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RESEARCH ARTICLE

A STUDY ON VINCA ROSEA LINN. ROOTS ASSAYING ANTIOXIDANT AND ANTI-BACTERIAL ACTIVITY WITH REFERENCE TO ITS PHYTOCHEMICAL SCREENING

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ABSTRACT

Introduction: Plants are one of the fundamental core elements of contemporary medicine. Vinca rosea Linn. is a rich source of alkaloids and is distributed in all parts of the plant which gives the scientific platform to this drug. Besides alkaloids it is also rich in phenolic compounds, organic acids, and amino acids of pharmacological significance in its different parts, however, there have been limited studies on the phytochemical and biological activities of its root. Therefore, this study aimed to explore the identity of Vinca roots and elucidate their phytochemical profile and assess their antioxidant and antibacterial properties. ***Methods:*** Gravimetric analysis was done to evaluate total alkaloid content in the root and ORAC assay and Agar well diffusion assay were performed for analysing its antioxidant potential and anti-bacterial activity respectively. ***Results:*** The phytochemical analyses showed the presence of carbohydrates, flavonoids, saponins, phenols, alkaloids and terpenoids in the extract. The hydroalcoholic extract showed a zone of inhibition of approx.10 mm against S. typhi and approx.2 mm zone of inhibition against E. coli thus, discloses the antibacterial potential of Vinca roots. The hydroalcoholic root extract of Vinca rosea Linn. was found to be rich in antioxidant activity with an Orac value of 1046.85 µmol of TE/g suggesting a potential source of natural antioxidants. ***Conclusion:*** Therefore, research on novel phytochemicals should be done to identify and isolate them from the roots of Vinca rosea and investigate their diverse applications for bioactive substances.

Key words: *Vinca rosea Linn. Root, Phytochemical, Antioxidant, Antibacterial.*

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INTRODUCTION

Medicinal plants contain physiologically active components which over the years have been used in traditional medical practice for the treatment of a wide range of illnesses (Adebanjo, 1985). As a result, plants are one of the fundamental core elements of contemporary medicine. According to the World Health Organization (WHO, 2001), phytomedicines are herbal preparations, produced by subjecting plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes which may be produced for immediate consumption, or as the basis for herbal products. The plant products may contain recipient or inert ingredients, in addition to the active ingredients. Phytomedicines can also be naturally occurring substances, usually of plant origin, used in the prevention and treatment of disease. Madagascar periwinkle or rosy periwinkle is the common name for Vinca rosea. It is an evergreen herb and one of the famous medicinal herbs in cancer treatment. It is native and endemic to Madagascar but is frequently grown as an ornamental and medicinal plant. The storehouse of the famous oncolytic alkaloids - vincristine and vinblastine, Vinca is a boon against cancer. Vinca rosea is a rich source of alkaloids and is distributed in all parts of the plant which gives the scientific platform to this drug. The study aims to analyse the presence of different phytochemicals present in the roots of Vinca rosea Linn.

This plant has been extensively studied for its therapeutic potential (Cheng, 2016 and Sukhdev, 2017) due to its rich alkaloid content (Abouzeid, 2019; Boga, 2011; Boyadzhiev, 2004; Liu, 2015). As a novel approach, total alkaloid content was determined using gravimetric analysis and successfully optimised in this study. The roots were also investigated for their antioxidant capacity and anti-bacterial activity which can be exploited further for their therapeutic potential. The root extract was examined against Gram-positive and Gram-negative bacteria through Agar well diffusion assay.

MATERIALS AND METHOD

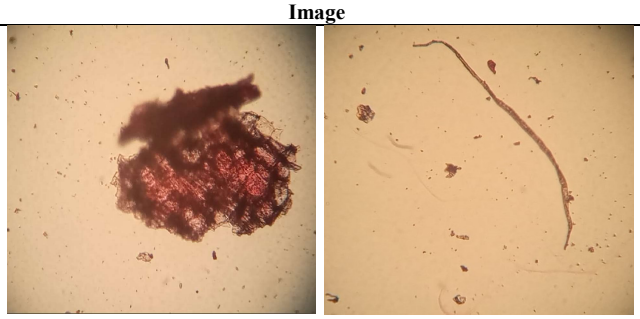
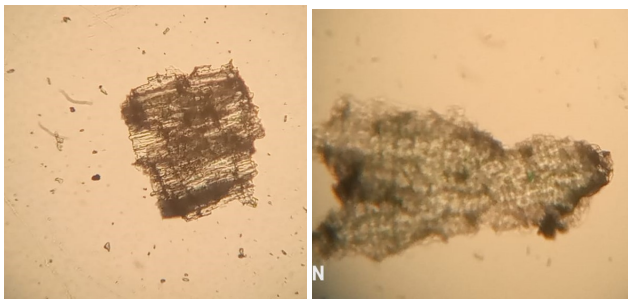
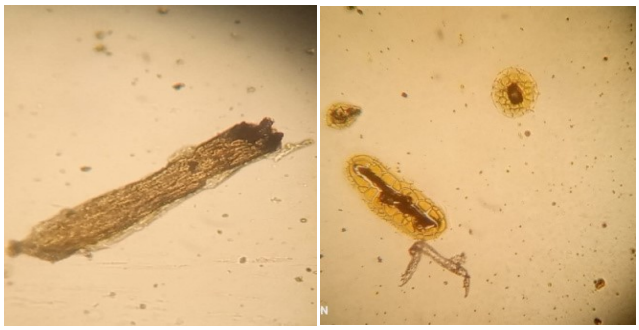
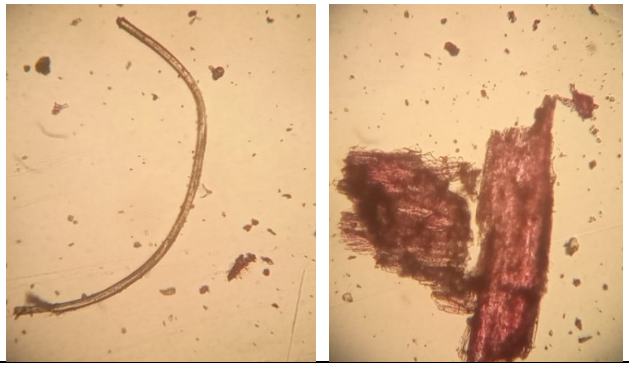
1. ***Plant materials:*** The selected plant of Vinca rosea Linn. was identified and the roots were collected. The plant was taxonomically identified by the Department of Dravyaguna, Banaras Hindu University, Varanasi, UP. The roots were further studied for its authenticity by powder microscopy and Transverse section study. Later, the roots of the plant were washed out in tap water, dried in the shade, crushed to its powderform, and stored in airtight bags.
2. ***Transverse section of Root:*** TS of the fresh root of Vinca rosea Linn. revealed lenticels in the cork and simple and compound starch granules in the cortex. The majority of the root's xylem was in the centre. Here, it was noted that xylem and phloem were both

traversed by uniseriate and biseriate medullary rays. The photomicrograph of TS is shown in Figure No. 1.

3. ***Powder microscopy:*** The fine powder of roots was subjected to powder microscopy using different staining reagents as mentioned in Table 1.
4. ***Preparation of plant extract****:* A bulk of 50 g of powder drug was fully extracted with hydroalcoholic solvent (50:50) using the Soxhlet apparatus. The solvent obtained after filtration was kept in a lyophilizer for 2-3 days to procure powdered extract [Figure 2].
5. ***Phytochemical Analysis (Qualitative method)****:* Phytochemical analysis of the root extract was essayed using standard qualitative methods as per the guidelines of ASU drugs, CCRAS (2010). The root extract of the plant was screened for the presence of biologically active compounds such as alkaloids, flavonoids, carbohydrates, proteins, tannins, saponins and so on (Table 2).

Determination of total alkaloid content: The oldest group of plant alkaloid groups that are used to treat cancer are the vinca alkaloids[8]. Vinca alkaloids were discovered in the 1950s by Canadian researchers, Robert Noble and Charles Beer. Restorative uses of this plant lead to the observation of these mixes for their hypoglycemic movement, which is of little significance contrasted with their cytotoxic effects (Gidding, 1999). Vinca rosea is a rich source of alkaloids and is distributed in all parts of the plant which gives the scientific platform to this drug. Major bioactive phytoconstituents of Vinca species are Vincristine Vinblastine, Vindoline, Serpentine, Reserpine, 16-Epi-Z-isositsirikine, α-Amyrin acetate, Oleanolic acid (Gupta, 2007). These alkaloids have growth inhibition and affect some human tumours. Vinblastine is used experimentally for the treatment of neoplasms and is recommended for Hodgkin's disease, choriocarcinoma. In vitro tests on various cell types revealed considerable anticancer efficacy for V. rosea.

Table 1. Powder Microscopy of Vinca Rosea

S. no.	Staining dye	Image	Character
1.	Safranine		Lignified trichomes, fibres and lignified red stained parenchyma
2.	Methylene blue		Parenchyma cluster with rectangular and polygonal stained cells
3.	Ferric chloride		Parenchymatous cells, Polyphenol and tannins containing dark red stained cells with clusters and fibres
4.	Eosine		Lignified fibre and Lignified parenchyma with horizontal cell arrangement

Vinca alkaloids also called mitotic spindle poisons, inhibit the assembly of the spindle forms from microtubules, thereby inhibiting mitosis in the cell cycle. Consequently, vinca alkaloids successfully stop cancer cells from proliferating. Different Vinca alkaloids have their unique properties (Asma, 2016). The alkaloid content was determined using gravimetric analysis to judge the quality and purity of the sample and as a standardization tool exhibiting the therapeutic efficiency of the plant.

The alkaloid content was easily extracted by chloroform regarding acid-base extraction and precipitation. The precipitated mass is analysed as the total alkaloid content of the plant sample. The calculated alkaloid content was found to be 6.61 % (w/w) in the roots considered to be a rich source of alkaloids possessing diverse biological properties as evinced in other parts (leaves, stem, seeds) of the Plant (Pham, 2020 and Barrales-Cureño, 2015).

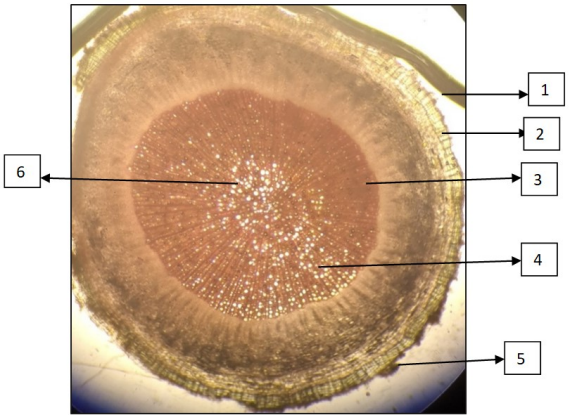


Figure 1. Diagram of TS of Vinca rosea Roots {Representing 1-cork, 2-cortex, 3-xylem, 4-xylem vessels, 5-lenticels, 6-medulary ray}

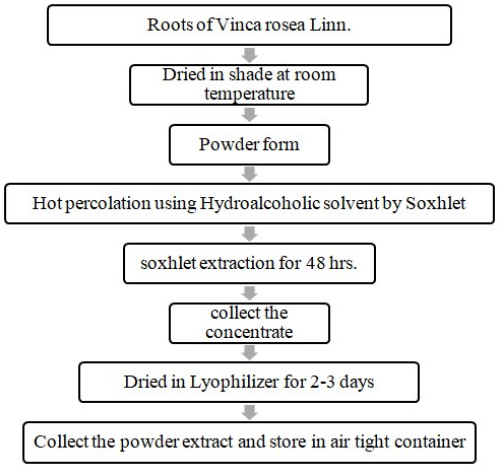


Figure 2. Flow chart of Preparation of Extract

Table 2. Preliminary Phytochemical Analysis of Hydroalcoholic Fraction of Vinca Rosea Linn. Extract

S.No.	Phyto-Compounds	Tests	Vinca rosea Root Extract
1.	Carbohydrate	Molisch's test Benedict's test Fehling's test	Present
2.	Tannin	Lead Acetate Test Ferric chloride Test	Absent
3.	Alkaloid	Dragendorff's test Mayer's test Wagner's test	Present
4.	Phenols	Litmus test Liebermann's test	Present
5.	Flavonoids	Shinoda test Sulphuric acid test	Present
6.	Steroids	Salkowski test Liebermann- burchard test	Absent
7.	Protein & Amino acids	Biuret test Milon's test	Present
8.	Saponins	Foam test Haemolytic test	Present

ORAC Antioxidant activity assay: The detrimental consequences of oxidative stress on human health have ramped up to a critical level. Excessive formation of free radicals and reactive oxygen species has been shown to develop a variety of ailments, including cancer and atherosclerosis, extremely difficult to treat. The plant sources are rich in antioxidants and Phyto-constituents which are useful to alleviate oxidative stress induced by reactive oxygen species and possess therapeutic value in treating these chronic diseases (Machlin, 1987). The Oxygen Radical Antioxidant Capacity (ORAC) assay was performed to evaluate the antioxidant potential of the plant extract (Glazer, 1990). This method is based on the capacity of the antioxidants to scavenge radicals in competition with the probe, resulting in the suppression of the decay of the probe as measured by fluorescence or visible absorption (Takashima, 2012).

Reagents & Chemicals: Fluorescein Solution (Remove from box and store at 4°C), Trolox Standard and AAPH Reagent (Remove from box and store at -20°C), AOX Assay Buffer and black assay plate (Store at Room Temperature), DPPH Solution (Diphenyl Di picryl hydrazine solution 5 mM), Phosphate buffer solution (7.0 pH), Methanol, Distilled Water.

Procedure: Compounds were diluted to a final concentration of 100 µg/mL, with a total reaction volume of 200 µL. Subsequently, 150 µL of working fluorescein solution was added to 60 well of the assay plate. The plate was incubated at 37°C for at least 5 min. Twenty-five µL of AAPH working solution was added to the wells, making up a total volume of 200 µL. Fluorescence was recorded with an excitation wavelength of 485 nm and an emission wavelength of 528 - 538 nm. Data were collected every 2 min for a duration of 2 hrs. and were analysed by calculating the differences of the area under the fluorescence decay curve (AUC) of samples and blank. The whole procedure is elaborated in Figure 3. The values were expressed as Trolox Equivalent (TE). From our results, the hydroalcoholic extract of Vinca rosea Linn. was found to be rich in antioxidant activity with an Orac value of 1046.85 µmol of TE/g suggesting a potential source of natural antioxidants (Bhutkar and Bhise, 2011).

Anti-bacterial assay: Bacterial resistance to antibiotics is a significant therapeutic issue. Vinca leaf extract has been screened against microorganisms to establish its antibacterial and antifungal potential. The antibacterial activity of the hydroalcoholic extract of Vinca rosea root was evaluated using Agar well diffusion assay (Russell, 2002). Anti-bacterial screening was done against common pathogenic micro-organisms gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli.).

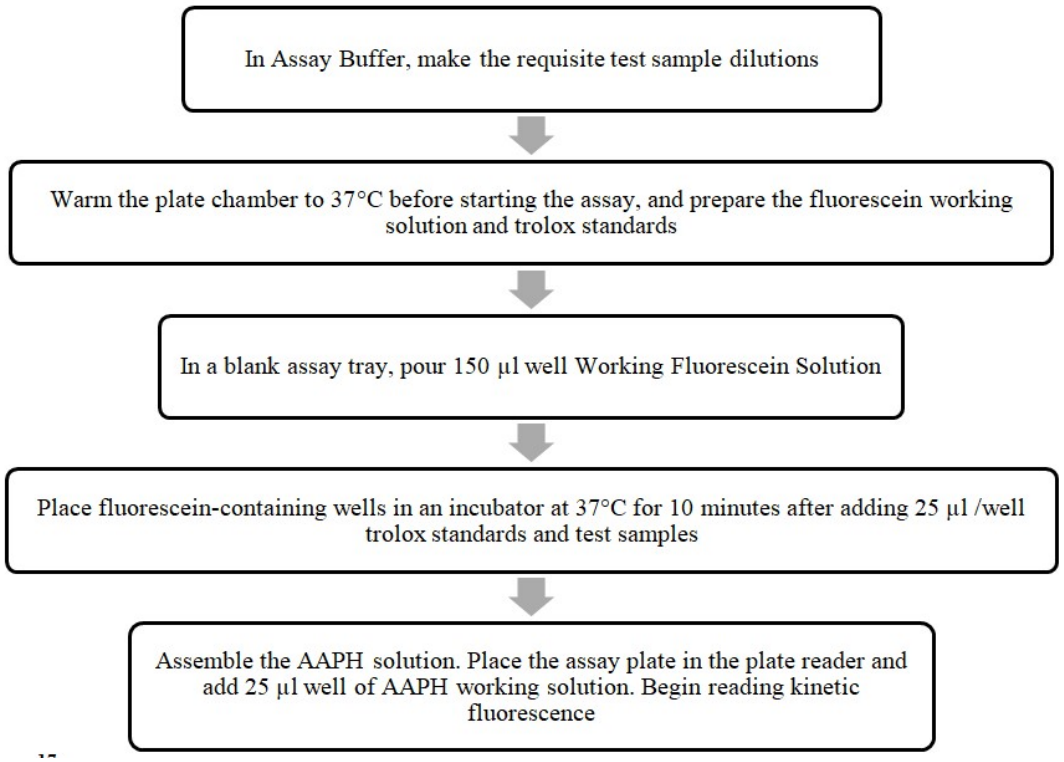


Figure 3. Flowchart of process of ORAC antioxidant activity assay

Table 3. Anti-bacterial activity Vinca rosea Linn. root extract

Anti-bacterial activity	Zone of inhibition (mm)
Name of the bacteria	Hydro-alcoholic root extract
Staphylococcus aureus	10
Rifampicin (Antibiotic)	18
Escherichia coli	2
Rifampicin (Antibiotic)	6

The agar plate surface is inoculated by spreading a volume of the microbial inoculum over the Muller Hinton plate. Then, using a sterile cork borer or tip, an aseptic hole is punched with a diameter of 6 to 8 mm. To compare the antibacterial activities, Rifampicin (20 µg/disc) used as standard antibiotic and as a negative control, a blank disc impregnated with H_2O was used. A volume of 80µL extract solution at desired concentration was introduced into the well. Then, depending on the test microorganism, agar plates are incubated under the appropriate conditions. The studied bacterial strain's growth is inhibited by the antibacterial agent, which diffuses in the agar media. The antibacterial potency of the test samples was measured by determining the diameter of the zones of inhibition in millimetre (Figure 4). The tested root extract showed moderate to strong anti-bacterial activity measured based on the zone of inhibition against the selected bacterial strains (Table 3).

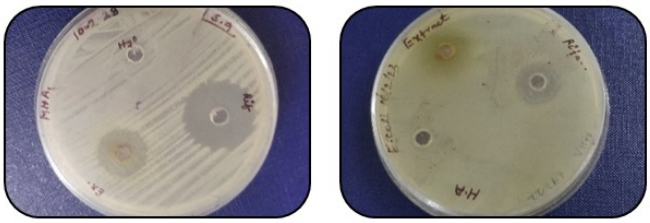


Figure 4. Anti-bacterial activity showing zone of inhibition

DISCUSSION

Vinca rosea is the most popularized medicinal plant that has been traditionally used as a medicine since ancient times. Vinca alkaloids were found by serendipity during diabetes research, in which extracts from the periwinkle plant Vinca rosea were initially studied due to folklore that indicated they had oral hypoglycaemic properties (Beck, 2000). Since that time, Vinca has served as the scientific probe for analysing phytochemicals, secondary metabolites, and the therapeutic outcomes they produce. However, there have been limited studies on the phytochemical and biological activities of its root (Pereira, 2010). Recently, the root has been found to possess high levels of bioactive compounds with strong antioxidant capacity (Pereira, 2010 and Pham, 2017). All parts of the plant contain more than 100 alkaloids in variable proportions, the maximum concentrations are found in the cortex of the roots, particularly when blooming (Jaleel, 2008 and Soleimani, 2013). As per the study roots of Vinca were found to be rich in alkaloids juxtaposed with the quantum availability of alkaloids in leaves, stem, fruit, seeds, and pericarp as asserted by the researchers previously (Karthikeyan, 2008). Many individual alkaloids have been extracted which are found to present abundantly and/or solitarily in roots of the plant. Vinblastine (Kumar, 2018), Vinpocetine (Kumar, 2018), Ajmaline (Kumar, 2018), Yohimbine (Kumar, 2018), vindesinen (Kumar, 2018), Tabersonine (2002), Trytamine (2002) are richly present in Vinca roots. Vinca rosea root extract has been phytochemically analysed as mentioned previously and it has been found that it contains carbohydrates, flavonoids, saponins, phenols, alkaloids and terpenoids (Ataei Azimi, 2008). It lacks tannins, cardiac glycosides, and steroids (2004). Polyphenols act as powerful antioxidants which interfere with the oxidation process as free radical terminators and sometimes also as metal chelators (Van Der Heijden, 2004). Also, according to a research high level of saponins and phenolics were found to have strong correlation with antioxidant power. As saponin-enriched fraction had the highest antioxidant capacity, followed by the root extract, while the aqueous fraction had the lowest antioxidant capacity (Ngoc Thuy Pham, 2019). Additionally, Vinca extracts and isolated alkaloids including vindoline, vindolidine, vindolicine and vindolinine were found to possess antioxidant properties (Ngoc Thuy Pham, 2019; Tiong, 2013; Pham, 2018; Pham, 2018) which could reduce and prevent the oxidation of other molecules. This evidence substantiates the findings in the study venturing Vinca roots as a natural antioxidant and rich in alkaloids which serve as an indicator for their potential application in food and pharmaceutical industries. Also, this study discloses the antibacterial potential of Vinca roots. Methanolic and ethanolic extracts of the leaves and stem have been reported with antibacterial activity against both gram-negative and gram-positive micro-organisms (Govindasamy, 2012). Hydro-alcoholic extract of roots exhibits strong antibacterial activity against positive microorganism and moderate activity against gram negative microorganism might be due do the presence of unique phytochemical constituents that need to be scrutinized to identify the compounds to use as a drug as main ingredient in the traditional medicine.

CONCLUSION

Vinca rosea Linn. root is incredibly rich in alkaloids with a wide range of therapeutic uses. The roots also possess a good antibacterial property and antioxidant activity which in turn is because of various poly phenols engulfed within the identified phytochemicals. As a result, research on novel phytochemicals should be done to identify and isolate them from roots of Vinca rosea. Furthermore, it is necessary to investigate diverse applications for bioactive substances obtained from this material for the pharmaceutical and nutraceutical industries.

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Conflicts of Interest: The authors declare no conflict of interest.

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