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In-Vitro Evaluation of Antibacterial Activity Using Crude Extracts of *Catharanthus Roseus*

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Abstract
Growing rate of antibiotic-resistantbacteria becomes a serioushealth issue most parts of the world, which has prompted the need to find other effective and efficient antimicrobial agents. They have been used as herbs with known medicinal value sources of bioactive compounds with potential therapeutic effects because of their broad-spectrum effect, lowtoxicity, and a low cost. Catharanthus roseus (L.) G. Don is a famous medicinal plant that is a member of Apocynaceae family and are widely researched in the field of pharmacology, particularly as an ant cancerous drug. Over the last few years, there had been a scientific interest in its antibacterial potential owing to the occurrence of various phytochemicals including alkaloids, flavonoids, phenolic compounds and terpenoids.This is a review of the literature, which summarizes and critically evaluates existing literature on the antibacterial activity of crude extracts of Catharanthus roseus. The main stress has been laid on the widely used extraction methods, phytochemical compounds, and methods of in vitro antibacterial testing such as agar diffusion tests and minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The variables affect the efficacy of antibacterial (i.e. extraction solvents, plants parts used and bacterial strains tested) are also discussed. The results in the review outline the potential to use Catharanthus roseus as an antibacterial agent and reinforce its usefulness as a future provider of plant-derived antimicrobial agents. More investigations on isolation of active compounds and suggested in order to make it applicable in the development of antimicrobial drugs.

Keywords: Catharanthus roseus, Madagascar periwinkle, Medicinal plants, Phytochemicals, Crude plant extracts, alkaloids, Ethnomedicinal use, in vitro antibacterial activity.

I. Introduction

The increased issue of antibiotic resistance has now become a critical health problem in the world, as most of the frequently used antimicrobial drugs are not as effective as they were before.Thebacterial infections are still a significant source of morbidity in the worlds, and the need to findnew and effective bacterial agents has thus become an area of great concern. In this regard, other sourcesof therapeutic methods are also being considered that are harmless and cost-efficient[16].The traditional medicine system has been practicing the uses of medicinal plants to treat infectious diseases for centuries. They are highly endowed with variety of bioactive compounds, and their antimicrobial action is usually broad-spectrum. Research in the field of plant-based antimicrobials has gained scientific attention in recent years, especially because the potential exists for new action mechanisms, fewer side effects, and lower prices. This has challenged researchers to reconsider the traditionally applied plants by employing contemporary experimentation [23].

Catharanthus roseus (L.) G. Don, also known as the Madagascar periwinkle, is a highly widespread medicinal herb that has been used therapeutically for a long period [21]. The plant is also known to produce very useful indole alkaloids, but it has other significant phytochemicals like flavonoids, terpenoids and phenolic compounds. In addition to its widely known anticancer uses, C. roseus has been reported with greater frequency to shows antibacterial efficacy against numerous Gram-positive and Gram-negative bacteria.The review is aimed at summarising and discussing literature on the antibacterial potential of Catharanthus roseus[22]. It has focused on its phytochemical profile, typically used antibacterial assessment techniques, such as agar diffusion and minimum inhibitory concentration, and considerations that determine its antimicrobial performance. The reviewed article will support the significant of Catharanthus roseus as a future source of plant-based antimicrobials and help to determine what needs more research by synthesising the results of other studies [4].

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II. Botany of Catharanthusroseus

The Madagascar periwinkle, or Catharanthus roseus, a commonly known flowering plant which belongs to the Apocynaceae family. The plant developed in Madagascar, as its name implies, but it is now cultivated all over the world. It is commonly used as a plant for decoration due to its lovely flowers, low maintenance requirements, and ability to spread. It is also very valuable because of its therapeutic value[16]. Catharanthusroseus is a part of a miniature evergreen herb which will normally measure up to 30 to 100 cm in height. The tender, juicy twigs of the plant might appear somewhat hairy. Its oval, oblong, smooth and glossy leaves are set in small stalks facing each other [4]. The ends of the leaves are rounded and, or a little pointed and the leaf margins are smooth.The flowers are produced in the axils of the leaves either individually or in little groups of one to four [20].They are available in various colours ranging between white and red, pink or purple depending on the type. The flowers have a long corolla with five broad petals and short calyx. A little way above the stamens the inside of the plant is a bit hairy[22].

2.1 Geographical distribution

Catharanthusroseus is also widely planted in India, Australia, South Africa, Vietnam, the Philippines, and the United States although it is native to Madagascar [21]. They are widely distributed in India in Tamil Nadu, Karnataka, Gujarat, Odisha [20]. The plant is also facing an endangered situation in the wild in certain areas due to excessive harvesting and destruction of habitats [22],[4].

2.2 Medicinal compounds

A major factor in the significance of Catharanthus roseus in medicine is that this plant generates significant quantities of alkaloids[5]. This plant has over 100 varieties of alkaloids [4]. These substances are nitrogen substances that cause most of the therapeutic effects of the plant [20]. The vincristine, vinblastine, ajmalicine, reserpine, serpentine, catharanthine and vindoline are some of the most valuablealkaloids. Among them, the most useful are vincristine and vinblastine that are highly useful in cancer chemotherapy, even in childhood leukaemia [17].

2.3 Ethnomedicinal utilization

The use of Catharanthus roseus in traditional medicine has a long history in most cultures [4]. The leaf, or the plantsare boiled in water and the extract is orally ingested for the treatment of diabetes mellitus in parts of Europe, Vietnam, Northeast India, and Jamaica [6]. In Tamil Nadu the whole plants are blended with cow milk in their powder form to be administered orally as methods of managing diabetes [16],[17].

Table 1. Significant phytochemicals and medical value of Catharanthus roseus

S. No	Phytochemical	Plant Part	Occurrence	Medical significance	Reference
1.	Tannins	Whole Plant	High (++)	Antioxidant	[4]
2.	Saponins	Whole Plant	Present (+)	Anti-inflammatory	[5]
3.	Flavonoids	Whole Plant	High (++)	Antioxidant	[5]
4.	Steroids	Whole Plant	Present (+)	Anti-inflammatory	[20]
5.	Terpenoids	Whole Plant	Absent (-)	Antimicrobial	[5]
6.	Triterpenoids	Whole Plant	Present (+)	Anti-inflammatory	[21]
7.	Alkaloids	Whole Plant	Present (+)	Anticancer	[20]
8.	Anthraquinones	Whole Plant	Present (+)	Laxative	[5]
9.	Polyphenols	Whole Plant	High (++)	Antioxidant	[4]

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10.	Glycosides	Whole Plant	Present (+)	Cardioprotective	[4]
11.	Coumarins	Whole Plant	High (++)	Anticoagulant	[21]
12.	Ajmalicine	Leaf / Root	Present	Antihypertensive	[5]
13.	Vindoline	Leaf / Root	Present	Anticancer Precursor	[4]
14.	Catharanthine	Leaf / Root	Present	Anticancer Precursor	[20]

Table 2. Large amounts of phenolic compounds were reported in various sections of Catharanthus roseus.

Plant Part	Phenolic Compound	Content (mg/kg dry weight)	Reference
Stem	3-O-caffeoylquinic acid 4-O-caffeoylquinic acid Quercetin glycosideKaempferol glycoside	769.9±12.7 2874.6 ±151.6 190.5± 3.1 190.8± 5.3	[6] [18] [18] [7]
Seeds	Kaempferol glycoside Quercetin glycoside Isorhamnetin glycoside	2714.2 ± 4.3 582.7 ± 6.6 354.1± 8.2	[6] [7] [18]
Petals	4-O-caffeoylquinic acid Kaempferol glycoside Quercetin glycoside Isorhamnetin glycoside	11153.2 ± 126.4 8120.8 ± 74.4 1027.9 ± 7.0 1330.4 ± 10.8	[17] [18] [6] [6]
Leaf	3-O-caffeoylquinic acid 4-O-caffeoylquinic acid Quercetin glycoside Kaempferol glycoside	2971.6 ±15.6 5156.8 ±137.2 310.9 ± 5.0 52.7 ±1.0	[6] [18] [17] [7]

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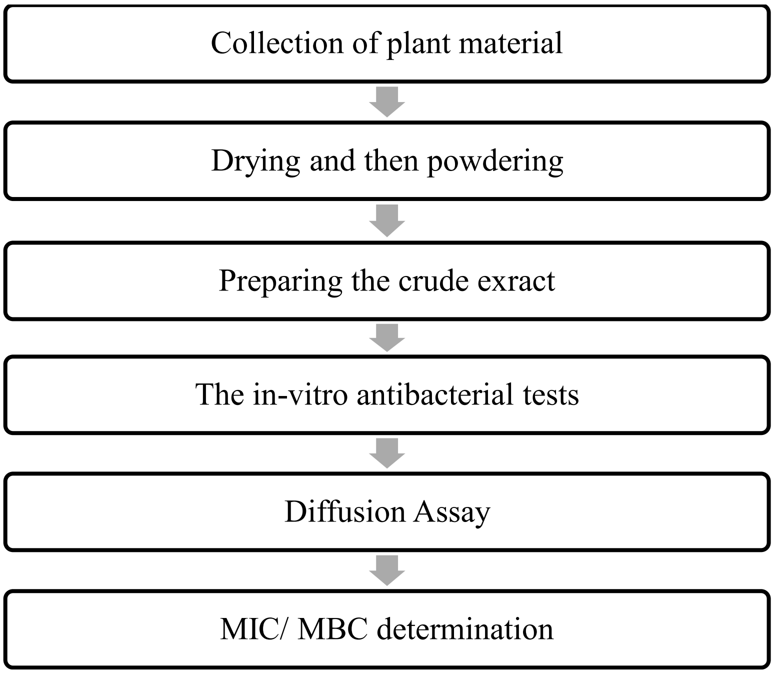

Figure 1. Extraction and antibacterial screening of Catharanthus roseus workflow.

III. Preparation of crude plant extracts

Catharanthus roseus plant materials were washed under running tap water after which, they were dried undershade to a constant weight and powdered to form a course material that could be extracted. Methanol and ethanol were the organic solvents that were generally used to prepare crude extracts. The powdered plant material was moistened in the corresponding solvents at a proportion of 10 g to 100 mL and allowed to react overnight in roomtemperature[7]. Soxhlet extraction was used in a number of studies in order to increase extraction efficiency. The extraction procedurewas reiterated by combining the extracts being concentrated (reduced pressure) to arrive at semi-solid crude extract that was kept at 4 ºC awaiting use [6].

To conduct in vitro assessment of the antibacterial ability of the crude extracts, stock solutions were prepared by reconstituting the crude extracts in appropriate solvents which in most were 5% Dimethyl Sulfoxide (DMSO). The stock concentrations presented in the literature go up to 1000 mg/mL whereby on serial dilution, a range of working concentrations (100-3.125 mg/mL) was obtained. Additional concentrations of 100-300 mg/mL were also made using ethanol, methanol or aqueous solvents depending on the study design[5], [8].

IV. In-vitro antibacterial screening methods

4.1 Agar well diffusion

The diffusion technique on an agar well is one of the most commonly used methods of testing the antibacterial activity of plant extracts. The principles of this assay rely on the fact that bioactive compounds can diffuse through solid growth medium, e.g., nutrient agar or Mueller Hinton agar, and suppress bacterial growth. The resulting clear zones around the wells reflect inhibition, which is a visual determination of the antimicrobial activity[11].An agar diffusion test is a common test used in Gram-positive bacteria, Gram-negative bacteria, so that plant extracts can be screened in advanceregarding their possible antibacterial effects. The size of the inhibition spot is most often taken as measure of activityand in certain studies, can be taken with respect to standard antibiotics to make comparisons between efficiency [13], [2].

Although the technique is straight forward, fast and very common, it is relatively qualitative. The extraction concentration,diffusion properties, and agar properties may have an effect on outcomes, and itwas found difficult to compare the results of different studies. Irrespective of these shortcomings, agar diffusionis an indispensable ingredient of the assessment of the antibacterial potential of medicinal plants, including Catharanthus roseus, and gives a valuable clue on which extracts to further

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quantify, such as minimum inhibitory concentration (MIC) [14].

Figure 2. Diagrammatic illustration of agar well diffusion technique to be applied in the evaluation of antibacterial.

4.2Minimum bactericidal concentration

It is said that the least concentration of an antimicrobial agent that causes complete killing of the test microorganism and no further proliferation of the microorganism. MBC is typically determined by the subculture of MIC assay tubes or wells that do not harbour any observable growth on antimicrobial-free agar plates [10]. A streak or plaqueof the small volume of the broth (typically 10-100 µL) is inoculated onto an agar medium of the same type as the one being evaluated in the MIC assay and is incubated at 35-37̊C, 18-24 h. The plates are then incubated until they form bacterial colonies. The lowest concentration of extract, which does not result in bacterial transference on the agar plates, is considered the MBC.[15], [2]

This methodmay be applied in determining whether the bacterial cells have been killed or simply stalled and capable of growing back to their normal size with the right conditions. In accordance with the overall standard procedures, MBC may be defined by the concentration which will be resulted on the reduction of the original population of bacteria by99-99.9%.[13]

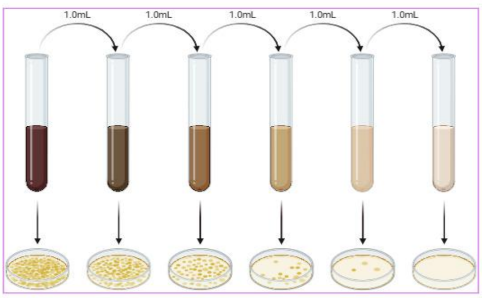

Figure 3. Practical broth dilution test with demonstration of MIC determination and MBC confirmation.

4.3 Minimal inhibitory concentration

Minimal inhibitory concentration (MIC) of pharmaceutical plant extracts remains highly dispersed with most of them ranging within the low to medium level of milligram per millilitre. These values differ depending on the species of the plant, solvent of extraction, part of plant that is beinganalysed and strain of bacteria under test [2]. Therefore, the extracts that have adjacent inhibitory areas in diffusion tests any differ substantially in actual antibacterial activity on the foundation of MIC.Some of these researches have also employed MIC-based assaysthat are applicablein the complement diffusion assays to determine the antibacterial activity of Catharanthus roseus. The results have revealed as the antimicrobial properties on Catharanthusroseus extracts are highly related to the extraction procedures as well as the section of the plant used that subsequentlydictates the levels of bioactive phytochemicals such as indole alkaloids and phenolic compounds.[12]

The determinationof MIC is therefore extremely significant in testing and measuring the antibacterial potential of Catharanthusroseus against Gram-positive and Gram-negative bacteria.Reported values of MIC in the literature have inconsistent units with sone being in mg/mL and other in ug/mL andtherefore difficult to compare the literature studies directly. Regardless of the variation as per the experimental methods, MIC assays are a standardand objective procedure of establishing the antibacterial effect and provide a reasonable clue on potential of Catharanthus roseus as a source of antimicrobial action as a result of plants.[10], [9]

V. Antibacterial activity against gram positive and gram negative

For the antibacterial effects on plant-based crude extracts, several studies have tested their effect on reference bacterial strains and food-borne bacteria isolates, such as Gram-positive and Gram-negative bacteria, as well. The bacterial isolates are typically pooled on the contaminated food by incubating and growing on solid agar medium that is subsequently recognised using the Gram staining systems. Most commonly, formal reference strains are also added, including Escherichia coli and Staphylococcus aureus, so that a comparison and validation of the results may be made. The agar well diffusion or disk diffusion tests are usually applied to determine the antibacterial screening of leaf stem extracts, whereby the presence of the inhibition zone is regarded as a measure of the capability to prevent the growth of bacteria by extracts.[5]

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Common positive controls to assess the comparative effectiveness of plant extracts are commonly standard antibiotics, e.g. ampicillin. The tests are usually conducted in three formats, and the standard deviation. The differences related to the treatments are usually determined by using statistical tests that are usually based on the one-way analysis of variance, with the p-values of less than 0.05 considered statistically significant.There are several plant species that are indicated to exhibit diverse antibacterial activity against food-poisoning bacteria, which include Bacillus cereus, Staphylococcus aureus, Escherichia coli, Salmonella typhi, and Pseudomonas aeruginosa. Some of these extracts are selectively active against strains of bacteria, whereas other extracts are broad-spectrum active.[9]

As a rule, Gram-negative bacteria (such as Salmonella typhi and Escherichia coli) are usually more resistant to plant extracts, whereas Gram-positive bacteria (particularly, Staphylococcus aureus) are usually more sensitive.As per the outcome of the diffusion assay, extracts that have strong antibacterial effects are normally subjected to further quantitative analysis by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays. These studies prove helpful to determine the growth-inhibitory or bactericidal action of potential plant extracts under the most susceptible types of bacteria. [16], [17]

Table 3. Antibacterial activity of Catharanthus roseus extracts that were reported against various bacterial strains.

Bacterial Group	Bacterial species	Reported antibacterial response	General susceptibility	Reference
Gram-positive bacteria	*Staphylococcus aureus*	Moderate to strong inhibition zones	Highly susceptible	[11]
	Bacillus cereus	Variable to moderate inhibition zones	Moderate susceptible	[18]
Gram-negative bacteria	*Escherichia coli*	Low to moderate inhibition zones	More resistant	[11]
	Salmonella typhi	Low inhibition zones	More resistant	[19]
	Pseudomonas aeruginosa	Low to moderate inhibition zones	Highly resistant	[18]

VI. Future prospects

Increasing incidence of antibiotic-resistant bacterial infection has to a large extent reinstated medicinal plants as a subject of interest due to the need to identify alternative sources of antibacterial agent. Most of the production of bioactive phytochemicals in Catharanthus roseus makes it have a bright future as a source of plant-based antibacterial in the future. It is hoped that further research should be conducted to improve extraction methods and methodologies of formulation to concentrate its crude extracts as safer, more stable, and effective against antimicrobial activities. Future studies should aim to standardize in vitro antibacterial tests like diffusion, MIC, and MBC testsand therefore it will be possible to compare studies across different studies. [1]

The scientific validity and reproducibility of the antibacterial tests will be improved through adherence to the established protocols and a number of complementary tests. Furthermore, to learn the mechanisms that are involved in the antibacterial effect of Catharanthus roseus extracts, phytochemical profiling will be required, as well as, active compounds isolation.The discovery of new antibacterial compounds can become easier with the help of the development of biotechnology, metabolomics, and other analytical procedures, which can facilitate the production of the new compounds on a mass scale. Moreover, toxicity tests, in vivo and clinical validation should also be done to determine the therapeutic importance of promising extracts. [2],[4]

In the modern world, it is possible to consider Catharanthus roseus which can be used in the design of safe, efficient and cheap plant-based antibacterial agents in the health care sector and contribute to sustainable cultivation strategies and intensive scientific investigation.[3]

VII. Conclusion

Growing problem on antibiotic resistance had strengthened the necessity by considering alternative sources of antimicrobials, especially the ones based on medicinal plants. Catharanthus roseus has been developed as a potentially useful plant with a high potential of antibacterial because there are numerous in vitro studies illustrating its applicability in controlling Gram-positive and Gram-negative bacterial strains. The high levels of phytochemicals in Catharanthus roseus, alkaloids, flavonoids, phenolics,terpenoids, they are considered to be the main reason of the antibacterial activity of this plant as they may work independently or in combination to prevent bacterial growth.

As discussed in this review, differences in extraction procedures, solvent polarity, part of the plants utilized, and type of bacterial species utilized has a great effect on the antibacterial activity of Catharanthus roseus extracts.Agar diffusion methods, MIC/MBC are common in vitro methods used to give crucial initial indicators of the antimicrobial potential of crude extracts. Nevertheless, with their promising outcomes, the majority of researchers are confined to laboratory assessment. Future studies ought to be directed toward standardization of extraction procedures, isolation and characterization of bioactive compounds as well as comprehensive study of the mechanisms of action.Moreover, in vivo experiments and toxicological tests have to be conducted to determine the safety and clinical use of Catharanthus roseus-derived antibacterial agents. Altogether, the results that are analysed in the present paper promote the perspectives of Catharanthus roseus as an efficient candidate in the creation of new plant-based antimicrobials and promote the advancement of the given research.

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