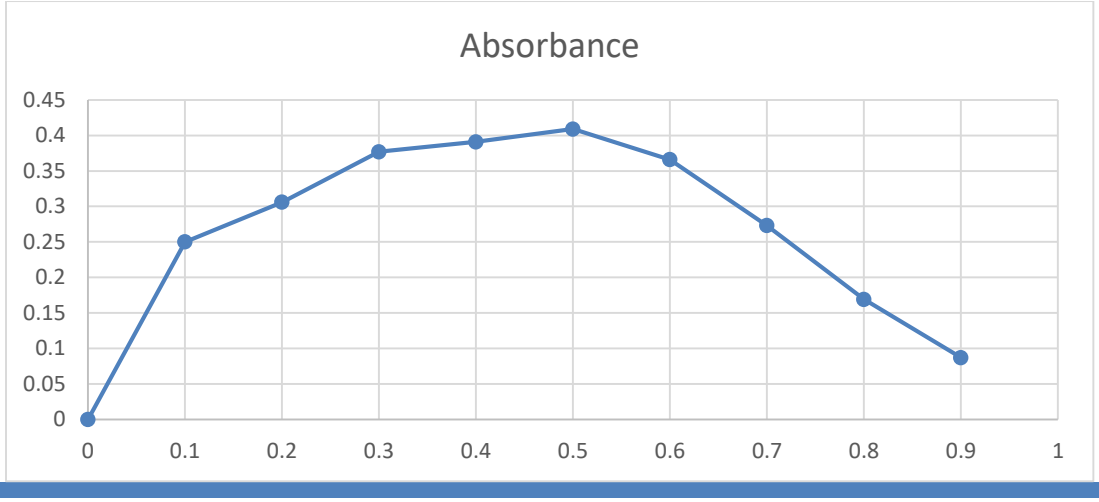


Table 3: Job's method for the determination complex molar ratio (ligand: Hg (II))

L:M	XL=VL /(Vm+VL)	Absorbance of Hg (II) complex
0:1	0	0
1:9	0.1	0.088
2:8	0.2	0.113
3:7	0.3	0.137
4:6	0.4	0.159
5:5	0.5	0.196
6:4	0.6	0.104
7:3	0.7	0.070
8:2	0.8	0.058
9:1	0.9	0.021
1:0	1	0

Graph 2: The relationship between Absorbance and pigment mole fraction XL for Hg (II) complex

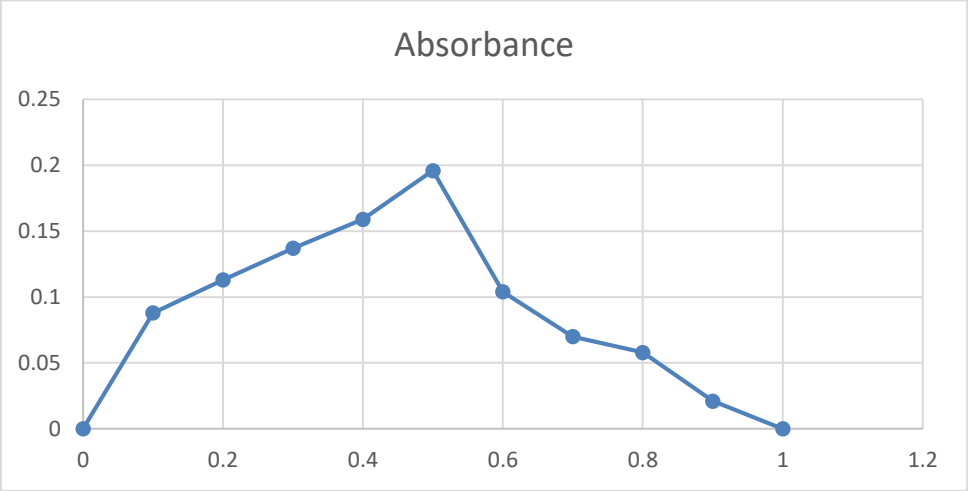


Table 4: Job's method for determination complex molar ratio (ligand: Ag (II)

L:M	XL=VL /(Vm+VL)	Absorbance of Ag (II) complex
0:1	0	0
1:9	0.1	0.173
2:8	0.2	0.252
3:7	0.3	0.374
4:6	0.4	0.461
5:5	0.5	0.559
6:4	0.6	0.640
7:3	0.7	0.528
8:2	0.8	0.413
9:1	0.9	0.327
1:0	1	0

Graph 3: The relationship between Absorbance and pigment mole fraction XL for Ag (II) complex

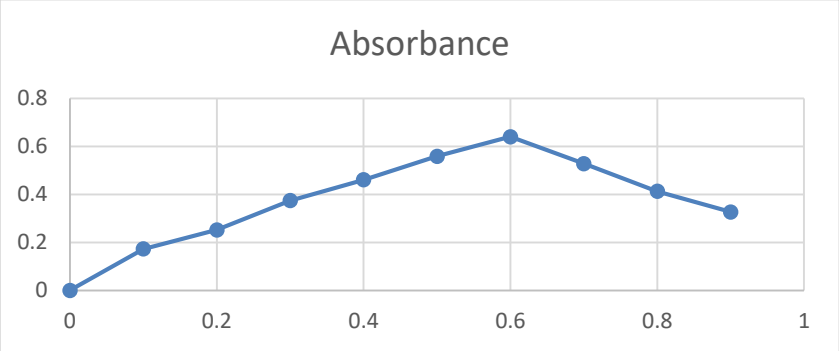


Table 5: Anthocyanin as pH indicator

S. No	pH value	Colour observed
1.	pH 1	Pink
2.	pH 2	Pink
3.	pH 3	Pink
4.	pH 4	Light pink
5.	pH5	Light Yellow
6.	pH 6	Yellow
7.	pH 7	Greenish Yellow
8.	pH 8	Greenish Yellow
9.	pH 9	Light Green
10.	pH 10	Green
11.	pH 11	Green
12.	pH 12	Green
13.	pH 13	Green
14.	pH 14	Yellowish Green

Fig 4: The colours produced by Anthocyanin from *C. roseus* at pH 1-7

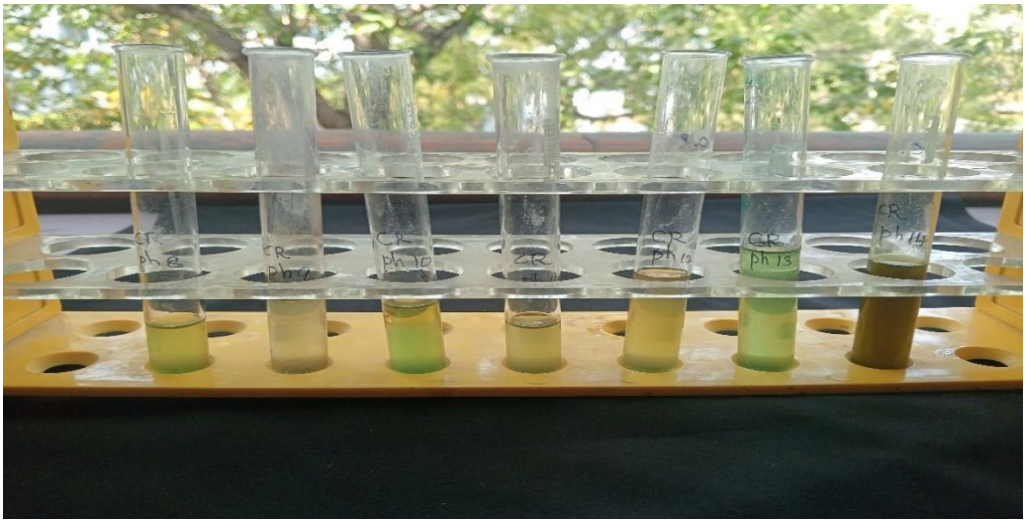


Fig 5: The colors produced by Anthocyanin from *C. roseus* at pH 8-14

Isolation of Microorganisms: After 24 hours of incubation, there are four different colonies were observed on EMB agar and SS agar plates.

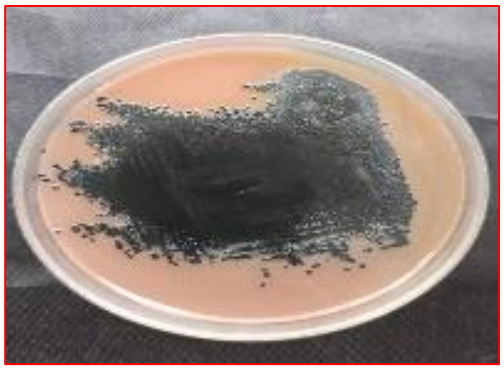


Fig 6: Smooth, black colour colonies with the production of H2S on SS Agar

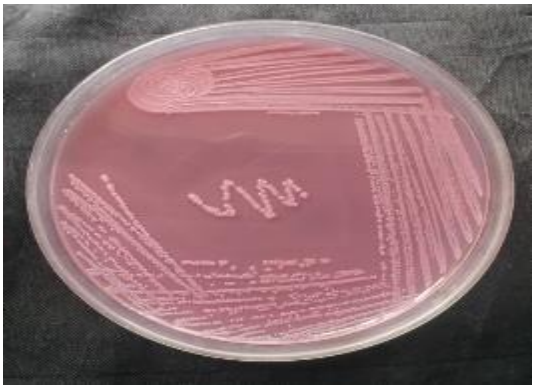


Fig 7: Smooth, translucent, moist colonies on SS Agar

Fig 8: Metallic sheen colonies appeared on EMB Agar

Fig 9: Pink mucoid colonies appeared on EMB Agar

Table 6: Biochemical test Result

Test name	*Salmonella*	*Shigella*	*Klebsiella*	*E. coli*
Gram Staining	Gram negative Rod	Gram negative Rod	Gram negative Rod	Gram negative Rod
Indole	Positive	Negative	Negative	Positive
Methyl red	Negative	Positive	Negative	Positive
Voges Proskauer	Negative	Negative	Negative	Negative
Citrate	Positive	Negative	Positive	Negative
TSI	K/A with H2S &gas production	A/A with gas production	A/A with gas production	A/A,no gas
Catalase	Positive	Positive	Positive	Positive
Oxidase	Negative	Negative	Negative	Negative

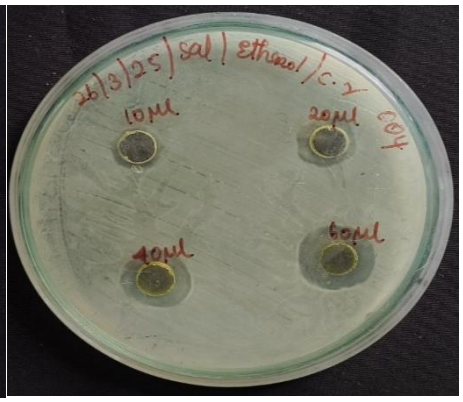
Anti-Bacterial Activity

Fig 10: Antibacterial activity of Ethanolic extracts of *C. roseus* against *Escherichia coli, Salmonella sp, Shigella sp, Klebsiella sp*.

E.coli

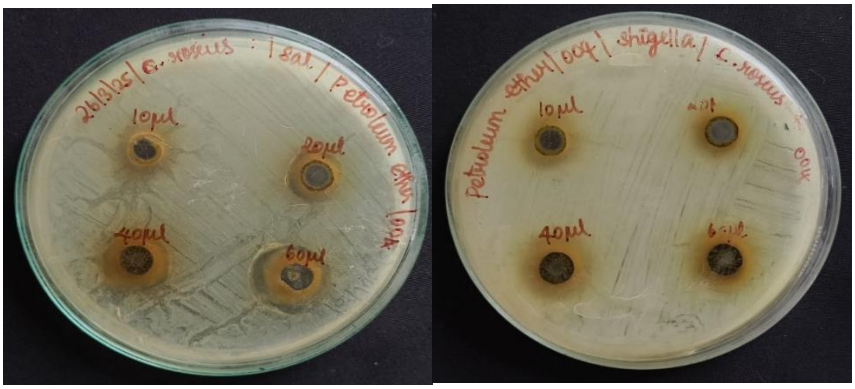
Klebsiella

Shigella

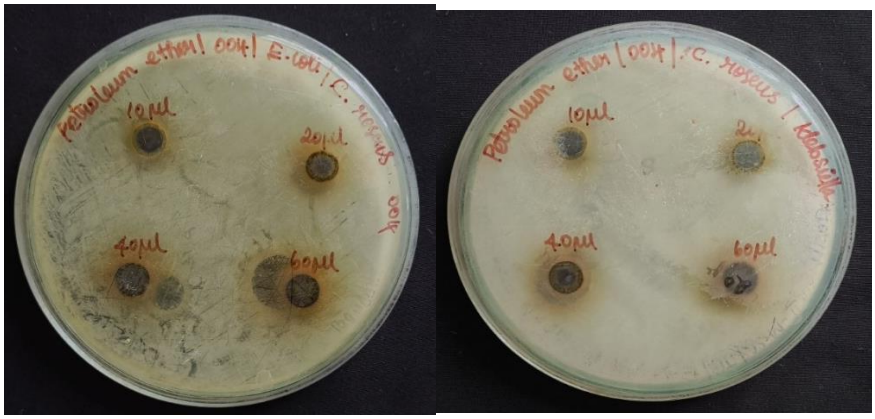


Salmonella

Fig 11: Antibacterial activities of Petroleum ether extracts of *C. roseus* against *Escherichia coli, Salmonella sp, Shigella sp, Klebsiella sp.*



Salmonella ***Shigella***



E.coli ***Klebsiella***

Discussion

Medicinal plants represent a critical source of life-saving medications for a significant portion of the global population. They remain a vital therapeutic resource for addressing various human ailments. India boasts a wide variety of flowering medicinal plants in its rich and diverse flora. Across all cultures, plants have been utilized for medicinal purposes from ancient times to the present. Medicinal plants are crucial in healthcare, with approximately 80% of the world's population relying on traditional medicine derived from plant sources. The plant *C. roseus* holds substantial medicinal significance. An extensive array of literature and publications, along with around 295 patents related to the plant and its derivatives, clearly underscores this importance. This plant has primarily been researched for its anticancer, antihypertensive, and antidiabetic effects. Consequently, there is a pressing need to develop alternative antimicrobial treatments for infections sourced from various medicinal plants. The antimicrobial properties observed in this study may be linked to the various types of secondary metabolites found in the plant material, whether individually or in combination. Finding

an effective treatment derived from plants would represent a significant progress in the therapy for microbial infections. The four bacterial strains (*Salmonella sp, Shigella sp, Klebsiella sp and E. coli*) utilized in this research are responsible for diseases in humans, including cholecystitis, urinary tract infections, and skin diseases. Nevertheless, these pathogenic bacterial strains were notably inhibited by the methanolic leaf extracts from the medicinal plants (Sarabjot K and Poonam M., 2014). Thus, this research supports the traditional and alternative use of the plants in treating a range of diseases and infections. Additionally, the bioactive molecules contained in the extract that are effective against these microbes require further characterization. The utilization of natural products is encouraged because they often have fewer or no side effects, are cost-effective, and help combat the development of resistance to standard synthetic antibiotics. Therefore, this study underscores the significance of employing medicinal plants as a viable alternative for treating various ailments. Anthocyanin pigments present in *C. roseus* flowers exhibit color variations based on pH levels. The aim of this study was to suggest an economical and straight forward indicator. Among the numerous pigments found in flower petals, anthocyanins, which are a category of flavonoids, have their chemical structures mainly characterized by their coloration, specifically the quantity of hydroxy groups on the B-ring and/or the presence of aromatic acyl groups, which modify anthocyanins and cause a bathochromic shift .The color of anthocyanins alters according to the pH within the vacuole where they are concentrated; they appear more blue in slightly acidic or neutral conditions, and more red in acidic conditions. Co-pigments, generally flavones and flavanols, lead to a bathochromic shift of anthocyanins when they interact with them. This property can be effectively utilized to demonstrate various chemical concepts such as acid-base equilibria, pKa, light absorption, and the effects of alterations in conjugated double bonds, among others. The species utilized in this research was *Catharanthus roseus*, from which the crude extract of the flowers was employed to acquire molecular absorption spectra, confirm the Lambert-Beer law, and serve as an indicator in acid-base titrations. The color shifted from dark pink to mehndi green as the pH varied from 2 to 9. To identify the peak absorption wavelength and illustrate how the spectra shape altered in relation to acidity, absorption spectra in the UV and visible ranges were recorded across different pH levels. The inclusion of stannous salt in the extract further amplified the pH changes and the absorption wavelengths.

Conclusion

In the current research, the phytochemical and antimicrobial activities were evaluated using ethanolic and petroleum ether extracts of *Catharanthus roseus*. Among the two extracts tested, the ethanol extract showed superior results in all assays. The findings regarding the metal scavenging activity indicate that anthocyanins possess significant potential for reducing metal concentrations. In the future,

it could serve as an effective treatment for diseases resulting from the unwanted accumulation of heavy metals in the body due to soil, water, air, fruits, vegetables etc. This serves as a natural solution for various health issues. Additionally, this study demonstrates that anthocyanins from *Catharanthus roseus* can function as acid- base indicator due to their capacity to exhibit different colors at varying pH levels. So, it will be used in various chemical and food industries.

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