



CODEN [USA]: IAJPBB

ISSN : 2349-7750

INDO AMERICAN JOURNAL OF PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187

<https://doi.org/10.5281/zenodo.5337081>Online at: <http://www.iajps.com>

Research Article

ANALYSIS OF POTENTIAL ANTIMICROBIAL ACTIVITY OF VINCA ROSEA AND ASSESSING ITS ROLE AS AN ALTERNATIVE AGAINST EMERGING ANTIBIOTIC RESISTANT MICROORGANISMS

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Article Received: July 2021

Accepted: July 2021

Published: August 2021

Abstract:

Background: Overuse of antibiotics is a major cause for the emergence of multidrug resistant strains of bacteria therefore, it has become imperative to find new alternatives and effective antimicrobial agents from natural resources available.

Aim: Screening and analyzing the antimicrobial activity of Vinca rosea plant solvent extracts against common gram negative and positive pathogenic strains and determining minimum concentration of plant solvent extract required for inhibitory action.

Material And Methods: Preparing solvent extract of Vinca rosea using Soxhlet Apparatus and determining antimicrobial activity by Disc diffusion method.

Results: Among the six organisms tested, significant antimicrobial activity was observed in methanol root extract against *Klebsiella pneumonia* (15mm zone at 100 mg/ml) followed by *Staphylococcus aureus* (12mm zone at 100mg/ml). For leaf extract, inhibition zone ranged from 5 - 6mm compared to gentamicin, penicillin G and chloramphenicol, root extract showed distinct results than all three in case of *Klebsiella pneumoniae* (gram negative) and effective results than penicillin G and chloramphenicol in case of gram-negative *Escherichia coli* and gram-positive *Bacillus subtilis* and *Staphylococcus aureus*.

Discussion: The present study revealed the antibacterial potential of crude extracts prepared from various dried parts of Vinca rosea, root extracts displayed strong. The difference in antimicrobial activity of extracts can be attributed to its chemical components.

Conclusion: The present study on Vinca rosea further lends support to the use of plant-based compounds as an alternate antimicrobial treatment in the future.

Key Words: Antimicrobial, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, Vinca rosea.

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Please cite this article in press D. Suneetha Devi et al., Analysis Of Potential Antimicrobial Activity Of Vinca Rosea And Assessing Its Role As An Alternative Against Emerging Antibiotic Resistant Microorganisms., Indo Am. J. P.

Sci, 2021; 08(08).

BACKGROUND:

The plant *Vinca rosea* is an herb used in many traditional medicines, its leaves, flowers and root extracts are used in Ayurvedic medicine as well as Chinese medicine. Its traditional uses include leaf juice to treat wasp stings and flower extract used as eye wash in infants. Periwinkle tea is used to treat diabetes and cough. Its extract is used to treat mouth ulcers and sorethroats, also used internally for loss of memory, hypertension, cystitis, gastritis and enteritis, diarrhea and raised blood sugar levels. Use of *Vinca rosea* has displayed dose dependent reduction in blood glucose of both normal and diabetic rabbits and comparable with that of the standard drug, glibenclamide ^[1, 2]. Plant extract and active compounds from it have been used to treat numerous diseases, including diabetes, malaria, and Hodgkin's disease. Several research groups have also shown that *Vinca rosea* has numerous high potential medicinal properties attributed to the presence of many active compounds found in the plant. It shows presence of various alkaloids and studies have revealed that *Vinca rosea* contains more than 130 of different alkaloids, many of which are biologically active, vinblastine and vincristine extracted from the plant are used in the treatment of leukemia. Vincristine is known to bind to tubulin dimers, inhibiting assembly of microtubule structures ^[3, 4].

Antibiotics provide the main basis for the therapy of microbial infection. However, overuse of antibiotics has become the major cause for the emergence and dissemination of multi-drug resistant strains of several groups of microorganisms. In the last few decades, bacterial resistance to antibiotics has become a serious therapeutic problem and the rate at which new antibiotics are being produced has slowed down ^[5]. Bacterial resistance to antibiotics has led to an increased risk of mortality, likelihood of hospitalization and the length of stay in the hospital. Therefore, the research and review for new and effective antimicrobial agents with a broad spectrum of activity from natural sources is increasing day by day. Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate, or cure several human diseases ^[6]. The quest for plants with antimicrobial properties continue to receive attention due to development of microbial antibiotic drug resistance, awareness of the limitations of synthetic pharmaceutical products to control major diseases and lastly the adverse side-effects of synthetic products on host such as hypersensitivity, immunosuppression and allergic reactions. Thus, the search

for novel antimicrobial agents is of utmost importance ^[7,8].

Various studies performed using *Vinca rosea* plant extracts have identified it for several antibacterial, antifungal and antiviral activities possessed by its active compounds ^[9, 10 & 11]. The present study is aimed at screening and analyzing the antimicrobial activity of *Vinca rosea* plant solvent extracts as an alternative to antibiotics. *Vinca rosea* root and leaf extracts were screened for their inhibitory action against some common human pathogenic bacterial strains. As both Gram negative and positive are potentially pathogenic strains, strains from both categories have been selected for this study. This study aims at determining and evaluating the minimum concentration of plant solvent extracts required for inhibitory action of various strains, using the disc diffusion method.

MATERIALS AND METHODS:**PREPARATION OF PLANT EXTRACTS**

The plant material, leaves and roots were washed thoroughly with tap water and then with sterile distilled water for the removal of dust and sand particles. The leaves were shade dried and powdered by hand crushing. The crude dry powder extracts of hand crushed material were prepared by using sterilized mortar and pestle under strict aseptic conditions. The powder was further subjected to aqueous and organic solvent extractions using standard protocol.

ORGANIC SOLVENT EXTRACTIONS

Approximately 10 gm of leaf powder/root was thoroughly mixed with 50 ml organic solvent, like methanol, ethanol, acetone, respectively. The mixture thus obtained was filtered through Whatman No.1 filter paper. The filtrate was then concentrated by complete evaporation of solvent at room temperature to yield the pure extract. Stock solutions of crude extract were prepared by mixing well the appropriate solvent to obtain a final concentration of 100 mg/ml and stored at 4°C after collecting in sterilized glass tubes until use.

DISC PREPARATION

The 5 mm discs were prepared from Whatman No.1 filter paper. The discs were autoclave sterilized. Then various solvent extract discs and control discs were prepared.

NUTRIENT AGAR FOR GROWTH OF MICROORGANISM

Nutrient agar was used for the culturing bacteria, the composition used was 0.5% peptone, Beef extract 0.3%, NaCl 0.5%, 1.5% agar agar, distilled water, pH was adjusted to 7.2.

A total of 6 bacterial strains including both Gram negative and Gram-positive bacteria were selected. *Bacillus subtilis* and *Staphylococcus aureus* Gram positive and *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Escherichia Coli* Gram negative bacteria were selected to assess susceptibility patterns against the extracts prepared in the present study. All strains were collected from the microbial type culture collection, Chandigarh, India. The bacterial cultures were maintained on nutrient agar at 28±2°C, each of the microorganisms were reactivated prior to susceptibility test by transferring them into a separate test tube containing nutrient broth and 24 hrs cultures were used for the antibacterial susceptibility assay.

ANTIBACTERIAL SUSCEPTIBILITY ASSAY

Extracts obtained by various processes were evaluated for their potential antibacterial activities by the standard agar well diffusion assay. All extracts were sterilized by sterile membrane syringe filter.

Table 1. Antimicrobial activity (zone of inhibition in mm) of various *Vinca rosea* extracts on the selected strains of pathogenic bacteria.

Name of the Solvent Extract				
Organism	Ethanol Leaf extract	Methanol Leaf extract	-	Control Acetone
<i>Bacillus subtilis</i>	4 mm	5 mm	-	3 mm
<i>Proteus vulgaris</i>	6 mm	5 mm	-	2 mm
<i>Klebsiella pneumonia</i>	5 mm	5 mm	-	-
<i>Pseudomonas aeruginosa</i>	3 mm	3 mm	-	2 mm
<i>Staphylococcus aureus</i>	3 mm	3 mm	-	1 mm
<i>Escherichia coli</i>	2 mm	2 mm	-	-
Name of the Solvent Extract				
Organism	Ethanol Root extract	Methanol Root extract	Acetone Root extract	Control Methanol
<i>Bacillus subtilis</i>	3 mm	12 mm	12 mm	1 mm
<i>Proteus vulgaris</i>	4 mm	3 mm	3 mm	-
<i>Klebsiella pneumonia</i>	5 mm	15 mm	8 mm	2 mm
<i>Pseudomonas aeruginosa</i>	3 mm	3 mm	3 mm	1 mm
<i>Staphylococcus aureus</i>	6 mm	12 mm	2 mm	-
<i>Escherichia coli</i>	4 mm	4 mm	2 mm	1 mm
Name of the Organism	Antibiotics			
	Gentamicin	Penicillin-G	Chloramphenicol	
<i>Bacillus subtilis</i>	20 mm	5 mm	10 mm	-
<i>Proteus vulgaris</i>	20 mm	15 mm	25 mm	-
<i>Klebsiella pneumonia</i>	10 mm	5 mm	5 mm	-
<i>Pseudomonas aeruginosa</i>	10 mm	1 mm	15 mm	-
<i>Staphylococcus aureus</i>	20 mm	2 mm	10 mm	-
<i>Escherichia coli</i>	13 mm	3 mm	2 mm	-

Antibiotics used were Gentamicin (HIMEDIA gentamicin GEN10 Susceptibility test Discs 10 mcg/disc), Penicillin (HIMEDIA-GP 10 Susceptibility test Discs 10 units/disc), Chloramphenicol (HIMEDIA chloramphenicol C 30 Susceptibility test Discs 30 mcg/disc).

RESULTS:

The results of antimicrobial activity of *Vinca rosea* leaves/roots extracts tested against pathogenic microorganisms such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Escherichia coli* are presented in Table 1. The tested extracts showed moderate to strong antimicrobial activity measured based on the zone of inhibition against the selected bacterial strains. The *Vinca rosea* plant leaves/root extracts were prepared in organic solvents such as acetone, ethanol and methanol. Methanol solvent showed maximum zone of inhibition with active metabolites. Extracts of *Vinca rosea* showed moderate antimicrobial activity at a concentration of 100mg/ml.

Leaf extracts showed zone of inhibition ranging from 5 to 6mm in diameter (Fig. 1). Amongst all the six organisms tested, higher inhibitory activity was observed against the organisms *Klebsiella pneumoniae* and *Staphylococcus aureus*. The ethanol leaf extract at the dose level of 100mg/ml showed inhibition zone of 6mm against *Proteus vulgaris*, followed by 5mm against *Klebsiella pneumoniae*, 4mm against *Bacillus subtilis*, 3mm against *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and 2mm against *Escherichia coli*. The leaf extract of methanol at the dose level of 100mg/ml showed the inhibition zone of 5mm for *Bacillus subtilis* and *Proteus vulgaris*, and 3mm for *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli*.

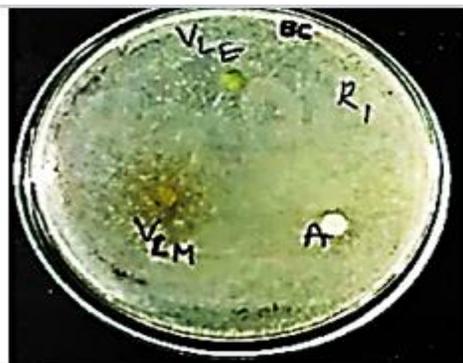
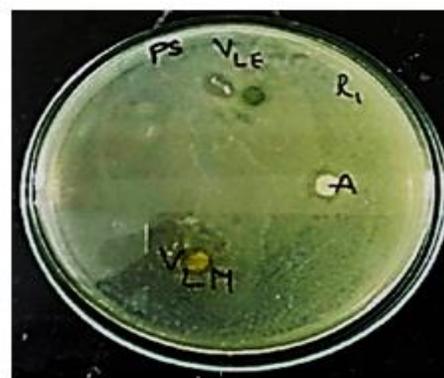
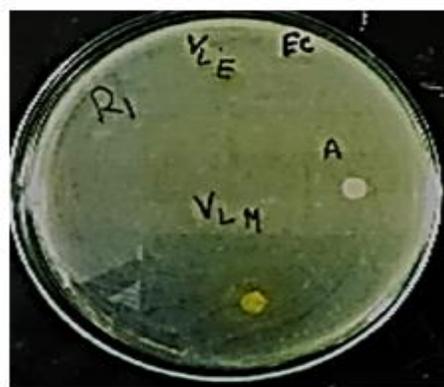
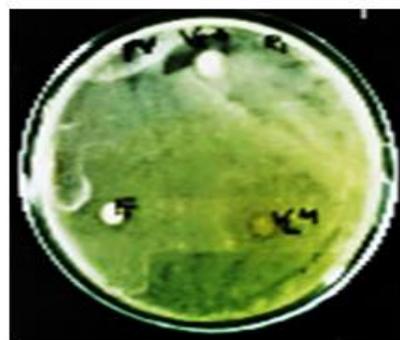
*Bacillus subtilis**Proteus Vulgaris**Klebsiella pneumoniae**Pseudomonas aeruginosa**Staphylococcus aureus**Escherichia coli*

Fig 1. Antibacterial activity exhibited by *Vinca rosea* leaf extract -ethanol extract (VLE) and methanol extract (VLM).

The root extracts of ethanol (Fig. 2) the dose level of 100 mg/ml showed the inhibition zone of 3mm against *Bacillus subtilis* and *Pseudomonas aeruginosa*, followed by the inhibition zone of 4mm against *Proteus vulgaris* and *Escherichia coli*, 5mm and 6mm against *Klebsiella pneumoniae* and *Staphylococcus aureus* respectively. The root extracts of methanol (Fig. 3) the dose level of 100mg/ml showed the inhibition zone of 15mm against *Klebsiella pneumoniae*, 12mm against *Bacillus subtilis* and *Staphylococcus aureus*, 4mm against *Escherichia coli* and 3mm against *Proteus vulgaris* and *Pseudomonas aeruginosa*. The root extracts of acetone with the dose level of 100mg/ml showed the inhibition zone of 12mm against *Bacillus subtilis*, *Klebsiella pneumoniae* 8mm, *Pseudomonas aeruginosa* and *Proteus vulgaris* inhibition zone of 3mm, *Staphylococcus aureus* and *Escherichia coli* 2mm. Inhibitory activity of both methanol leaf extract ethanol leaf extract against *Proteus vulgaris* ranged around 5mm and 6mm respectively. In case of *Escherichia coli* both ethanol and methanol extracts showed minimum inhibitory activity with the zone of 2mm.



Vinca rosea Root extract -Antibacterial activity against *Bacillus subtilis*

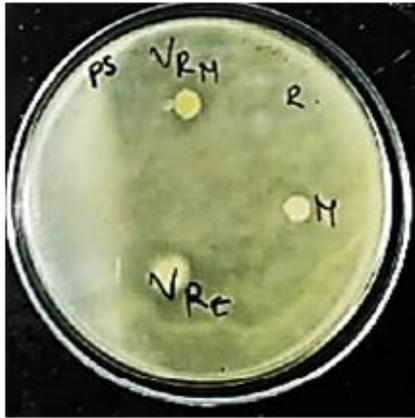


Vinca rosea Root extract -Antibacterial activity against *Proteus Vulgaris*



Vinca rosea Root extract -Antibacterial activity against *Klebsiella pneumoniae*

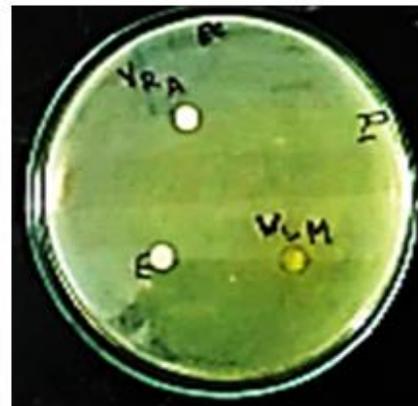
Fig. 2. *Vinca rosea* methanol root extract (VRM), *Vinca rosea* ethanol root extract (VRE) and acetone root extract (VRA) and control methanol (M).



Vinca rosea Root extract- Antibacterial activity against *Pseudomonas aeruginosa*



Vinca rosea Root extract- Antibacterial activity against *Staphylococcus aureus*



Vinca rosea Root extract- Antibacterial activity against *Escherichia coli*.

Fig 3. *Vinca rosea* methanol root extract (VRM), *Vinca rosea* ethanol root extract (VRE) and acetone root extract (VRA) and control methanol (M).

Commercially available antibiotic (Fig. 4) gentamicin showed the most potent at a dose level of 10 mcg/disc, displaying the inhibition zone of 20mm for *Bacillus subtilis*, *Proteus vulgaris* and *Staphylococcus aureus*, 13mm for *Escherichia coli*, 10mm for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Penicillin-G a dose level of 10units/disc showed the inhibition zone of 15mm for *Proteus vulgaris*, 5 mm for *Bacillus subtilis* and *Klebsiella pneumoniae*, *Escherichia coli* 3mm, *Staphylococcus aureus* 2mm, and 1mm for *Pseudomonas aeruginosa*. Chloramphenicol a dose level of 30mcg/disc showed an inhibition zone of 25mm against *Proteus vulgaris*, 15 mm for *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis* 10mm, *Klebsiella pneumoniae* 5mm, *Escherichia coli* 2mm.

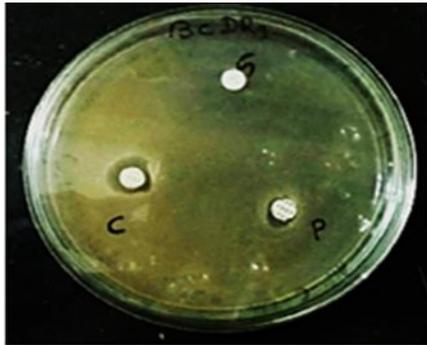
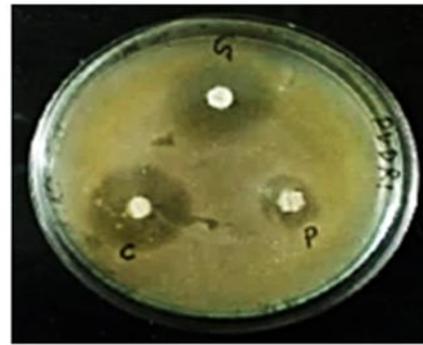
*Bacillus subtilis**Proteus Vulgaris**Klebsiella pneumoniae**Pseudomonas aeruginosa**Staphylococcus aureus**Escherichia coli*

Fig. 4. Antimicrobial activity of broad-spectrum antibiotics: G- gentamicin, P- penicillin-G, C- chloramphenicol.

The antimicrobial activity of methanol root extracts showed enhanced inhibitory action when compared with leaf solvent extracts particularly against *Bacillus*, *Klebsiella* and *Staphylococcus* (Fig. 5). The activity of *Vinca rosea* plant extracts (Fig. 6) showed distinct results than all three antibiotics in case of *Klebsiella pneumoniae* (gram negative) and slightly effective results than penicillin and chloramphenicol in case of *Escherichia coli* and more effective results than penicillin and chloramphenicol, in case of *Bacillus subtilis* and *Staphylococcus aureus* (gram positive). The roots extracts showed strongest inhibitory activity against *Klebsiella pneumoniae* (15mm zone) at 100mg/ml of root extract, followed by *Staphylococcus aureus* which showed 12mm inhibition zone at 100mg/ml root extract.

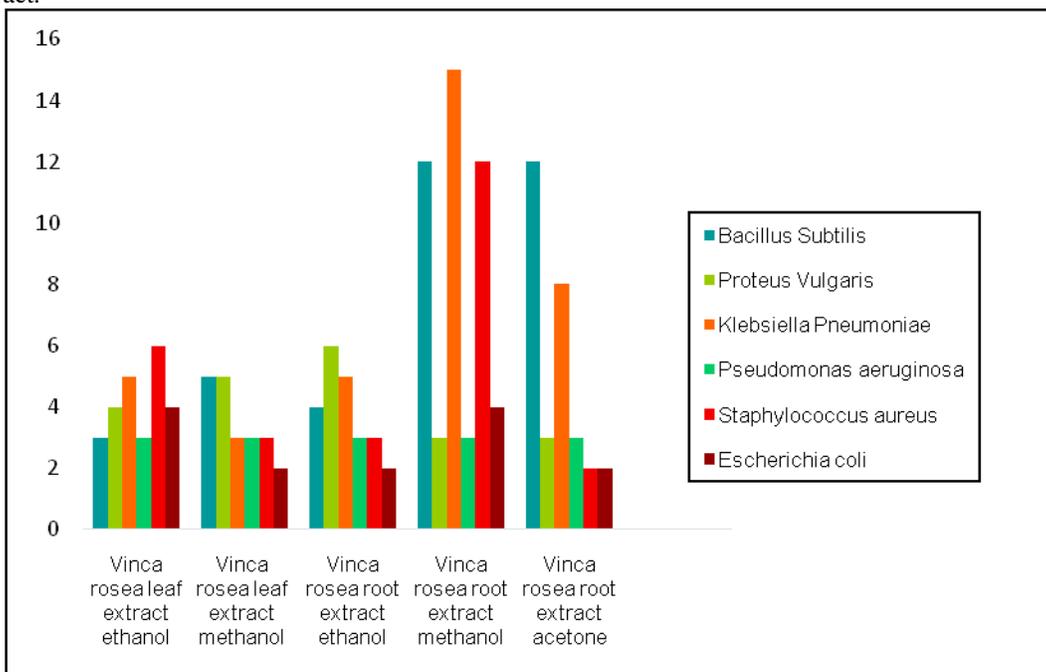


Fig. 5. Graphs comparing the activity of various extracts of *Vinca rosea*.

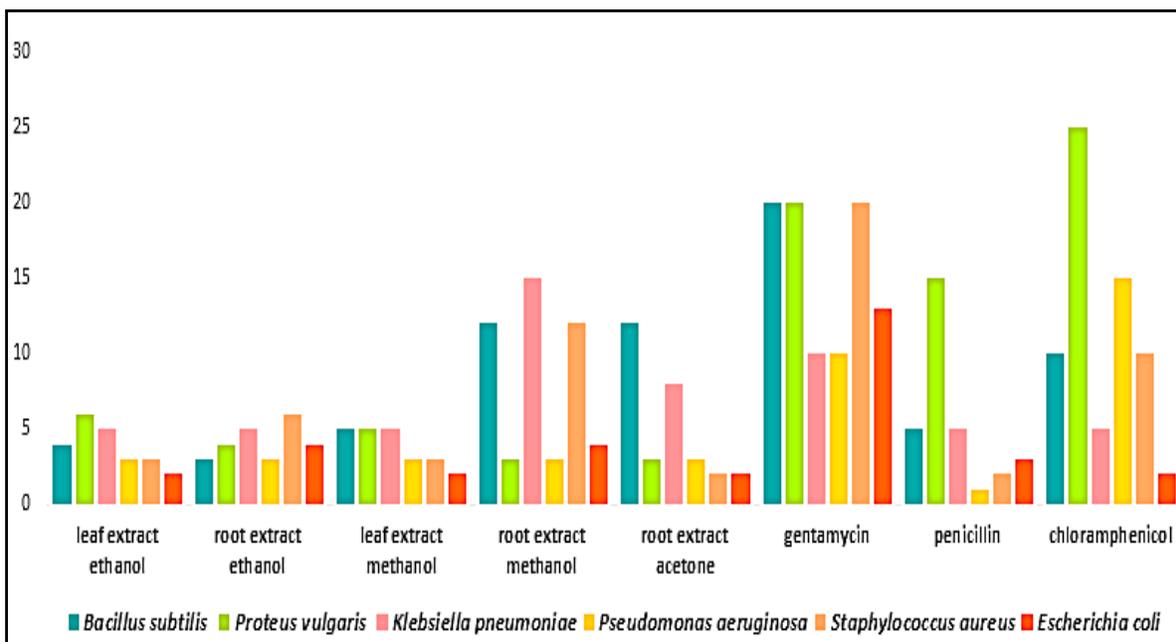


Fig. 6. Comparison of antimicrobial activity of *Vinca rosea* extracts and various antibiotics.

DISCUSSION:

The plant *Vinca rosea* Linn (Periwinkle) plant synonyms include *Catharanthus roseus*, *Ammocallis rosea* and *Lochnera rosea*, Other English names occasionally used include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle, and “Old-maid”. In India it is known as “Nithyakalyani” in Telugu. The plant grows mainly in tropical and sub-tropical regions and is indigenous to Madagascar and cultivated as an ornamental plant in Southern Florida, other geographical sources include Africa, India, Thailand, Taiwan, Eastern Europe and Australia. It is extensively cultivated in Northern India in order to meet their commercial and ever-increasing demand in the indigenous systems of the medicine and also their need to the pharmaceutical industry. It is an Apocynaceous, erect 40-80cm high, woody at the base, ever-blooming, pubescent herb or shrub which has been shown to be a source of many alkaloids. The leaves are petiolate acute base, rounded apex, entire margin and oppositely arranged. The flowers are axillary, violet, rose or white, 4-5cm in diameter. It has enjoyed a popular reputation in indigenous medicine in various parts of the world and has been used both in Ayurvedic and Chinese medicine.

Phytochemical compounds e.g., glycosides, saponin, tannin, flavonoids, terpenoid alkaloid have variously been reported to have anti-microbial activity; the two classes of active compounds in *Vinca rosea* are alkaloids and tannins⁽¹²⁾. According to Russell *et al* most of the bacterial pathogens have been developing resistance against many of the available anti-microbial drugs and because the rate of antibiotic production is much lower than the rate at which the microbes are becoming resistant to them.

The present study shows that the acetone, ethanol, methanol extracts have inhibitory activity against most of the pathogenic microorganisms. The inhibition of the growth of these organisms *in-vitro* by the plant extracts may be due to the presence of some active constituents in the extracts. These active principles act alone or in combination to inhibit the growth of the bacterial organisms⁽¹³⁾. The antimicrobial activity can be enhanced if the phyto-active components are purified and adequate dosage determined for proper administration.

In previous studies extracts from leaves of this plant have demonstrated maximum antibacterial activity. Further studies undertaken to determine the active components of leaf extract displayed significant antibacterial activity⁽¹⁴⁾. Previously in a study, the antimicrobial activity of *Vinca rosea* leaf extract was tested against two pathogenic bacteria,

Staphylococcus aureus and *Pseudomonas aeruginosa*, similar results were obtained in the present study. The methanol extracts of *Vinca rosea* viz., leaves / root showed maximum antibacterial activity compared to extracts of acetone and ethanol. The complication in the antimicrobial activity of propolis could be due to the differences in its chemical components. It has also been reported that the samples collected from different geographic origin with different climates and vegetation show different antibacterial activities⁽¹⁵⁾.

In the present study root extracts displayed stronger antimicrobial activity than the leaves. The present study reveals the antimicrobial activity of methanol / ethanol extracts of leaves and roots of *Vinca rosea* and the antibacterial potential of crude extracts of different parts of *Vinca rosea*. The plant extracts showed antibacterial activity against both Gram-positive and Gram-negative organisms, in confirmation with the earlier study reports. According to the study the antimicrobial activity of the leaf extracts of the plant evaluated in both gram positive and negative bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Bacillus subtilis*, etc. *Staphylococcus aureus* were found to be more susceptible when compared to other bacteria such as *Escherichia coli*, and *Pseudomonas aeruginosa*. Gram-positive and Gram-negative bacteria have been implicated as causative agents of various human infections. The current study illustrates that Gram positive bacteria were more susceptible to this extract compared to Gram-negative bacterial species, previous studies by Pankaj *et al* suggested this could be due to the differences in chemical composition and structure of cell wall of both types of microorganisms.

CONCLUSION:

Medicinal plants are an important resource for primary healthcare and complementary healthcare systems. Use of plants as a source of medicine has been inherited and is an important component of the healthcare system. These readily available and culturally important traditional medicines form the basis of an accessible and affordable healthcare regime and an important source of livelihood for indigenous and rural population. The literature in this area of research showed that plant kingdom has many species of plants harboring substances of medicinal value that are yet to be discovered. Even though research for new therapeutics for various disease conditions is expanding, the identification of substances of medicinal value harbored by plants is still in nascent stages; though large numbers of plants are constantly being screened and have proved to be

significant natural resources for broad spectrum antimicrobial activity with greater emphasis on the preventive action, more pharmacological investigations are necessary. In the current scenario the active constituents present in the plant material need to be identified and isolated to develop medicinal drugs. The acceptance of medicines from plant origin as an alternative form of healthcare is increasing because they are serving as promising sources of novel antibiotic prototypes and a vital source of new lead compounds for drug discovery and development^(16, 17, 18 & 19).

The discovery of various antibiotics against bacterial infection resulted in misuse and overuse of antibiotics and this led to development drug resistant bacteria, the recent surge in drug resistant strains of superbugs has led to a potential global health crisis⁽²⁰⁾. Medicinal plant is the most exclusive source of life saving drugs for majority of the world's population. They continue to be an important therapeutic aid for alleviating the ailments of human beings. The search for defense mechanism, longevity and remedies to relieve pain and discomfort drove early man to explore these immediate natural surroundings. It led to the use of plants, animal products and minerals etc., and the development of a variety of therapeutic agents. Today, there is a renewal interest in traditional medicine and an increasing demand for more drugs from plant sources because green medicine is safe and more dependable than costly synthetic drug, many of which have adverse side effects.

Vinca rosea was investigated from the ancient time for their phytochemical components and their therapeutic effect. The plant contains enormous phytochemical constituents of various medicinal applications. This plant also possesses various properties such as anti-cancer, anti-diabetic, anti-helminth, anti-diarrheal, anti-microbial etc. Hence most work could be done on the above plant to reveal the unknown mysteries which would help the need of the present pharmaceutical world. The medicinal uses of these plants to heal diseases including infection and has been extensively applied by people. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, the development of modern drug from *Vinca rosea* should be emphasized for the control of pathogenic microorganisms. The results of present study *Vinca rosea* has further strengthened the scientific evidence supporting the need for the development of antibacterial products and their use as an alternate route for the treatment of bacterial infection in the future.

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