

EVALUATION OF BIOACTIVITY OF *CATHARANTHUS ROSEUS*

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ABSTRACT

Catharanthus roseus is a flowering plant belongs to Apocynaceae family, has a wide use towards Ayurveda, traditional Chinese medicine, etc. In the present study *Catharanthus roseus* leaf extracts were prepared using 20% Acetone and Isopropyl alcohol. The prepared extracts were analysed for its various medicinal properties such as Antiamyolytic property, Antimicrobial activity, etc., from the findings it was clear that the test plant extracts are potential anti diabetic and antimicrobial agent which was identified by using Plate assay and Agar diffusion method further confirmed by using Minimal inhibitory concentration and swarming motility of the test pathogens. From the Phytochemical study it was clear that It carries various phytochemicals such as phenols, alkaloids, saponins, foam etc., which is Bioactive in nature and further confirmed by using Thin Layer chromatography. It is concluded that these biological activities may be due to the presence of the phytochemicals present in them.

Key words: Antimicrobial, Antidiabetic, MIC, Swarming Motility, Phytochemicals etc.,

INTRODUCTION

India has a rich biodiversity of medicinal plants many of them are not yet examined completely. There is a great demand for the plant based pharmaceutical products due to various reasons such as the cost, rate of recovery, side effects, availability etc., *Catharanthus roseus* (Apocynaceae), which is a potent medicinal plant with many pharmacological actions such as antimicrobial, antioxidant, anthelmintic, antifeedant, antisterility, antidiarrheal, antidiabetic effect etc and their chemical extraction has a role in cancer treatment.

Traditionally it has been used for treating various diseases.. Alkaloids were the major phytochemical constituent of the above medicinal plant and have different types maintaining various medicinal uses. The pharmacology of the plant was found to be associated especially with the alkaloids that occupy most of the parts of the plant. Gajalakshmi and Devi Rajeswari 2013). Diabetes mellitus (DM) is a group of metabolic disorders characterized by high blood sugar levels. If the hyperglycemia is not controlled, adverse metabolic changes lead to the continuous development of serious complications. Formation of reactive oxygen/nitrogen species and inflammatory responses are principal mechanisms that have been involved in the development of hyperglycemia-induced tissue damage.

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The commercially available drugs used in the treatment of diabetes have harmful side effects hence the need to discover medicines mainly from medicinal plants. Since ancient years *Catharanthus roseus*, ornamental plant is used as holy medicine to cure many diseases (Mediline Goboza, 2019). Vindoline is a bioactive compound derived from *Catharanthus roseus* used against type 2 diabetes induced complications and the leaf juice or water decoction of *Catharanthus roseus* is used as a medicine for the treatment of diabetes. The leaves of this plants play an important role in cancer treatment (Kabesh et al 2015). This plant is a potential source for antileukemic alkaloids (Ramya et al 2008). Leaf extracts of this plants has antimicrobial potential against *Staphylococcus spp*, *E.coli*, *Pseudomonas spp*, and *Streptococcus spp*. (Shanmugaraju and Bhagyaraj 2016). *Catharanthus roseus* plant is a "natural herbal resource" can be used widely in the pharmaceutical and pharmacological industry due to its antimicrobial, antitoxic and anticancer property due to the presence of various phytochemical compounds (Somashékara Rajashekara, et al., 2022).

Historically and in contemporary times, along with supporting literature citations. The information provided is supported by various literature sources such as Chakraborty et al. (2008) and Nameer et al., (2015), Historical references are also cited, including Green (1913), Wroughton (1907), Hutton (1949) and Fletcher (1911). Solution for enhancing pollinator habitat and fostering biodiversity conservation. By creating landscapes rich in flowering plants and nesting sites, pollinator gardens can provide vital resources for bees, butterflies, birds, and other pollinating insects. Moreover, these green spaces offer educational opportunities and aesthetic benefits, making them valuable assets for communities and urban environments. In this article, we examine the scientific basis and practical considerations for establishing pollinator gardens as a conservation strategy.

The preferred habitat of *P. nudiventris* includes semi-arid scrub forests, thorny areas, drylands, cattle grounds, bushy desert tracts, grasslands, edges of cultivated areas, and dried

ponds (Srinivasulu and Nagulu 2002, Kumar and Nijman 2016). Hedgehogs are nocturnal and usually eat at night. Their diet consists predominantly of insects, with occasional plant materials.

METHOD

PREPARATION OF PLANT EXTRACTS

The leaf samples of *Catharanthus roseus* were plucked and washed to remove dirt and dust, air dried to ground into powder. The powder was mixed with acetone and isopropyl alcohol solution separately at a concentration of 20 %. Test tubes mouth were covered with aluminum foil and maintained at room temperature for 3 days and then into a platform shaker for 1 day. The supernatant was collected and dried at 50 ° C after centrifugation for 10 minutes at 4000 rpm at 25 ° C. The extracts was collected and stored at 4 ° C (i).

DETECTION OF PHYTOCHEMICALS

Qualitative analysis of glycosides, phenols, terpenoids, saponins were done using method of Samrot et al (j).

THIN LAYER CHROMATOGRAPHY:

Thin layer chromatography was done using 10 µl of extracts loaded on the TLC plates and eluted using Ethanol as a mobile phase after the separation the plates were dried by placing it in iodine saturated container. Rf value was calculated further.

ANTI-AMYLOLYTIC ACTIVITY

PLATE ASSAY

ENZYME PREPARATION:

Bacillus sp was inoculated to media containing (K)- Soluble starch-1g, Yeast extract - 0.2g, Peptone - 0.5g, MgSO₄ - 0.05g, NaCl -0.05g, CaCl₂ - 0.15g, Agar - 2g, Distilled water - 100mL, pH 7.0 and allowed to incubate for 24 h at 37 ° C. After incubation period, the broth was centrifuged at 4000 rpm for 10 minutes and the supernatant was collected and was used as crude amylase source.

PLATE ASSAY

Sterile starch casein agar plates were prepared and 5 wells were Punctured. 100 µl of crude enzyme (obtained from the above mentioned process) was loaded into the centre well and the surrounding 4 wells were loaded with 100 µl of enzyme and varying amounts of plant extract (25 µl, 50 µl, 75 µl, 100 µl). The plates were incubated at 37 ° C for 24 h. After incubation, the zone of clearance was observed by adding iodine solution to the plate.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity was performed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus licheniformis*, *Klebsiella pneumonia* for the plant extract. A plate without plant extract was kept as constant. After incubation, the diameter of the zone of clearance was recorded²³.

SWARMING MOTILITY

Nutrient agar plates with 2 % agar were prepared which was followed by 0.75 % of agar solution on top. After that was poured on the solid agar plate, it was allowed to solidify. 1 µl of 24 h old culture of *Proteus sp.*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus licheniformis* was placed on the top of the agar plate and incubated at 37 ° C for 24 h. After incubation, the measurements of the movement were recorded²⁴.

MINIMAL INHIBITORY CONCENTRATION

100 µl of *Escherichia coli* culture was added into all the test tubes as fixed volume to which different concentrations of plant extract were also added. The entire volume of test tube was made up to 5 ml using nutrient broth. The tubes were incubated at 37 ° C for 24 h. The optical density at 620 nm was checked and compared with control.

RESULTS AND DISCUSSIONS:

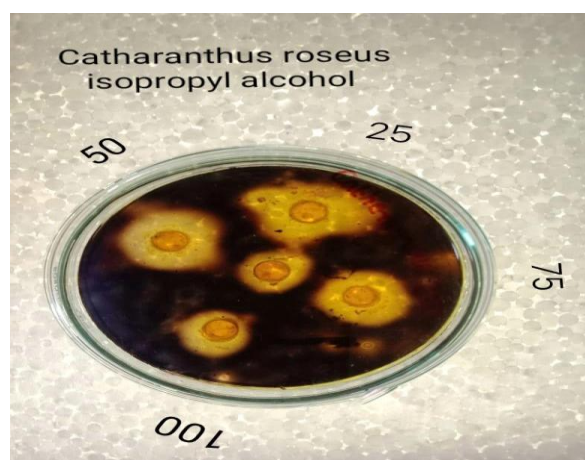
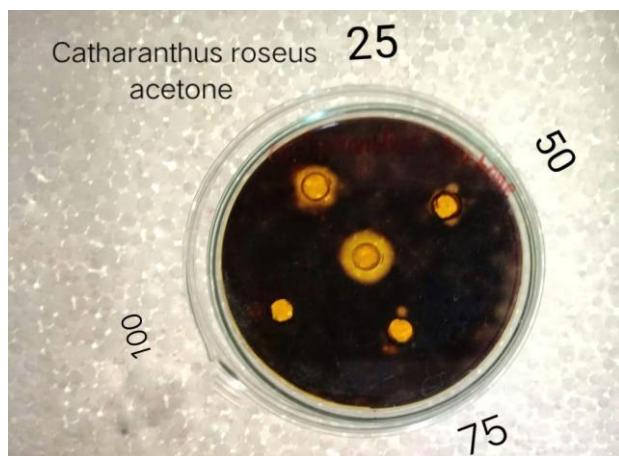
ANTIMICROBIAL ACTIVITY

The antimicrobial activity of *Catharanthus roseus* extracts was studied against *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus*

licheniformis and *Escherichia coli*. Acetone extracts of *Catharanthus roseus* showed a zone of inhibition ranges of 8 mm, 12 mm, 22 mm, 10 mm respectively. Among these pathogens *Bacillus licheniformis* showed highest sensitivity against acetone extracts of *Catharanthus roseus*. Whereas Isopropyl alcohol extracts of *Catharanthus roseus* showed a inhibition range of 6 mm, 10 mm, 12 mm, 5 mm respectively. Among these pathogens *Bacillus licheniformis* showed highest sensitivity against Isopropyl alcohol extracts of *Catharanthus roseus*. The methanolic extract of *C. roseus* highly inhibited *M. luteus* and *E. coli* and less inhibition against *S. aureus* was found by Kamal Raiz *et al.*, in 2021 In a similar study by Ologundu *et al.*,2019 found that the alkaloids present in the roots of *Catharanthus roseus* is antibacterial against *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Micrococcus roseus*, *Micrococcus luteus* and *Bacillus subtilis*. It is ineffective against *Bacillus licheniformis*.

ANTIAMYLOLYTIC ACTIVITY

High Blood glucose level is the characteristics of Type 2 diabetes mellitus which is one of the reason for high morbidity and mortality globally (m). Antiamylolytic effect of both the leaf extracts were tested against the crude amylase enzyme extracted from *Bacillus sp.* the amylase activity on the culture plate was decreased as the concentration of acetone and isopropyl alcohol extracts increased, it was indicated by zone of clearance around the well. *Catharanthus roseus* is used in treatment of several diseases like diabetes, cancer, high blood pressure, asthma, inflammation, dysentery etc. Jayanthi *et al.*,2019 found that *Catharanthus roseus* is a widely used medicinal plant treating many diseases. It also carries many phytochemical compounds such as amino acids, terpenes, lipids, steroids, flavonoids, ascorbic acids, tannins, vitamins and alkaloids. This plant carries various health benefits such as insecticidal, anti-inflammatory, antidiabetic, anti ulcer, anti oxidant, anti malarial and antibacterial.



Antimicrobial activity of Acetone extracts of *Catharanthus roseus*

Pathogens	Zone of inhibition (in mm)			
	50	100	150	200
<i>Pseudomonas</i>	-	2	5	8
<i>Klebsiella</i>	-	5	7	12
<i>B.liqueficiens</i>	-	9	13	22
<i>Escherichia coli</i>	-	2	5	10

Antimicrobial activity of isopropyl alcohol extracts of *Catharanthus roseus*

Pathogens	Zone of Inhibition in mm			
	50	100	150	200
<i>Pseudomonas</i>	-	0	3	6
<i>Klebsiella</i>	-	3	8	10
<i>B.liqueficiens</i>	-	6	10	12
<i>Escherichia coli</i>	-	0	2	5

SWARMING MOTILITY

The swarming motility for *Pseudomonas aeruginosa*, *E.coli*, *Proteus* and *Bacillus licheniformis* against acetone and isopropyl alcohol extracts of leaves of *Catharanthus roseus* were investigated (Table 2). The movement and distance travelled by the microorganism against the extract was measured and recorded as 10mm,13mm,37mm,21mm respectively against acetone extract and 16mm,15mm,16mm and 40mm respectively against isopropyl alcohol extract.

Famuyide et al, 2019, found that the plant extracts are potential biofilm formation inhibitor and pre-formed biofilms inhibitor, which indicates the antimicrobial nature of the phytochemicals present in the plant. Djihane, et al., 2017 stated that the Mode of action of plant extract on gram positive and gram negative bacteria may be attributed to their Morphology of cell wall which includes LPS, enzymes present in the periplasmic space. The extract of *Catharanthus roseus* leaf possesses antibacterial properties. (Dharmalingam et al., 2015) which would have arrested the movement of Bacterial pathogens in the semisolid agar.

Table 2. Distance travelled by microorganisms against acetone and isopropyl alcohol extract of *Catharanthus roseus*.

Plant extracts	Pseudo monas aeruginosa	E. coli	Proteus sp.	B. liqueficans
Acetone extract	10mm	13mm	37mm	21mm
Isopropyl alcohol extract	10mm	15mm	16mm	40mm

There is a gradual increase in the inhibitory effect on each test pathogens were observed, which was demonstrated by the elevation in the OD values. At 50µl concentration of the extract the inhibition was very less when the concentration reaches 100µl Marked increase in

the inhibition was observed, which increased upto 200µl concentration. When MIC results were compared with the zone of inhibition in the agar diffusion method.

Apurv Gaur et al. 2016 found that different concentrations of acetone and ethyl acetate extracts of *Catharanthus roseus* inhibited *E.coli* at a concentration of 25µg/ml and 12.5µg/ml *Proteus vulgaris* at 12.5µg/ml for *P. vulgaris* and *K. pneumoniae* at 6.25µg/ml and 50µg/ml for in acetone and ethyl acetate extracts respectively. *Catharanthus roseus* is a potent antibacterial agent effective against *E.coli*, *B. subtilis* and *S. aureus* (Syeda, and Riazunnisa, 2020). Amjad Khalil 2012 ethanol leaf extract of *Catharanthus roseus* at 100mg/ml concentration has strong inhibition of 15 mm against *Staphylococcus aureus* and *E.coli* at 11 mm.

Table 3. MINIMAL INHIBITORY CONCENTRATION

Amount of plant Extracts	<i>Escherichia coli</i>		<i>Pseudomonas</i>		<i>Klebsiella</i>		<i>B.liquefacians</i>	
	AE	IE	AE	IE	AE	IE	AE	IE
50	0.12	0.13	0.13	0.14	0.13	0.13	0.12	0.14
100	0.11	0.17	0.10	0.11	0.10	0.10	0.10	0.11
150	0.14	0.07	0.07	0.08	0.07	0.07	0.08	0.09
200	0.16	0.18	0.05	0.06	0.05	0.05	0.06	0.07

DETECTION OF PHYTOCHEMICALS

The phytochemical compounds in acetone and isopropyl alcohol extracts of *Catharanthus roseus* were screened using a qualitative analysis (Table 4). The acetone extract revealed the presence of phenols, glucosides and terpenoids. Isopropyl alcohol extracts of *Catharanthus roseus* contained glucosides, phenols, saponins and terpenoids., *Catharanthus roseus* contains phytochemicals such as alkaloids, phenols, saponins, tannins, terpenoids, steroids, flavonoids etc. (Kaur .H, 2019). Methanolic and Ethanolic extract of *Catharanthus roseus* leaves are found to have Flavonoids, Tannins, Alkaloids and

Terpenoids, which is essential for drug development (Prakash Solanki *et al.*,2018). Kamal Raiz *et al.*, 2021, identified the presence of flavonoids, terpenoids, glycosides, anthraquinone, tannins, proteins, carbohydrates, saponins, and alkaloids in the methanol extracts of *C. roseus* leaves.

Table 4.Detection of phytochemicals in acetone and isopropyl alcohol extracts of *Catharanthus roseus*

Plant extracts	Phenols	Glycosides	Terpenoids	Saponins
Acetone extracts	+ve	+ve	+ve	-ve
Isopropyl alcohol extracts	+ve	+ve	+ve	+ve

THIN LAYER CHROMATOGRAPHY

A Similar study by Vanitha varadarajan *et al* 2012 Also supported the above finding in which Phytochemicals were qualitatively determined using TLC, HPLC HPTLC. Dharmalingam *et al.*, in 2015 found that the methanolic leaf extract of *Catharanthus roseus* was used for the detection of alkaloid using the thin layer chromatography Thin Layer chromatography (TLC) is a standard technique opt to separate phytochemicals such as Alkaloids, Phenol, Saponins, Protein , tannins terpenoids, etc., (Kabesh *et al.*, 2015).

Table. 5 .Thin layer chromatography of acetone and isopropyl alcohol extracts of *catharanthus roseus*

Plant extracts	Rf value
Acetone extract	0.922
Isopropyl alcohol extract	0.711

PLATE 1- ANTIBACTERIAL ACTIVITY

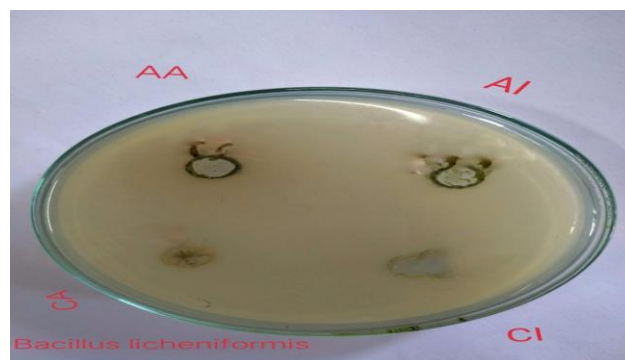
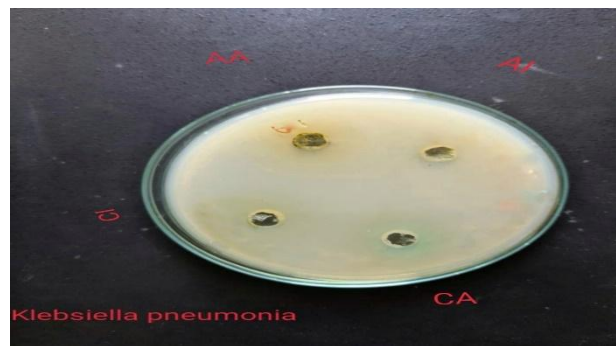
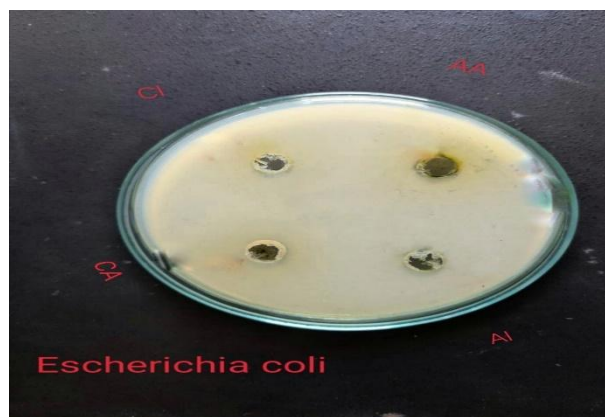
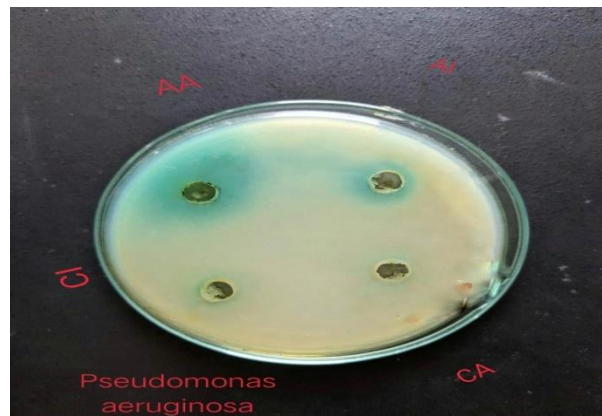


PLATE 3- SWARMING MOTILITY

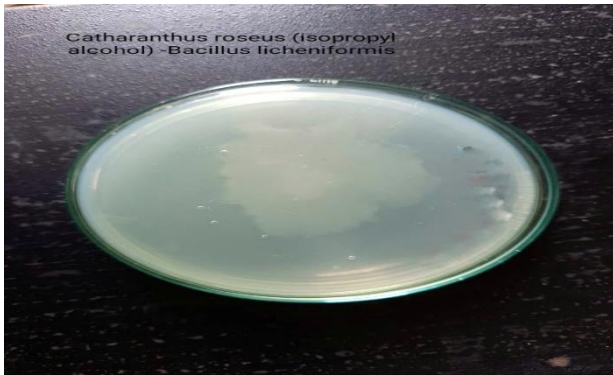
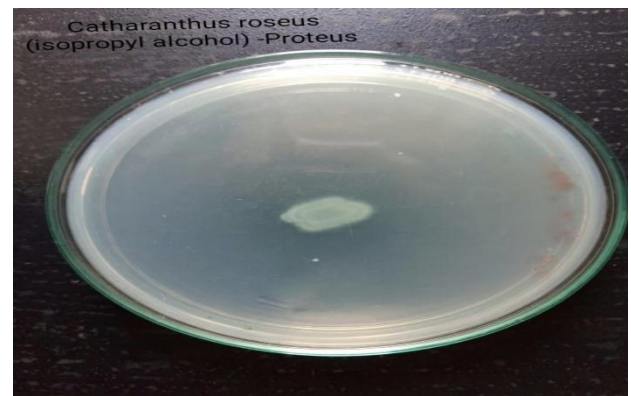


PLATE 4- SWARMING MOTILITY



PLATE 5. SWARMING MOTILITY



Summary and conclusion:

From the study it is very clear that the Leaf *Catharanthus roseus* has various phytochemical which are effective in exhibiting various bioactivities such as antimicrobial against various pathogenic microorganisms, antidiabetic in nature. It is not only a ornamental plant and also a medicinal herb.

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